ANNUAL REPORT OF THE
LABORATORY OF BIOCHEMICAL GENETICS
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1985 through September 30, 1986

annual report

Two λgt11 cDNA libraries from human brain were screened with 3 oligodeoxynucleotide probes for recombinants coding for α subunits of G signal transducing proteins, which couple receptors activated by hormones or light to effectors such as adenylate cyclase or cGMP phosphodiesterase. Fourteen of the 575,000 recombinant clones screened from a human basal ganglia cDNA library and 12 of the 400,000 clones screened from a human cerebral cortex library were detected with 2 or 3 of the ^{32}P-probes used. DNA inserts from 13 positive clones were sequenced partially; 11 clones were identified as α_s cDNA and 2 clones as α_i. The DNA insert from one of the α_s clones was sequenced completely and additional partial sequences were obtained for 10 α_s clones. Four species of α_s cDNA were found that differ in nucleotide sequence in the region that corresponds to α_s amino acid residues 71-88. The clones differ in the codon for α_s amino acid residue 71 (glutamic acid vs. aspartic acid), the presence or absence of codons for the next 15 amino acid residues, and the presence or absence of an adjacent serine residue. A mechanism was proposed for generating 4 species of α_s mRNA by alternative splicing of precursor RNA transcribed from a single gene.

cDNA from one of the two human α_i clones was sequenced completely (BG-4), and a partial sequence was obtained for the second clone. The first nucleotide residue of BG-4 α_i cDNA corresponds to the 14th residue of the bovine α_i coding sequence and the last residue of BG-4 (1261) is in the 3'-untranslated region. The amino acid sequence derived from the nucleotide sequence of human BG-4 α_i cDNA is highly homologous to bovine and rat α_i sequences reported by others. In addition, the 3'-untranslated region of BG-4 α_i cDNA is highly homologous to the 3'-untranslated regions of bovine and rat α_i cDNA. The 3'-untranslated nucleotide sequences of human, bovine, and rat α_s cDNAs also are highly conserved, but differ markedly from α_i 3'-untranslated sequences. These results suggest that the 3'-untranslated regions of α_s and α_i genes and/or mRNA are needed for functions that have not been identified thus far.

In previous studies we have shown that elevation of cAMP levels of NG108-15 neuroblastoma-glioma hybrid cells or neuroblastoma cells for several days results in 10-100 fold increases in the activity of voltage-sensitive calcium channels, 15-45 fold increases in spontaneous secretion of acetylcholine at synapses, and 5-15 fold increases in the abundance of synapses with cultured striated muscle cells. In addition, the number of molecules of the voltage-sensitive calcium channel protein subunit that binds [^{3}H]-nitrendipine increases 12-fold. We previously obtained about 100 cDNA clones that hybridize to species of mRNA that are more abundant in NG108-15 or NS20-Y cells that had been treated with dibutyryl cAMP for several days then in untreated control cells. Quantitative studies on the extent of increase in abundance of the species of mRNA that respond to dibutyryl cAMP were performed using the cloned cDNA as probes. Twenty cDNA clones were obtained that hybridize to species of poly A$^+$ RNA that increase in abundance 10-90 fold due to treatment of cells with dibutyryl cAMP. Northern blots also were performed and the number of bands of poly A$^+$ RNA that hybridize to each cloned cDNA probe and their chain lengths were determined.

Affinity purified antibodies to the α, β, and γ protein subunits of voltage-sensitive calcium channels were used to screen a λgtll cDNA library prepared from poly A^+ RNA from rat skeletal muscle. Approximately 20 recombinant clones were found that were identified tentatively as calcium channel α subunit cDNAs. Other cDNA clones were obtained that are putative γ subunit clones.

In previous studies a putative cDNA clone for choline acetyltransferase was found. We now have determined the nucleotide sequence of the 1118 bp DNA insert. Partial amino acid sequences of several peptides derived from choline acetyltransferase by the action of peptidases were obtained in collaborative studies by Lou Hirsh and his colleagues in Dallas. The λgtll cDNA library was screened again with 2 new oligodeoxynucleotide probes to different regions of choline acetyltransferase and cDNA clones were obtained that were recognized by both probes. Further studies with these clones are in progress.

Antigenic molecules termed TOP, which are distributed in a dorsal > ventral concentration gradient in chicken retina, are expressed early in development (by 48 hr after fertilization) in the optic cup of chicken embryos and continue to be expressed in retina thereafter. ^{35}S-labeled-TOP-antibody complexes were purified by protein A-Sepharose column chromatography and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. TOP also was purified from dorsal retina by anti-TOP IgG-Affigel 10 affinity column chromatography. Both purification methods yielded one major band of protein with an M_r of approximately 47,000. A protein of M_r approximately 47,000 also was purified from chicken embryo brain. Cultured cells dissociated from 8-day chicken embryo retinas accumulated the amount of TOP expected of cells in the intact retina, depending on the position of the cells in the retina. TOP accumulations by cells dissociated from dorsal or ventral retina, mixed in different proportions and cocultured were additive. These results show that TOP is a protein, that the gradient of TOP is established early in development, and that perpetuation of the gradient does not depend on the continuous presence of an extracellular gradient of diffusable molecules or on maintenance of interactions between cells. Synapses and neurites in the retina of developing chick embryos were reduced markedly by injection of anti-TOP antibody into the eye.

The addition of bradykinin to NG108-15 cells was shown in previous studies to increase cellular levels of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. The newly synthesized IP_3 in turn stimulates the release of stored calcium ions into the cytoplasm, thereby activating calcium-dependent K^+ channels. The increased efflux of K^+ ions results in cell hyperpolarization. This is followed by cell depolarization due to inhibition of M channels, thereby decreasing the rate of K^+ efflux from cells via M channels. Additional results now show that inhibition of M channels is due to diacylglycerol and Ca^{2+} dependent activation of protein kinase C. Several phosphoproteins were detected by two dimensional gel electrophoresis whose synthesis is dependent upon the addition of bradykinin to cells. Whereas, injection of inositol 1,4,5-trisphosphate inside NG108-15 cells results in the release of stored calcium into the cytoplasm, injection of inositol 1,3,4-trisphosphate or inositol 1,3,4,5-tetrakisphosphate has little or no effect on calcium mobilization, but instead results in the activation of nonspecific cation channels. Calcium ions are not required for the activation of the nonspecific

cation channels. The nature and significance of these findings warrant further investigation in light of recent reports that inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate are present in some tissues and that inositol 1,3,4,5-tetrakisphosphate is synthesized by phosphorylation of inositol 1,4,5-trisphosphate, catalyzed by an appropriate kinase, and that inositol 1,3,4-trisphosphate is formed by dephosphorylation of inositol 1,3,4,5-tetrakisphosphate.

Immunofluorescence staining on cryostat sections prepared from embryonic brain extract-treated myotubes revealed a precise colocalization of a 43,000 M_r cytoplasmic protein (distinct from actin) with newly-formed ACh receptor aggregates. This result is consistent with a role for the 43,000 M_r protein in receptor immobilization, as suggested indirectly by studies from other laboratories on fish electric organ and the neuromuscular junction.

We previously showed that partially purified and highly purified fractions from the extracellular matrix of the Torpedo electric organ induce ACh receptor aggregation in cultured myotubes with a time course similar to that of embryonic pig brain extract. We now have found that antiserum against a partially purified fraction from Torpedo (700 units/mg protein) can absorb about 60% of the receptor aggregation activity of brain extract. Under the same conditions, 90% of the activity in the Torpedo fraction was absorbed. This result is consistent with the presence of immunologically related aggregation factors in electric organ and brain.

We previously showed that neural factor induced formation of ACh receptor aggregates on tetrodotoxin-treated myotubes is associated with the localized deposition of basal lamina. We now find that embryonic brain extract and ciliary ganglion explants induce a widespread deposition of basal lamina on non-tetrodotoxin-treated myotubes. Ascorbate oxidase blocks this deposition of basal lamina, suggesting that ciliary ganglion and embryonic brain extract contain ascorbate-like factors that promote muscle basal lamina formation. The extensive induction of ACh receptor aggregates by ciliary ganglion explants was only partially inhibited by ascorbate oxidase, and basal lamina deposition still occurred at the ACh receptor aggregate sites. These results suggest that the ascorbate-like factor contributes to, but is not primarily responsible for the induction of receptor aggregates. In addition, they suggest that deposition of basal lamina at receptor aggregates can occur independently of the ascorbate-like factor.

We have been studying hormonal and neurotransmitter-dependent mechanisms that regulate the gene for proenkephalin (pEnk), the precursor of the opioid peptides methionine- and leucine-enkephalin, in clonal cell lines of neural origin, as well as in rat brain. NG108-15 neuroblastoma-glioma hybrid cells and C6 rat glioma cells contain pEnk mRNA, quantitated by blot hybridization. C6 cells contain a much higher abundance (3-6 pg/μg RNA) but lower enkephalin content than NG108-15 cells. Treatment of C6 cells with compounds that activate adenylate cyclase and raise the cAMP concentration (e.g. by a beta-adrenergic receptor agonist such as (-)-norepinephrine or by forskolin) elevate the pEnk mRNA abundance. Glucocorticoid hormones such as dexamethasone or cortisol, while having no effect alone on the pEnk mRNA level, potentiate the effect of cAMP elevation, producing maximum elevations of 8-fold. C6 cells contain proenkephalin but do not process this precursor significantly. Treatment with

norepinephrine and dexamethasone raises the content of proenkephalin 11-fold. Treatment of cells with glucocorticoid and forskolin for 1-6 hr increases pEnk gene transcription at least 2.5-fold. These results suggest that glucocorticoids and neurotransmitters that elevate cAMP transcriptionally regulate enkephalin biosynthesis in enkephalinergic cells.

Studies have been initiated on the regulation of expression of the gene for proneuropeptide Y (pNPY), the precursor of neuropeptide Y, a putative regulator in the autonomic nervous system. pNPY mRNA is relatively abundant in NG108-15 hybrid cells. Treatment of these cells with glucocorticoids elevates pNPY mRNA 2-fold.

Two novel neuropeptides having anti-analgesic activity were recently isolated and sequenced by Dr. H. Y. Yang's group. Their structures are Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2 (A18F-NH_2) and Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2. A rat hypothalamus λgt11 cDNA library was screened with ^{32}P-oligodeoxynucleotides corresponding to portions of these peptides and putative A18F-NH_2 cDNA clones were obtained.

Nearly all prokaryotic genes use the translation initiation codon AUG. However, there are a few examples where GUG or UUG function as initiation codons in E. coli. The gene for E. coli adenylate cyclase, is one of the genes that uses the unusual UUG initiation codon. We have investigated the effect of this unusual initiation codon on the expression of the adenylate cyclase gene by changing the DNA sequence coding for the UUG initiation codon to ATG and GTG, using oligonucleotide-directed mutagenesis. A comparison of the activities associated with the three codons was made in three different environments: (1) in the normal environment, with the adenylate cyclase gene expressed from its own promoters, (2) in a transcription fusion with the adenylate cyclase gene under the transcriptional control of the phage lambda promoter, and (3) in a gene fusion with the adenylate cyclase gene fused to the E. coli galactokinase gene to generate a fusion protein with galactokinase activity. In each of the three environments, it was observed that the UUG initiation codon had the lowest efficiency of translation initiation and the AUG initiation codon had the highest efficiency, while the GUG initiation codon was intermediate. These results may provide a partial explanation for the finding that the cellular concentration of adenylate cyclase is very low.

In E. coli cAMP plays a crucial role in regulating the expression of inducible genes. The levels of this nucleotide are controlled primarily by a catabolite-dependent modulation of adenylate cyclase activity. Insight into the mechanism of regulation of the activity of this enzyme has come primarily from studies of permeable cells. Current information suggests that the phosphoenolpyruvate:glucose phosphotransferase system (PTS) is intimately involved in the regulation. Additionally, potassium and phosphate ions play key roles in modulating adenylate cyclase activity. A model for interaction of adenylate cyclase with PTS proteins and potassium phosphate to form a regulatory complex was proposed previously by us. The purpose of the present study was to test the proposed model for adenylate cyclase regulation using a reconstitution approach. We found that all of the unique features of adenylate cyclase characteristic of the regulatory complex observed in permeable cells were reconstituted in cell-free extracts. The results strongly support the proposal that adenylate cyclase activity is regulated by PTS proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00009-12 LBG

PERIOD COVERED

October 1, 1985 - September 30, 1986

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)*

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Patricia Bray, Biologist, LBG, NHLBI
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Koh Yano, Visiting Fellow, LBG, NHLBI
David Trisler, Staff Fellow, LBG, NHLBI

COOPERATING UNITS *(if any)*

Allen Spiegel, Chief, MDB, NIADDK
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LAB/BRANCH

Laboratory of Biochemical Genetics

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Section of Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
11	9	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.)*

λgtll cDNA libraries derived from human brain poly A^+ RNA were screened for recombinants that code for α-subunits of G signal transduction proteins. Eleven α_s and two α_i clones were characterized. Four species of α_s cDNA were found. A mechanism for generating the four species of α_s mRNA by alternative splicing of precursor RNA was proposed.

Treatment of NG108-15 neuroblastoma-glioma hybrid cells cAMP for several days results in thee appearance of voltage-sensitive calcium channels and other ions channels. Twenty cDNA clones were obtained that hybridize to species of poly A^+ RNA that increase in abundance 10-90 fold due to treatment of cells with dibutyryl cAMP. Affinity-purified antibodies to the α or Y protein subunits of voltage-sensitive calcium channels were used to screen a λgtll cDNA library. Twenty putative voltage-sensitive calcium channel α subunit cDNA clones and 29 putative Y subunit clones were found.

Antigenic molecules termed TOP, which are distributed in a dorsal > ventral concentration gradient in chicken retina, were purified. TOP was shown to be a protein with an M_r of 47,000. The gradient of TOP in the retina is formed early in embryonic development. Thereafter, perpetuation of the gradient does not depend on the continuous presence of an extracellular gradient of diffusable molecules or on maintenance of interactions between cells. Synapses and neurites in the retina of developing chick embryos were reduced markedly by injection of anti-TOP antibody into the eye.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL00017-11 LBG

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Acetylcholine Receptors

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

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Laboratory of Biochemical Genetics

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2	1.5

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☐ (a1) Minors

☐ (a2) Interviews

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

Our aim is to study the organization of neurotransmitter receptors on nerve and muscle cells in relationship to the development and function of synapses. Our recent work has focused upon the factors, extinsic and intrinsic to the developing skeletal muscle fiber, which regulate the distribution of nicotinic acetylcholine receptors. Acetylcholine receptor aggregation is induced on cultured myotubes by neuronal factors, and this system is used to study the mechanisms of receptor aggregation, as well as the stabilization or elimination of aggregates which occur in developing neuromuscular junctions. Our major findings in the past year are as follows: 1) A 43 kilodalton nonactin protein is precisely colocalized with newly formed receptor aggregates. This protein may play a role in receptor immobilization. 2) 60% of the receptor aggregating activity of embryonic brain extract is absorbed by antiserum against a receptor aggregating fraction from the extracellular matrix of Torpedo electric organ, suggesting the presence of immunologically related aggregation molecules in the two preparations. 3) The formation of muscle cell basal lamina induced by embryonic brain extract and ciliary ganglion explants is blocked by ascorbate oxidase, suggesting that nerve induced formation of basal lamina is mediated by an ascorbate-like factor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00018-09 LBG

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Regulation of the Biosynthesis of the Opioid Peptides and Other Neuropeptides

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

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Laboratory of Biochemical Genetics

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2.5	2.5	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.)*

This project consists of several studies on the molecular genetics of protein precursors of neuropeptides in the mammalian nervous system.

A major study concerns regulation by hormones and neurotransmitters of the gene for proenkephalin (pEnk), the precursor of the opioid peptides methionine- and leucine-enkephalin, in clonal cell lines of neural origin, as well as in rat brain. NG108-15 neuroblastoma-glioma hybrid cells and C6 rat glioma cells contain pEnk mRNA, quantitated by blot hybridization. C6 cells contain a much higher abundance (3-6 pg/ug RNA) but lower enkephalin content than NG108-15 cells. Treatment of C6 cells with compounds that raise the cyclic AMP concentration by activation of adenylate cyclase (e.g. beta-adrenergic receptor agonists such as (-)-norepinephrine or forskolin) elevate the pEnk mRNA abundance. Glucocorticoid hormones such as dexamethasone or cortisol, while having no effect alone on the pEnk mRNA level, potentiate the effect of cyclic AMP elevation, producing maximum elevations of 8-fold. C6 cells contain proenkephalin but do not process this precursor significantly. Treatment with norepinephrine + dexamethasone raises the content of proenkephalin 11-fold. Treatment of cells with glucocorticoid + forskolin for 1-6 hr increases pEnk gene transcription at least 4-7-fold. These results suggest that glucocorticoids and neurotransmitters that elevate cyclic AMP transcriptionally regulate enkephalin biosynthesis in enkephalinergic cells.

Studies have been initiated on the regulation of expression of the gene for proneuropeptide Y (pNPY), the precursor of neuropeptide Y, a putative regulator in the autonomic nervous system. pNPY mRNA is relatively abundant in NG108-15 hybrid cells. Treatment of these cells with glucocorticoids elevate pNPY mRNA 2-fold. Probable rat pNPY cDNA clones are being characterized.

In collaboration with Dr. H.-Y. Yang (NIMH) efforts have continued to clone cDNA for precursor(s) to two anti-analgesic neuropeptides isolated by her group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00151-16 LBG

PERIOD COVERED

Oct. 1, 1985 - Sept. 30, 1986

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)

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5.6	4.6	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.)

A. Translational Efficiency of the Adenylate Cyclase Gene.

Since the analysis of the structure of the adenylate cyclase gene revealed that the initiation codon was UUG rather than the usual AUG, it was suggested that this feature of the gene structure might play a regulatory role. We tested the possibility that the UUG codon decreases the efficiency of translation. By the use of recombinant DNA procedures, we constructed plasmids containing the gene for adenylate cyclase in which the initiation codon was UUG, GUG or AUG. Tests of the expression of adenylate cyclase activity by these plasmids revealed that the AUG initiation codon promoted from three to five times as much gene expression as did the UUG initiation codon. These results support the hypothesis that the UUG initiation codon limits the expression of the adenylate cyclase gene.

B. Reconstitution of Regulatory Properties of Adenylate Cyclase in Escherichia coli Extracts.

Based on studies carried out using intact or permeabilized cells, it has been proposed that the regulatory properties of E. coli adenylate cyclase require an interaction of this enzyme with proteins of a unique multienzyme sugar transport system. In addition, evidence has been presented that a functional interaction is only observed in the presence of inorganic phosphate. These ideas were put on a firmer basis by a reconstitution approach, using a purified preparation of E. coli adenylate cyclase and homogeneous preparations of the transport proteins. In these experiments, we were successful in reconstituting all the regulatory properties of adenylate cyclase observed in intact cell preparations.

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Annual Report of the
Laboratory of Biochemistry
Section on Enzymes
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

Role of Oxygen Radical Mediated Inactivation of Enzymes in Protein Turnover, Neutrophil Function, and Gliosis

The covalent modification of enzymes by mixed-function oxidation (MFO) systems may be implicated in diverse physiologic and pathologic processes, including: protein turnover, aging, neutrophil function, pulmonary damage, and oxygen toxicity.

(a) Protein Turnover. Results of earlier studies showed that the oxidation of E. coli glutamine synthetase (GS) and several other enzymes by MFO systems leads to the modification of one or more histidine residues and the generation of carbonyl groups. These changes are accompanied by a loss of catalytic activity and to an increase in susceptibility of the oxidized enzymes to proteolytic degradation. Oxidation of glutamine synthetase is not associated with significant changes in protein conformation as measured by changes in sedimentation velocity, viscosity, electrophoretic mobility, intrinsic fluorescence, or CD spectrum. However, as the time of exposure of GS to a MFO system is increased, the number of histidine residues in the enzyme decreases, the number of sulfhydryl groups that will react with alkylating reagents increases, the susceptibility to proteolytic degradation increases, the ability to bind Mn^{2+} decreases, the number of carbonyl groups increases, the heat stability of residual catalytic activity decreases, and significant changes in the surface hydrophobicity of the enzyme take place.

During exposure to the MFO system, the enzyme is converted first to a catalytically inactive, more hydrophilic form, which contains one less histidine residue than the native enzyme. This form is not susceptible to proteolytic degradation by a highly purified protease isolated from E. coli. It is, however, degraded by the neutral cysteine protease isolated from rat liver cytosol. With further oxidation, the hydrophilic intermediate is converted to a form which is more hydrophobic than the native enzyme; this form is readily degraded by the E. coli protease. Susceptibility of the enzyme to oxidation by MFO systems is completely blocked when the active site of the enzyme is occupied by the transition substrate analog methionine sulfoximine. This confirms earlier observations that the substrates of enzymes protect them from oxidation by MFO systems, and provide the basis of a mechanism by which cellular metabolites may regulate protein turnover.

Studies carried out in collaboration with J. F. Hare (University of Oregon) have shown that oxidative modification of E. coli GS "marks" it for proteolytic degradation in vivo. When native GS or a preparation which had lost only one histidine residue was introduced into hepatoma cells by microinjection, the enzymes were only slowly degraded. However, microinjected enzyme which had lost two histidine residues per subunit was degraded about ten times faster than the native enzyme control.

(b) Techniques for the Detection and Separation of Oxidized Protein. To facilitate the visualization and quantitation of low levels of carbonyl groups

in proteins, a highly fluorescent hydrazine reagent has been synthesized by reaction of fluorescein isocyanate with hexamethylenedihydrazine. Reaction of the reagent with carbonyl compounds yield highly fluorescent hydrazones which are stable at 4°C and neutral to acid pH.

With this reagent, it was demonstrated that most proteins in freshly prepared tissue extracts contain significant amounts of carbonyl groups, attesting to the fact that the oxidation of proteins in vivo is a general phenomenon. Results of preliminary studies indicate that antibodies directed against the fluorescence moiety of the protein hydrazone derivatives may be used to separate oxidized from native proteins. Methods are being developed also for the separation of oxidized and native proteins by differential reaction with the immobilized carbonyl reagent, polyacrylamide hydrazide.

(c) Identification of Oxidation Products. It has been established that arginine, histidine, proline, and lysine are among the amino acid residues in proteins that are oxidized to carbonyl derivatives by MFO systems. Arginine and proline are both oxidized to 5-oxo-2-amino pentanoic acid. Proline is oxidized also to pyroglutamic acid and to a substance tentatively identified as either 3-oxo- or 4-oxo-proline. Results of studies with a model MFO system comprised of H_2O_2, Fe^{2+} and chelating agents (the Fenton reagent) have shown that free amino acids undergo oxidative decarboxylation/deamination reactions in which the α-carbon atom is converted to an aldehyde which can be oxidized further to a carboxyl group. For example, phenylalanine yields CO_2, NH_3, and a mixture of phenylacetic acid and phenylacetaldehyde. In addition, small quantities of compounds are formed which are potent inhibitors ($K_i = 10^{-2}$-10^{-9}) of horse liver alcohol dehydrogenase. The inhibitors which were derived from phenylalanine and leucine oxidation were isolated as pure compounds and were identified as the oxime derivatives of phenylacetaldehyde and isovaleraldehyde, respectively. The kinetics of alcohol dehydrogenase inhibition by these compounds is biphasic. Primary binding to the dehydrogenase is competitive with respect to the substrate ethanol, but the binding step is followed by an NAD-dependent reaction leading to essentially irreversible inhibition of the enzyme. Detailed kinetic analyses of this phenomenon were initiated and then discontinued when it was learned that the inhibitory action of oximes on alcohol dehydrogenase was recently studied extensively by other workers.

The ability of bicarbonate ion to stimulate the oxidation of amino acids by Fenton reagent may be related to its effect on the oxidation and reduction of iron ions. Bicarbonate was found to directly stimulate the auto-oxidation of Fe^{2+} to Fe^{3+} and also the reduction of Fe^{3+} to Fe^{2+} by hydroxylamine.

(d) Studies with Hepatocytes. A model in vitro system comprised of freshly isolated rat hepatocytes is being developed for investigations on the role of protein oxidation in aging, exercise, diet restriction and oxygen toxicity. Results of preliminary experiments show that the carbonyl content of proteins in hepatocytes from 1 and 9-month old rats is about the same, but is lower than the level in hepatocytes from 19-month old animals. When hepatocytes from all three age groups were exposed to a MFO system comprised of ascorbate, ferric iron and oxygen or menadione, the levels of oxidized protein increased, and in all groups, the levels of oxidized protein were enhanced by pretreatment of the hepatocytes with inhibitors of endogenous oxidative stress defense systems such

as catalase or the glutathione reductase cycle.

(e) Neutrophil Function. During the period of phorbolester-induced respiratory burst, human neutrophils were found to catalyze covalent attachment of free tyrosine to a large number of endogenous proteins by a pathway that does not involve protein synthesis. Tyrosine incorporation occurs in neutrophils from patients with myeloperoxidase deficiency but not in neutrophils from patients with chronic granulomatous disease. This suggests that cytochrome b559-NADPH oxidase but not myeloperoxidase is involved. Following extensive proteolytic digestion or strong acid hydrolysis of the tyrosine derivatized proteins, dityrsine was tentatively identified among the amino acid products. Positive identification awaits the availability of an authentic sample of dityrosine for comparison.

(f) Gliosis. Studies (in collaboration with Dr. Halks-Miller) on the role of MFO catalyzed protein oxidation in neuronal damage in gliosis have continued. It was found that treatment of injured spheres with α-tocopherol depresses gliotic index, malondialdehyde formation and protein oxidation (as judged by the generation of carbonyl groups). Moreover, the effect of mechanical injury in this system is mimicked by treatment with a MFO system comprised of ferrous iron, ADP, and oxygen.

Cellular Regulation

Tyrosine Kinase Activity. The discovery that a number of hormone and growth factor receptors, and a retrovirus transforming gene product catalyze the phoshorylation of tryosine residues in proteins has focused attention on the role of protein tyrosine kinases in eucaryotic cellular function. To facilitate research in this area, highly sensitive immunochemical procedures have been developed for the detection, isolation and quantitation of protein containing phosphorylated tyrosine residues. In one procedure, proteins containing tyr-P residues are incubated with sheep anti•tyr-P antibodies; the antigen•antibody complexes thus formed are adsorbed to protein A•sepharose containing rabbit•sheep IgG antibodies, after which the proteins containing tyr-P residues were selectively desorbed by incubation with tyr-P. Among other tyr-P-containing proteins, the phosphorylated insulin receptor and the EGF receptor have been isolated and quantitated by this method.

A modification of this technique employed anti•sheep IgG conjugated with horseradish peroxidase. Used in conjunction with electro blotting techniques, it was possible to examine the subcellular localization of Tyr-P containing proteins and to identify them on SDS gels. In collaboration with workers at Johns Hopkins University, it was shown that 30 minutes after incubation with ATP, the binding of EGF to a receptor protein and the phosphorylation of tyrosine residues on the protein occurs primarily in the Golgi apparatus. This suggests that EGF binding activity and tyrosine phosphorylation occur prior to incorporation of the receptor into plasma membrane.

In collaboration with workers in the Laboratory of Vision Research, NEI, it was found that in a mouse lens cell line, the level of phosphorylated tyrosine residues in protein is stimulated by orthovanadate. This stimulation appears to be due to inhibition of protein tyr-P phosphatase activity.

Annual Report
Section on Intermediary Metabolism
and Bioenergetics
Laboratory of Biochemistry
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

A continuing research project deals with the roles of selenocysteine in selenium-dependent enzymes and the mechanism of incorporation of this unusual amino acid in proteins. The biological mechanism of formation of selenocysteine residues in proteins is investigated in two different bacterial systems. Selenoprotein A, a 12,000 M_r protein component of clostridial glycine reductase, contains a single selenocysteine residue. Analysis of peptides containing ^{75}Se-labeled selenocysteine isolated from this protein established the amino acid sequence of 16 residues flanking the selenocysteine to be:

-(Glu)-Cys-Phe-Val-Secys-Thr-Ala-Ala-Gly-Ala-Met-Asp-Leu-Glu-Asn-Gln-Lys-

Based on this information complementary synthetic nucleic acid probes will be used to locate the cDNA encoding the protein. The corresponding cDNA can then be cloned and sequenced in order to determine the identity of the precursor to the selenocysteine residue.

In a collaborative project with the research group of Professor August Böck of the University of München, the cDNA encoding the selenoprotein subunit of Escherichia coli formate dehydrogenase has already been sequenced and our part of the project is to furnish the complementary amino acid sequence of a peptide containing the selenocysteine residue. E. coli cells labeled with ^{75}Se are used as the source of the hydrophobic, membrane bound formate dehydrogenase which is partially purified by hydrophobic column chromatography prior to cleavage by proteases. Tryptic, chymotryptic, and Staphylococcus aureus V8 protease peptides have been prepared and procedures for isolation of ^{75}Se-labeled selenocysteine peptides have been developed. Because of problems inherent in dealing with seleno-peptides and hydrophobic peptides we are now engaged in the scale-up of these methods in order to obtain sufficient amounts of the final pure peptides for sequence analysis.

Revised isolation procedures for the two other protein components of clostridial glycine reductase were developed and are now ready for scale-up. For these proteins (B and C), which are hydrophobic, membrane-associated proteins, the use of phenyl and octyl sepharose chromatographic steps has proven to be of great advantage. Availability of substrate levels of proteins A, B, and C will allow enzyme mechanism studies on the nature of glycine dependent ATP synthesis to be investigated.

One aspect of current studies on amino acid transfer ribonucleic acids (tRNAs) involves the synthesis of selenonucleoside analogues of thionucleosides that occur in tRNAs. Two such nucleosides recently synthesized are 5-methylaminomethyl-2-selenouridine and 5-aminomethyl-2-selenouridine. These were used as proof of identity of two selenium-modified nucleosides isolated

from bacterial $tRNA^{Lys}$ and $tRNA^{Glu}$. The availability of the synthetic selenonucleosides is invaluable for chemical characterization of these interesting seleno-compounds. Also, they can be used as antigens to elicit antibodies for study of the mechanism of introduction of selenium into tRNAs. Based on a recent report that 5-carboxymethylaminomethyl-2-thiouridine occurs in Bacillus subtilis $tRNA^{Lys}$, attempts to synthesize the selenium analogue of this nucleoside were undertaken. This has proven to be a more difficult problem but, if successful, availability of this compound should facilitate identification of other unknown selenonucleosides that we have found in our bacterial tRNAs.

The general applicability of a purification procedure for tRNAs using a monoclonal anti-AMP antibody affinity column matrix was tested. The method takes advantage of the affinity of the 3'-AMP group of uncharged tRNAs for the antibody column. When an amino acid is esterified to the 2' or 3'-hydroxyl group of this terminal AMP residue, the tRNA no longer binds and the charged tRNA passes through the column. Non-esterified tRNAs originally present in the mixture are then eluted as a separate fraction. Using successive acylation and deacylation cycles highly purified preparations of several different tRNAs were isolated from bulk tRNA mixtures.

In a research project on the conversion of biomass to methane supported by the Gas Research Institute of Chicago, we are studying at the enzyme level the conversion of acetate to methane and carbon dioxide. Methanosarcina barkeri, a methane-producing organism that can grow anaerobically on acetate as sole carbon source, was used as biological material. Various lines of evidence indicate a role of carbon monoxide dehydrogenase in the acetate fermentative reaction. We, therefore, have isolated in pure form and determined a number of the properties of the enzyme. Inhibition by glyoxaldehyde, a compound bearing vicinal carbonyl groups, depended upon enzyme turnover suggesting that a reactive group on the enzyme is exposed during catalysis. Cyanide, also an inhibitor, appears to react at the same site on the enzyme as carbon monoxide, the substrate. Unlike the carbon monoxide dehydrogenase isolated from Clostridium thermoaceticum by Wood and coworkers, the M. barkeri enzyme fails to catalyze an exchange of carbon monoxide and the carboxyl carbon of acetyl-CoA. This difference between the biosynthetic and biodegradative enzymes indicates that in the methane fermentation of acetate there is an activated species of acetate other than acetyl-CoA.

During studies on the mechanism of conversion of guanosine and its nucleotides to 8-hydroxy-5-deazaflavin, a cofactor abundant in methane bacteria, it was observed that several purine and pyrimidine compounds were actively decomposed by cells and extracts of Methanococcus vannielii. In fact, guanine, xanthine, uric acid, hypoxanthine, uracil, and thymine all are metabolized to the extent that they can serve as sole nitrogen source for growth of M. vannielii. Studies on the interconversion and subsequent metabolism of the purines indicated that reaction pathways similar to those described for purine-fermenting clostridia are involved.

The commercially useful formation of acetone and butanol by Clostridium acetobutylicum is a two-stage process in which sugars are first fermented to acetic and butyric acids and then these acids are converted to ethanol, butanol, and acetone. Previous studies showed that butyrate kinase levels

uniquely varied over a very wide range during the overall process and thus the enzyme might be involved in the switching of the fermentation from acid to solvent production. The enzyme was purified 50-fold to homogeneity in 31% yield and its composition and properties were determined. Rabbit polyclonal antibodies produced to the pure enzyme will be used to study the time course of the expression of butyrate kinase as a means of optimizing the solvent production process.

The diol dehydratase from Clostridium glycolicum converts ethylene glycol or propylene glycol to the corresponding aldehydes. Unlike other dehydratases described previously the C. glycolicum enzyme is not cobamide coenzyme dependent but instead contains a novel, unidentified radical species. The enzyme is extremely oxygen sensitive and is tightly bound to the cell membrane. Since commonly used solubilization procedures failed to liberate the diol dehydratase, a systematic study was undertaken to develop a method. Sonication of crude membrane preparations in 0.1 M CHES buffer [2(N-cyclohexylamino)ethanesulfonic acid] at pH 8.5-9 containing 2 mM dithiothreitol with the further addition of 30% dimethyl sulfoxide and lysophosphatidylcholine allowed recovery of 45% of the initial activity in a soluble enzyme preparation. These preparations exhibited the same EPR radical signal as the membrane-bound enzyme species. This solubilization procedure liberated more than 95% of the membrane-bound formate dehydrogenase from Escherichia coli and thus may be generally useful for membrane-bound, oxygen-sensitive enzymes.

Annual Report of the
Section on Metabolic Regulation
Laboratory of Biochemistry
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The research projects of the investigators in the Section on Metabolic Regulation are mainly concerned with the physical, chemical, and biological approaches to resolve the mechanisms of enzyme action and its regulation. In the past year, research has been concentrated on (1) regulation of enzymic activity by cyclic cascade mechanisms, by Ca(II) and Ca(II)-calmodulin complex; (2) mechanistic studies of enzyme action and activation; and (3) theoretical analysis of energy transduction via a electro-conformational coupling mechanism. Together, these research programs will provide a better understanding on how enzymes in living cells are regulated and do work.

I. Regulation of Enzymic Activity

A. Regulation of the Mg(II)-ATP-dependent Protein Phosphatase

The Mg(II)-ATP-dependent protein phosphatase, inactive as isolated, is a major phosphorylase phosphatase in skeletal muscle and has been found in numerous other tissues. The enzyme is composed of two types of subunits. The catalytic subunit migrates at ~ 38K D_a during SDS gel electrophoresis and the modulator subunit at ~ 31K Da. This phosphatase activity is present in large quantity (0.9 unit/mg) relative to the spontaneously active protein phosphatase (0.7 unit/mg) in crude extract. The specific activity of this enzyme is protein concentration dependent. In ~ 0.1 nM enzyme concentration, the specific activity is 13,000 nmole/min/mg. This value decreases to about 3,500 nmole/min/mg at ~ 6 nM. These data suggest an association/dissociation mechanism which affects the activity of the activated enzyme.

Previously, we have shown that the activation mechanism involves a transient phosphorylation of the 31K Da modulator subunit catalyzed by kinase F_A (also known as glycogen synthase kinase-3); and the regulatory subunit of type II cAMP-dependent protein kinase inhibits both the activated form and the activation of the protein phosphatase. In the current studies, we showed that the Mg(II)-ATP-dependent protein phosphatase as isolated is a complex consisting of two modulator subunits and one catalytic subunit. This stoichiometry is supported by: (i) Gel filtration data showed that the molecular weight of the complex is about 100K Da while the sucrose gradient centrifugation experiment showed the complex migrates as 70K Da. This apparent value determined by the two methods described above would be larger and smaller, respectively, if the complex is asymmetric rather than globular. (ii) Densitometric scans of silver stained SDS-polyacrylamide gel yielding a molar ratio of the subunits based on the integrated peak areas is 2.08 modulator subunits per catalytic subunit. (iii) The phosphatase subunits have been separated by reverse phase HPLC on a C-18 column run in 0.05% TFA with an acetonitrile gradient elution. The modulator subunit elutes at 27.5 min and the catalytic subunit elutes as two resolved peaks at 39.4 and 41.9 min, labeled as CI and CII. Both CI and CII migrate at ~ 38K DA in SDS-PAGE. However, CII appears to be slightly smaller than CI, derived by cleavage of a small peptide from one end of the catalytic subunit.

The separated subunits were hydrolyzed in boiling HCl and the amino acid compositions were determined. The data for the modualtor subunit were normalized to residue per 22,100 daltons and are in good agreement with recently reported sequence data. The data for the CI and CII were normalized to 38K Da and 37K Da, respectively. The calculated molar ratio is 1.63 modulator subunit per catalytic subunit. (iv) The native phosphatase was denatured in 6 M guanidine and a deconvolution analysis of the UV spectrum was performed, allowing quantitation of Phe, Tyr, and Trp. The determined ratio of PHE and Tyr cannot be accounted for by a 1:1 stoichiometry of modulator to catalytic subunit but is in good agreement with a 2:1 stoichiometry. (v) Steady-state kinetic analysis shows that one modulator binds with very high affinity to the catalytic subunit, while the second modulator subunit functions as a competitive inhibitor for the substrate. In addition, the competitive inhibition mechanism was also confirmed by the kinetics of enzyme inhibition studies using added modulatory subunit. The presence of a second modulator subunit which functions as a competitive inhibitor for the substrate, phosphorylase a, imposes a second level of regulation. Because phosphorylase is present at very high concentration, ~ 50 μM, it can effectively compete for binding to the active site of the phosphatase, while other phosphoprotein if present in low concentration will be protected from dephosphorylation. In view of the fact that Mg(II)-ATP-dependent protein phosphatase is present in relatively high concentration (~ 50 nM) and the isolated catalytic subunit exhibits a broad specificity, the second modulator subunit could provide an additional substrate specificity by protecting other phosphoproteins which do not bind tightly to the phosphatases and are present in low concentration.

B. Glutamine Synthetase Cascade

(1) Quantitation of the proteins involved in glutamine synthetase cascade. Glutamine synthetase in E. coli and other enteric bacteria is rigorously regulated in response to the availability of the nitrogen source. The enzymic activity is modulated by (i) feedback control, (ii) covalent interconversion of the enzyme, and (iii) repression and derepression of the synthesis of the enzyme. Covalent modification involves two nucleotidylation cycles, namely, the adenylylation/deadenylylation of glutamine synthetase and the uridylylation/deuridylylation of a regulatory protein, P_{II}. Adenylylation and deadenylylation of glutamine synthetase, which leads to its inactivation and reactivation, respectively, is catalyzed at two separate catalytic sites of adenylyltransferase (ATase). The activity of this bifunctional enzyme is modulated by P_{II}, which undergoes reversible uridylylation/deuridylylation. The unmodified P_{II} stimulates the adenylylation activity of ATase, while the uridylylated P_{II} is required for the deadenylylation reaction. The enzyme which catalyzes the uridylylation and deuridylylation of P_{II} is also a bifunctional enzyme, the uridylyltransferase (UTase). The relative concentration of the four proteins involved in this bicyclic cascade was determined using sheep antibodies prepared against each protein, all of which was purified from strains which are capable of overproducing each component. The relative abundance of glutamine synthetase, P_{II}, ATase and UTase was found to be 411:42:2.6:1, respectively, for E. coli K-12 grown under nitrogen-limiting conditions.

(2) P_{II} regulating the in vivo synthesis of glutamine synthetase. The transcriptional regulation of glutamine synthetase is closely coupled to the

posttranslational modification cyclic cascade by the fact that the unmodified P_{II} is required to repress the synthesis of glutamine synthetase. DNA sequence of the glnB gene which encodes the protein P_{II} was established using the M13 cloning/dideoxy sequencing method. The result was confirmed by comparing it with amino acid sequences of N-terminal region and three peptides produced by proteolytic cleavage of P_{II}. The data show that P_{II} contains 103 amino acids which have a subunit molecular weight of 11,590 daltons. Two tyrosines were found at residue 46 and 51, and Tyr^{51} is the uridylylation site. P_{II} contains one cysteine^{73}, but no Ser, Asn, or Trp. The cysteine residue was available for reactions with Ellman's reagent only when the protein was denatured. The DNA sequence near the upstream region showed no obvious binding sites for RNA polymerase, indicating that the glnB gene belongs to an unidentified operon.

(3) Study of the in vitro reconstituted bicyclic cascade. The glutamine synthetase cascade was reconstituted by mixing four purified proteins in accordance with the ratio determined in vivo. The state of adenylylation ($\bar{n}_a$) of glutamine synthetase and of uridylylation ($\bar{n}_u$) of P_{II} were measured at steady-state under various concentrations of glutamine and α-ketoglutarate. At a fixed concentration of glutamine, the $\bar{n}_a$ values change from 0 to 12 when one increased the α-ketoglutarate concentration. The sensitivity indexes as defined in the cyclic cascade model were calculated, with respect to α-ketoglutarate, to be 3.26, 3.91, and 3.29 when glutamine concentration was set at 0.05, 0.1, and 0.2 mM, respectively. However, the $\bar{n}_u$ values plateaued before they reached the maximum value of 4, which was obtained only when glutamine was absent. When the concentration of α-ketoglutarate was fixed and the glutamine concentration was varied, both the $\bar{n}_a$ and $\bar{n}_u$ values changed from 0 to its theoretical maximum. The sensitivity indexes for the $\bar{n}_a$ with respect to changes in glutamine concentration are 4.07 and 5.95 when α-ketoglutarate was maintained at 0.02 and 0.1 mM, respectively. Thus, the state of adenylylation responds more sensitively to changes in the concentration of glutamine than of α-ketoglutarate. The different responses of $\bar{n}_a$ and $\bar{n}_u$ to glutamine and to α-ketoglutarate are due to the fact that (i) glutamine and α-ketoglutarate affect both directions of the adenylylation cycle in a reciprocal manner, (ii) they antagonize each other's binding to ATase, (iii) in the uridylylation cycle, glutamine stimulates the deuridylylation reaction but inhibits the uridylylation reaction, while α-ketoglutarate only stimulates the uridylylation reaction, and (iv) glutamine and α-ketoglutarate do not affect each other's binding to UTase. As a consequence, the bicyclic cascade responds with a higher sensitivity to changes in glutamine than to α-ketoglutarate concentration.

C. Regulation of Glutamine Synthetase in S. cerevisiae

Two forms of glutamine synthetase, active and inactive with respect to its biosynthetic activity, have been isolated from S. cerevisiae. Clear separation of the two forms was achieved by HPLC. The active form is stable and easily purified, while the inactive form which elutes as a broad peak is unstable and difficult to purify. The following results indicate that the two forms are products of the same gene: (i) an increase of the inactive form caused by the addition of glutamine was accompanied by a decrease of the active form, (ii) molecular weights of the two forms measured under native and denaturing conditions were identical and were found to be 360K Da and 45K Da, respectively, (iii) antibody directed against the active form cross-reacted with the inactive

form, and (iv) tryptic peptides derived from the two forms yielded identical elution profiles on a C-18 reverse phase HPLC column. Analysis of the active form revealed that the enzyme contains 6 sulfhydryl groups and no disulfide linkage. None of these -SH groups were available for reactions with Ellman's reagent except when the enzyme was completely denatured or dissociated into monomers. The N-terminal was blocked by an acetyl group. Amino acid sequence of several tryptic peptides including the N-terminal peptide were determined. The results show clear homology (> 90%) to mammalian glutamine synthetase but not to E. coli glutamine synthetase. Steady-state kinetic analysis was performed for three different reactions, γ-glutamyltransferase reaction and biosynthetic reaction with either NH_2OH or NH_3 as substrate, using the two forms of enzyme in the presence of either Mg(II) or Mn(II) as divalent cation. The data show that the inactive form exhibits about 10% of the maximal biosynthetic activity of that exhibited by the active form. However, the inactive form possesses a higher activity for catalyzing the nonphysiological γ-glutamyltransferase reaction.

In addition, glutamine synthetase is known to undergo irreversible inactivation by ATP and methionine sulfoximine, due to the formation of the tightly bound ADP and methionine sulfoximine phosphate. This inactivation process was studied using the active form of yeast glutamine synthetase. The reaction was monitored by the incorporation of [γ-^{32}P]ATP into glutamine synthetase, by the decrease in the γ-glutamyltransferase activity and in the biosynthetic activity. The first-order rate for the irreversible inactivation process deviated from the expected first-order rate constant indicating that an inactivated subunit retards the reactivity of its neighboring subunits. Moreover, when the remaining enzymic activity was plotted against the extent of incorporation of ^{32}P, one obtained a concave-up curve for the γ-glutamyltransferase activity and a concave-down curve for the biosynthetic activity. For example, when 50% of glutamine synthetase subunits is occupied by methionine sulfoximine phosphate and ADP, only 25% of γ-glutamyltransferase was detected while 65% of the biosynthetic activity still remained. These results clearly indicate the existence of subunit interaction in the octameric yeast glutamine synthetase.

D. Discovery of a Protein Which Protects Against Oxidative Inactivation of Enzymes

A number of enzymes, including glutamine synthetase, are known to undergo oxidative inactivation in the presence of Fe(III), O_2 and appropriate reducing agents such as dithiothreitol, ascorbate, xanthine/xanthine oxidase, or NAD(P)H/cytochrome P-450 reductase and cytochrome P-450, or NAD(P)H oxidase. Yeast extracts contain a protein which can provide protection against oxidative inactivation. This protector protein was purified to homogeneity. It appears that this protein consists of about 15 identical subunits of M_r 27.5K Da, and exhibits neither superoxide dismutase activity nor that of catalase. In addition, it does not function as an effective chelator for Fe(III). The protective capability of the purified protein is dependent on the reducing system used. The most effective protection was observed when a sulfhydryl reagent, such as dithiothreitol, β-mercaptoethanol, β-mercaptoethylamine, thioglycerol, or glycerol dimercaptoacetate, was involved. Partial protection was observed against the xanthine/xanthine oxidase system and it fails to protect oxidative inactivation involving ascorbate or NAD(P)H/mixed-function oxidase system.

Currently, the protective capacity of this protein appears to derive from its ability to inhibit the reduction of Fe(III) by sulfhydryl reagents. In separate experiments, it has been shown that this protein inhibits both the oxidative consumption of dithiothreitol and the formation of Fe(II) from Fe(III).

E. Phosphorylation/Dephosphorylation of Ca(II)-Calmodulin-dependent Protein Phosphatase by Protein Kinase C

We have shown that Ca(II)-calmodulin-dependent protein phosphatase isolated from bovine brain contains 0.2 to 0.6 mole of covalently bound phosphate per mole of enzyme and that it is phosphorylated by protein kinase C. To further demonstrate that protein kinase C is the phosphorylating enzyme, the extent of phosphorylation was shown to enhance greatly by the presence of Ca(II), phosphatidylserine, and especially phorbol ester or diacylglycerol. Stoichiometry of the phosphatase phosphorylation by protein kinase C was determined to be 2 mole phosphate per mole protein. The presence of calmodulin led to diminished incorporation of phosphate. This observation suggests that the calmodulin-phosphatase complex has a conformation that is less susceptible to phosphorylation or that the phosphorylation site coincides with the calmodulin binding domain. The effect of phosphorylation on the phosphatase activity has not been firmly established. Preliminary results indicate that phosphorylation may result in improved affinity of the phosphatase for calmodulin. It should be pointed out that two other protein kinases have also been investigated for their ability to phosphorylate the Ca(II)-calmodulin-dependent protein phoshatase. It was found that cAMP-dependent protein kinase can phosphorylate slowly the catalytic A subunit of the phosphatase, but calmodulin-dependent protein kinase is ineffective.

One of the phosphoryl groups on the Ca(II)-calmodulin-dependent protein phosphatase was found to be a good substrate for a Mg(II)-activated, Ca(II)-inhibited phosphatase previously reported by us. Dephosphorylation of the phosphatase in the presence of Mg(II) was at least 15 times faster than in the presence of Ca(II) at pH 7.6, 30°C. Since the new phosphatase has high substrate specificity, the fact that the Ca(II)-calmodulin-dependent protein phosphatase is phosphorylated in a Ca(II)-dependent manner by protein kinase C but dephosphorylated in the absence of Ca(II) suggests that any regulation of the calmodulin-dependent protein phosphatase by phosphorylation/dephosphorylation must be closely linked to Ca(II) level in vivo.

F. Purification and Characterization of Ca(II)-sensitive Protein Phosphatase

A Ca(II)-inhibited phosphatase from bovine brain has been purified about 1500-fold by a procedure which includes homogenation and centrifugation, 35-60% $(NH_4)_2SO_4$ cut, DEAE-cellulose chromatography, S-200 gel filtration, red dye A affinity column, and CaM-sepharose column. The enzyme is now ~ 60% pure. The preliminary estimation of the molecular weight yielded a value of about 40,000. This novel Ca(II)-inhibited phosphatase appears to play an important physiological role based on the following observation: (i) it exists in large quantity in the brain, (ii) it dephosphorylates phosphoryl groups that are incorporated into protein by Ca(II)-dependent protein kinases, i.e., its function seems to be the coordination of Ca(II)-regulated phosphorylation/dephosphorylation.

II. Receptor-mediated Signal Transduction and Regulation of Intracellular Calcium Concentration

A. The Role of Phospholipase C in Signal Transduction

Phosphatidylinositol-specific phospholipase C plays an important role in initiating signal transduction through cell surface receptor, by generating two second messenger molecules, diacylglycerol and inositol 1,4,5-triphosphate. The phosphatidylinositol-specific phospholipase C in bovine brain can be separated into two forms, PLC-I and PLC-II, on HPLC-DEAE column. PLC-II was purified to homogeneity. On SDS-PAGE, PLC-II migrates at 145K Da; however, when the same sample was subjected to native gradient polyacrylamide gel, four bands, one major band migrated at 200K Da and three minor bands with molecular weights corresponding to different oligomeric states of the 200K Da protein were visible. Western blot experiments using anti-PLC-II antibody indicated that PLC-I might be derived from PLC-II by proteolytic cleavage. PLC-I and PLC-II catalyze the hydrolysis of both phosphatidylinositol and phosphatidylinositol 4,5-diphosphate. These hydrolytic activities are pH dependent, and more active at pH 5.3 than at pH 7.2; however, the activity of PLC-II is more sensitive to pH changes than that of PLC-I. Ca(II) is a positive allosteric effector for both activities. At neutral pH, Ca(II) is not absolutely required for the hydrolysis of phosphatidylinositol 4,5-diphosphate, while Ca(II) is essential for the hydrolysis of phosphatidylinositol.

The purified PLC-II is phosphorylated by protein kinase C. The physiological significance of this phosphorylation is under investigation.

B. Regulation of Intracellular Calcium Concentration

Rapid intracellular changes in both calcium and pH concentration in sperm of sea urchin Strongylocentrotus purpuratus caused by (i) effects of a chemotactic peptide speract, isolated from egg; (ii) effects of a complex egg coat which causes the sperm acrosome reaction (this reaction includes exocytosis of the sperm acrosomal granule and is essential for fertilization), were monitored by fluorescent indicators. We first introduced the cell permanent ester form of the indicators into sperm. Intracellular esterase activities then regenerates the indicators in the cell. The calcium indicators used were fura-2, indo-1 and quin-2, and pH indicators were dimethylcarboxyfluorescein, biscarboxyethylcarboxyfluorescein and carboxyfluorescein. Using the calcium indicators, it was found that intracellular calcium concentration is about 100 nM in sperm swimming in sea water. Addition of speract causes a rapid increase in intracellular pH followed by a transient rise of intracellular calcium by 2- or 3-fold. Inhibition of the increase in pH inhibits the calcium entry. The increase in intracellular calcium is important because sperm chemotaxis does not occur in the absence of external calcium. The morphological changes of the sperm acrosome reaction also follow increases in intracellular pH and calcium. The increase in intracellular calcium is larger than that induced by speract and it is not transient. Inhibitions which block acrosome reaction partially inhibit both pH and calcium increases. The results suggest two possible mechanisms for increasing the intracellular calcium, they are: (i) calcium entry by means of Na^+/Ca^{2+} exchange activity or (ii) opening of a calcium channel which is coupled to Na^+/H^+ exchange. In addition, preliminary

results from studies using a monoclonal antibody to a specific sperm membrane protein of 210K Da indicates that this protein is important to calcium metabolism by the sperm. Binding of this monoclonal antibody causes intracellular calcium to increase but it does not alter the intracellular pH . However, antibody binding alone does not initiate the acrosome reaction. Acrosome reactions do occur if the antibody effect is coupled with an increase in intracellular pH artifically enhanced by other means.

III. Mechanism of Enzyme Action and Activation

The calmodulin-dependent protein phosphatase consists of a 60K Da catalytic A subunit and a 19K Da Ca^{2+}-binding B subunit. It requires divalent metal ions such as Ni(II), Mn(II), Mg(II), etc. for expression of full catalytic activity. The mechanism of activation by Ni(II) has been studied in detail using p-nitrophenyl phosphate as the substrate. Two Ni(II) can be bound to the phosphatase. The first Ni(II) binds extremely tight and it gives rise to extensive activation in the presence or absence of calmodulin. The time course of activation by the first Ni(II) exhibits a lag phase which, upon analysis, conforms with a first-order kinetic process and is indicative of a conformational rearrangement. However, the second Ni(II) binding results in deactivation. Contrary to other investigators who suggested that the inactivation is the result of subsequent loss of the bound metal ion, our study established that the inactivation is due to the binding of a second Ni (II). The dissociation constant for the initial step is 2 mM and 21 mM followed by a conformational change step which has a rate constant of 4 min^{-1} and 0.078 min^{-1} for the first and second Ni(II) binding, respectively. The notion that the binding of the second Ni(II) was responsible for the deactivation of Ni(II)-activated phosphatase, was supported by the observation that when equimolar amounts of EDTA were added to remove unbound Ni(II) during the early phase of activation, the phosphatase remained essentially activated without undergoing the deactivation process. However, when excess EDTA was added to remove both Ni(II) and Ca(II), the calmodulin-dependent protein phosphatase became essentially inactivated. This inactivation was not due to dissociation of calmodulin in the absence of Ca(II) because a similar observation was found in the absence of calmodulin. Since the phosphatase-bound Ni(II) [first Ni(II)], Zn(II) and Fe(III) cannot be removed by excess EDTA within the duration of experiments, the inactivation must be due to removal of Ca(II) from the B subunit of the enzyme. Thus, Ca(II) binding to the B subunit is vital to Ni(II) activation of the catalytic A subunit.

IV. Model Analysis

A. Theoretical Description of Interfacial Reaction Dynamics

Biomolecular reactions in which one of the reactants is localized at an interface while the other reactant, such as ligand, is initially molecularly dispersed in homogenous phase can occur by two paths. One involves the direct interaction of a homogenous reactant with its interfacially localized reaction partner, and the other proceeds by initial absorption of the homogenous reactant and subsequent surface diffusion to reaction. We have previously obtained an equation for diffusion controlled association of a ligand to active sites localized at a surface. This theoretical formulation took into account both

the direct and surface diffusive mechanism for the association reaction. Recently, we have reformulated the expression for the diffusion controlled dissociation rate constant such that it is applicable to cases where the dissociating moieties have significantly disparate sizes (e.g., ligand dissociating from a cell). This correction is equivalent to mathematically acknowledging that the reactants have finite sizes and are not mutually interpenatrable. We have also shown analytically the equivalence of the branching method and the classical kinetic formulation for evaluating diffusion controlled reactions.

B. Influence of the Transmembrane Electric Potential on the Function of Membrane Proteins

Transmembrane potential plays an important role in determining the activity of membrane bound proteins. The rationale behind this derived from the fact that a modest physiological transmembrane potential of 50 mV across a 50 Å membrane represents an electric field strength of 100,000 V/cm, and that changes in membrane potential can perturb the conformational equilibria of many membrane proteins. Thus, the physiologically obtainable changes in the transmembrane potential can determine the function of many membrane proteins. A theory has been developed to describe the effects of shift in transmembrane potential on the equilibrium controlling the reaction between two conformational states of a transmembrane protein. It is proposed that modulation of the transmembrane potential may provide a general mechanism for the regulation of the activity of many signal- and energy-transducing proteins embedded in the membrane. We have shown that when this modulation is achieved in an oscillatory manner, as would be the situation with external modulation by an applied oscillating electric field, this energy can be absorbed directly by an energy transducing enzyme and converted to do chemical or transport work, if the catalytic cycle of the enzyme involved has at least one step sensitive to the electric field, and that there exists some intrinsic or field-induced asymmetry in the enzyme state stabilities. The results of the analysis revealed that the efficiency and efficacy for such energy transduction is comparable to experimentally measurable ones. Similarly, it was shown that under certain conditions, randomly fluctuating electric fields can also transduce energy through an appropriate membrane enzyme system to do work. This theory can be used to provide a rational interpretation for the observation showing that ATP synthesis occurs even when there is an apparently "insufficient" thermodynamic driving force contained in the proton electrochemical gradient. The proposed theory provides a very general mechanistic formulation through which energy transduction via macromolecules can be understood, and the extension of this theory can also provide a mechanistic explanation on how two a priori independent reactions can be coupled by an enzyme.

Annual Report of the
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October 1, 1985 to September 30, 1986

Research in the Section on Protein Chemistry consists of studies on the physical and chemical properties of macromolecules of biological interest and on 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 involves 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 the numerous examples of stabilization and destabilization of protein structures by ligands, metal ions, and other inorganic ions.

Glutamine synthetase, a strictly regulated enzyme in Escherichia coli, is a dodecamer; each subunit (50,000 M_r) contains a catalytic site with two essential divalent cation sites (n_1 and n_2) and a tyrosyl residue that is the site of covalent modification by enzymatically-catalyzed adenylylation-deadenylylation reactions. The 12 identical subunits of enzyme are arranged in 2 superimposed hexagonal rings of about 140 Å in diameter and centers of adjacent subunits are ~ 45 Å apart. Studies of the interactions of divalent cations, substrates, substrate analogs, and inhibitors with glutamine synthetase from E. coli have continued.

We have shown that the very tight binding of 2 Mn^{2+}, L-methionine-S-sulfoximine phosphate, and ADP ($K_A' > 10^{12}\ M^{-1}$) formed on each subunit of E. coli glutamine synthetase at pH 7 by phosphorylation of the L-glutamate analogue by ATP, stabilizes intersubunit bonding domains. Various analogues of ATP that are substituted at the 6- or 8-position of the adenine ring have since been shown to serve as substrates for the phosphorylation of L-met-S-sulfoximine and thereby can be introduced specifically into active-sites of the enzyme as structural probes. The distance between active site nucleotide probes of the enzyme has been measured by fluorescence energy transfer taking advantage of the essentially irreversible binding of various ADP analogues at neutral pH when bound with L-met-S-sulfoximine phosphate and Mn^{2+} at active sites. We used two fluorescent donors, either 8-mercapto ATP alkylated with N-(iodoacetylaminoethyl)-5-napthylamine-1-sulfo acid (AEDANS-ATP) or 1-N^6-etheno-2-aza-ATP (aza-ε-ATP) and two acceptors, 6-mercapto purine ribonucleoside triphosphate or 8-mercapto ATP alkylated with the chromophore 4(p-dimethylaminophenylazo)phenyl-4-iodoacetamide. The fluorescence yields of enzyme derivatives with 1 or 2 eq of fluorescent donor per dodecamer and either an acceptor or ADP at the remaining active sites were compared at pH 7.0. Excellent agreement was obtained with the different combinations of donor/acceptor probes on the dodecameric enzyme, resulting in a maximum range of ± 2 Å in calculated distances between active-site nucleotide probes. The results, together with the known geometry of the enzyme, indicate that active-site probes are widely separated and that energy transfer occurs from a single donor to 2 or 3 acceptors on adjacent subunits. The calculated distance between equidistant active-site probes on heterologously bonded subunits within the same hexagonal ring is 56-61 Å. Probes on isologously bonded subunits can be no closer than 60 Å and may be as far apart as 78 Å. Thus, nucleotides at active sites are away from the 6-fold axis of symmetry

toward the outer edges of the dodecamer and are located > 30 Å from the plane separating hexagonal rings.

The same fluorescent enzyme derivatives that were used for the determination of intramolecular fluorescence energy transfer distances could be induced by Zn^{2+} in the presence of $MgCl_2$ to form face-to-face aggregates of enzyme dodecamers along the 6-fold axes of symmetry. The Zn^{2+}-induced stacking of glutamine synthetase dodecamers also could be fully reversed by adding a Zn^{2+} chelator such as EDTA. The kinetics of the Zn^{2+}-induced stacking reaction was measured by time-dependent fluorescence and light scattering changes; the fluorescence quench was dependent on the presence of acceptors in layered dodecamers and correlated well with the degree of linear polymer formation as a function of time. The time-dependent fluorescence quench during Zn^{2+}-induced face-to-face aggregation at pH 7.0 and 25°C had a second-order rate constant of $\sim 5 \times 10^5\ s^{-1}\ M^{-1}$ at early stages, and reached a maximum when the average n-mer was 6 dodecamers. Thus, the approaches used in these studies also may be useful in studying the kinetics of other self-assembly systems when both a fluorescent donor and an acceptor are attached to the monomer species. Moreover, the maximum quench obtained by stacking fluorescent derivatives of glutamine synthetase indicated that the average intermolecular distance between donor and acceptor probes in layered dodecamers is ~ 36 Å. This intermolecular energy transfer distance confirms that active-site nucleotide probes are toward exterior surfaces away from the lateral plane between hexagonal rings of the dodecamer.

We began this study on fluorescence energy transfer distances between active sites to determine whether the kinetic and binding data that indicated some form of communication between active sites of glutamine synthetase could be explained by the proximity of pairs of active sites in the dodecamer. The stabilization of submolecular oligomers by active-site ligands implies that ligands binding at the active site alter the structure of the intersubunit bonding domains. These conformational changes may also extend to adjacent active sites leading to enhanced binding of inactivating ligands and thus to a nonrandom distribution of inactive subunits in partially inactivated dodecamers. However, fluorescence energy transfer measurements show that no two active sites of glutamine synthetase are closer to each other than to the rest of the active sites. These apparently anamolous results will be explained soon by the X-ray structural analysis being performed in David Eisenberg's laboratory at UCLA.

We have found that mercapto nucleotides can form very stable complexes with aquo glycyl-L-methionato·Pt(II) and these have been used for introducing an electron dense probe into active sites of glutamine synthetase. Also, we have found that we can adenylylate the enzyme using 6-S-ATP as a substrate of adenylyltransferase and then react the attached 6-S-AMP groups with the Pt(II) complex. These enzyme derivatives have been supplied to David Eisenberg at UCLA for X-ray crystallographic analysis. Although there have been problems in obtaining the correct crystal form of Pt(II)-enzyme derivative for X-ray crystallographic analysis, we are persisting in these studies since an electron dense probe at active sites or at adenylylation sites potentially can solve important aspects of the 3-dimensional structure of glutamine synthetase.

The stacking of glutamine synthetase dodecamers is being studied by calorimetry in order to investigate forces governing macromolecular assembly

reactions. Zn^{2+} binds to a site distinct from the active site of each subunit with $K_A' = 5 \times 10^6\ M^{-1}$ at pH 7.0 and deforms the enzyme in such a way that when 50 mM $MgCl_2$ also is present spontaneous face-to-face aggregation of enzyme dodecamers occurs. The rate of Zn^{2+} induced polymerization of glutamine synthetase increases with increasing temperature with an Arrhenius activation energy of 17.7 kcal/mol of dodecamers -- a rather small activation energy considering that Zn^{2+} binding deforms the enzyme and that 6 intermolecular contacts must formed in the stacking process. Enthalpy changes (ΔH) for (1) the binding of Zn^{2+} to the enzyme and protein conformational changes, and (2) the Zn^{2+}-induced aggregation of the enzyme were measured by calorimetry at pH 7.0 and 22.5, 30.0, and 38.0°C. In the absence of Mg^{2+}, the addition of 0.7 eq of Zn^{2+} per subunit produces no aggregation of the enzyme and $\Delta H = +83 \pm 3$ kcal/mol of dodecamer for (1) at 30°C. Lower ΔH values were measured in the presence of 50 mM $MgCl_2$ and 1.1 eq of Zn^{2+}/subunit for (1) + (2). Subtracting the ΔH values for (1) from those for (1) + (2) as a function of temperature gave an estimate for $\Delta Cp \simeq -850$ cal/K·mol for protein-protein reactions in (2). This large ΔCp value implicates a dominant role of water in the stacking process.

A reversible thermal transition of dodecameric glutamine synthetase from E. coli which involves the melting of active-site structures also is being investigated. The results of these studies should give some insight into the folding pathways involved during the assembly of this oligomeric enzyme.

Active-site ligand and metal ion interactions with mammalian octameric glutamine synthetase from bovine brain have been studied. The evidence obtained from binding and kinetic studies suggests that the enzyme has two essential metal ion binding sites per subunit, both of which must be filled for activity expression. The second Mn^{2+} binding site binds the nucleotide-metal ion complex ($K_A \simeq 10^6\ M^{-1}$) after the first site ($K_A \simeq 1.5 \times 10^5\ M^{-1}$) is occupied by Mn^{2+}. Filling the first site with Mn^{2+} or Mg^{2+} produces structural changes in the enzyme as evidenced by UV difference spectra and tryptophanyl residue fluorescence changes. Filling the second site requires the presence of nucleotide. Moreover, a Job analysis showed that all subunits of the bovine brain enzyme express γ-glutamyl transfer activity upon binding 1.0 eq of Mn·ADP complex per subunit. The affinity of the enzyme subunit for Mn·ADP is increased ~ 53-fold by the binding of arsenate or P_i (assay conditions). Although some of the features of Mn^{2+} binding of the brain enzyme are similar to those previously found for glutamine synthetase from E. coli, there are subtle differences. Furthermore, a specific binding of chloride ions to the brain enzyme ($K_A \simeq 10^4\ M^{-1}$) has been found to destabilize this enzyme and to promote both fluorescence and UV absorbance changes. The effects of chloride ions and those produced by an allosteric binding of L-glutamate on brain glutamine synthetase may be physiologically important.

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 and Zn^{2+} uptake by regulatory dimers upon displacement of the mercurial reagent with 2-mercaptoethanol. The properties of PAR-Zn^{2+} interactions make PAR a generally useful reagent for studying Zn^{2+} release from proteins. Current studies on the binding of Zn^{2+} to isolated regulatory subunits relate directly to the mechanisms of ATCase assembly in vivo. Newly initiated studies are on the role of Zn^{2+} in maintaining the quaternary structure of yeast arginase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00201-15 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism of the Branched-Chain Amino Acids

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

PI: J. Michael Poston Research Chemist LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Enzymes

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.05	0.85	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 study of the metabolism of the branched-chain amino acids has revealed a pathway of metabolism of leucine that is catabolic in bacteria and appears to be synthetic in humans. The pathway depends upon the activity of the enzyme, leucine 2,3-aminomutase, an enzyme dependent upon adenosylcobalamin as a cofactor. Other enzymes which function in the pathway are β-leucine transaminase/deaminase, coenzyme A transferase, and thiolase. The relative carbon flux through this pathway and the pathway which is independent of cobalamin greatly favors the independent pathway in brain, heart, kidney, and liver. In the testis, however, the cobalamindependent pathway accounts for over forty percent of the carbon flux. This suggests that the metabolism of leucine may play an important role in this organ. The nature of the transaminase/deaminase will be examined and purification of the enzyme will be attempted. The relationship between enzyme activity and various disease states such as pernicious anemia and inborn errors of metabolism will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00202-15 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Kinetics, Regulation and Mechanism of Biochemical Reactions

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

PI:	P. Boon Chock	Chief, Section on Metabolic Regulation	LB, NHLBI
Others:	Stewart R. Jurgensen	Staff Fellow	LB, NHLBI
	R. Dean Astumian	Staff Fellow	LB, NHLBI
	Pann-Ghill Suh	Visiting Fellow	LB, NHLBI
	Sue Goo Rhee	Research Chemist	LB, NHLBI
	Earl R. Stadtman	Chief, Laboratory of Biochemistry	LB, NHLBI

COOPERATING UNITS *(if any)*

E. Eisenberg, Lab. of Cell Biology, NHLBI; R.W. Schackmann, Washington University, Seattle; J. Vandenheede, Katholieke Universitiet, Belgium; T.Y. Tsou, Johns Hopkins University

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.25	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.)*

(1) The Mg(II)-ATP-dependent protein phosphatase is composed of two types of subunits. The catalytic subunit C migrates at 38K Da during SDS-gel electrophoresis and the modulator subunit M at 31K Da. This enzyme is inactive as isolated. It is activated by protein kinase FA which is the same as glycogen synthase kinase-3, and MgATP. Quantitation of this enzyme in crude extracts shows that it is present in high concentration (~ 50 nM). In addition, the maximal specific activity of the purified enzyme is enzyme concentration dependent. Further study shows that the enzyme is composed of two modulator and one catalytic subunit as evidenced by the data obtained from (i) gel filtration and sucrose gradient centrifugation experiments, (ii) densitometric scans of silver stained SDS-polyacrylamide gels, (iii) calculations based on amino acid analysis of the separated subunits; (iv) deconvolution analysis of the UV spectra of the denatured enzyme complex, and (v) steady-state kinetic analysis of the enzyme inhibition by added modulator subunit. One modulator binds very tightly to the catalytic subunit, while the other is dissociable and functions as a competitive inhibitor for the substrate. Thus, it provides a mechanism for protecting the phosphoproteins present in low concentrations.

(2) Development of a model for interactions between transmembrane proteins and the membrane potential. The theory can be used to provide a reasonable interpretation for energy transduction and for explaining how two a priori independent reactions can be coupled by an enzyme.

(3) Rapid intracellular changes in both calcium and pH concentration in sperm of sea urchin Strongylocentratus purpuratus caused by speract and by egg coat were measured.

4/

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00203-13 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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	Staff Fellow	LB, NHLBI
Others:	James Yan	Staff Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*
Jayasree Nath, Department of Hematology, Walter Reed Army Institute of Research

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 previously identified several biological systems including neutrophil activation, aging and gliosis in which proteins are oxidatively modified in reactions similar to the MFO-mediated reactions we have characterized in vitro.

In the past year, we have focused on the regulation of respiratory burst activity in an effort to understand the process of neutrophil activation and the mechanisms of protein modification. We have partially characterized a novel reaction in which tyrosine is incorporated into neutrophil proteins by a mechanism which is dependent on PMA-stimulated respiratory burst activity and independent of protein synthesis. At least one product of the reaction appears to be similar if not idenical to dityrosine. However, synthesis of the authentic product is required for verification. We have also investigated the possibility that activation of G-6-PD during neutrophil activation may be due to phosphorylation mediated by protein kinase C. However, no phosphorylation of the enzyme has been detected under a variety of conditions. Isoelectic focusing experiments revealed that multiple forms of G-6-PD are present in G-6-PD purified from control or from activated cells. These results suggest that increased activity and increased heat stability of the enzyme from activated cells may be due to limited proteolysis. Finally, in the gliosis model system, treatment of injured spheres with α-tocopherol depresses gliotic index, malondialdehyde formation and protein oxidation using DNPH-reactivity. Moreover, the effects of mechanical injury in this system can be mimicked by treatment with Fe/ADP/oxygen. Again α-tocopherol depresses both gliotic index and malondialdehyde formation. These results suggest that neuronal damage in gliosis may be due in part to MFO reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00204-19 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Structure: Enzyme Action and Control

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

PI:	Ann Ginsburg	Chief, Section on Protein Chemistry	LB, NHLBI
Others:	Marlana B. Blackburn	Staff Fellow (1/22/84-9/27/85)	LB, NHLBI
	Patrick J. McFarland	Chemist (10/14/84-5/11/86)	LB, NHLBI
	Julie A. Sahakian	Biotechnician (6/9/86-)	LB, NHLBI
	John R. Jefferson	Staff Fellow (6/22/86-)	

COOPERATING UNITS *(if any)*
M.R. Maurizi, Lab. Molecular Biology, NCI; J.B. Hunt, NSF (Chem. Div.); Susan Green and P. Hensley, Georgetown Univ. 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

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Laboratory of Biochemistry

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	3.0	0.3

CHECK APPROPRIATE BOX(ES)

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

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

(1) Nucleotide analogs have been introduced as structural probes into active sites of dodecameric glutamine synthetase from E. coli. Various analogs of ATP that are substituted at the 6- or 8-position of the purine ring have been further modified with spectrophotometric and fluorometric probes or an electron dense Pt(II) marker. Fluorescence energy transfer measurements were made with enzyme derivatives containing 1 or 2 eq of fluorescent nucleotide donor per dodecamer and ADP or acceptor analogs at the remaining active sites. Energy transfer measurements made last year showed that active site probes in the dodecamer are widely separated (56-60 Å). Using the same fluorescent derivatives, zinc-induced face-to-face stacking of enzyme dodecamers had a second-order rate constant of 50000/M·s at 25°C and fluorescent donor and acceptor probes in layered dodecamers were found to be ~ 36 Å apart. The zinc-induced stacking of enzyme dodecamers also is being studied by calorimetry at 22.5, 30.0, and 38.0°C in order to investigate the forces governing macromolecular assembly reactions. Polymerization rates increase with increasing temperatures with an Arrhenius activation energy of 17.7 kcal/mol. A value of $\Delta Cp = -850$ cal/K·mol has been estimated for polymerization.

(2) Glutamine synthetase from S. typhimurium has been labeled at active sites or adenylylation sites with mercapto nucleotide·platinum(II) and sent to UCLA for crystallization and X-ray analysis.

(3) Bovine brain glutamine synthetase has been found to have two essential divalent cation sites/subunit -- a structural site and a higher affinity nucleotide-metal ion site which is filled after the first site is occupied by Mn(II) or Mg(II). Although the enzyme is active with either Mg(II) or Mn(II) in vitro, only Mg(II) is bound to the brain enzyme in vivo. An allosteric site for chloride, L-glutamate, or arsenate is indicated.

(4) A reversible thermal transition of dodecameric glutamine synthetase from E. coli was found to involve a melting of active site structures.

(5) Studies of Zn(II) binding to regulatory proteins are in progress.

S/

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00205-31 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Occurrence and Biochemical Roles of Selenium in Selenoproteins 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:	Rong-xin Zhu	Fogarty Visiting Fellow	LB, NHLBI
	Gregory E. Garcia	NIH Staff Fellow (Started May 1, 1986)	LB, NHLBI
	Joe Nathan Davis	Laboratory Research Assistant	LB, NHLBI

COOPERATING UNITS *(if any)*

Gas Research Institute, Chicago, Illinois.
Dr. August Böck, University of München, München, West Germany.
Dr. Harlan Wood, Case Western Reserve University, Cleveland, Ohio.

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	4.0	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.)* Studies were continued on clostridial glycine reductase that catalyzes the reductive deamination of glycine and the concomitant synthesis of ATP. The protein C component, isolated in apparently homogeneous form, behaves as an associating-dissociating system of two dissimilar subunits. Marked loss of activity using certain types of chromatographic steps (e.g., hydroxyapatite) seems to be due to selective loss of one of the subunits. The catalytic activity of protein C is destroyed by alkylation and by brief heating at 50°C. Scale-up of the isolation procedure for protein C is in progress and antibodies will be produced to be used for studies on regulation of its biosynthesis. To study the mechanism of insertion of the selenocysteine residue in the selenoprotein A of glycine reductase, experiments have been initiated to isolate and clone the cDNA encoding this selenoprotein. Comparison of the DNA sequence with the known sequence of a 16-residue selenocysteine containing peptide isolated from selenoprotein A should provide information concerning a putative precursor amino acid. The collaborative program with A. Böck of München on the origin of the selenocysteine residue in a formate dehydrogenase of E. coli took a very interesting turn when the München group found that a stop codon within the cDNA that codes for this protein is suppressed and read-through occurs. This stop codon is within the DNA sequence corresponding to a large selenocysteine containing peptide located near the amino terminus of the selenoprotein subunit of the enzyme. The stop codon may be used here to specify selenocysteine insertion by some unknown mechanism. We have developed procedures for isolation of labeled peptides generated by treatment of 75-Se-labeled formate dehydrogenase with specific proteases. Scale-up of these methods is in progress in order to obtain sufficient amounts of the highly hydrophobic selenocysteine containing peptide(s) for amino acid sequence analysis. A method for isolation of pure amino acid transfer ribonucleic acids (tRNAs) was further developed using a monoclonal anti-AMP antibody affinity column obtained from Dr. Sue Goo Rhee. This procedure proved to be far superior to previous methods using a boronate affinity column matrix.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00206-27 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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
Others:	Si-Yu Xu	Visiting Fellow	LB, NHLBI
	Adolfo Amici	Visiting Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*

None

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Laboratory of Biochemistry

SECTION

Intermediary Metabolism and Bioenergetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	3.0	0.9

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

(1) Various approaches to the synthesis of 5-carboxymethylaminomethyl-2-selenouracil were explored.

(2) 5-hydroxy-2-aminovaleric acid was established as one of the oxidation products of polyproline and polyarginine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00211-13 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Protein Oxidation in Protein Turnover and in Aging

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

PI:	E. R. Stadtman	Chief, Laboratory of Biochemistry	LB, NHLBI
Others:	B. S. Berlett	Biological Laboratory Technician	LB, NHLBI
	A. Amici	Visiting Fellow	LB, NHLBI
	L. Tsai	Research Chemist	LB, NHLBI
	R. Levine	Senior Investigator	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Enzymes

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.6	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 was established that arginine, proline, histidine, and lysine residues of proteins are among those that are oxidized to carbonyl derivatives by mixed-function oxidation systems. Arginine and proline residues are both oxidized to 5-oxo-2-aminopentanoic acid. Proline is also oxidized to pyroglutamic acid and very likely to either 3-oxo- or 4-oxo-proline. Results with a model system comprised of hydrogen peroxide, ferrous iron and iron chelating agents (the Fenton system) have shown that phenylalanine is oxidized to phenyacetaldehyde and phenylacetic acid together with ammonia and carbon dioxide. Similarly, leucine is oxidized to isovaleraldehyde and isovaleric acid. In addition, small quantities of a potent inhibitor (Ki = 1 to 10 nanomolar) of horse liver alcohol dehydrogenase is produced from both amino acids. These were isolated and shown to be the oxime derivatives of the above aldehydes. In attempts to understand the role of bicarbonate ion in the oxidation of amino acid by the Fenton system, it was found that bicarbonate stimulates the auto-oxidation of ferrous iron to ferric iron and also the reduction of ferric iron to ferrous iron by hydroxylamine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00212-15 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Ammonia-Assimilatory Enzymes in E. coli K12

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

PI:	Mary Anne Berberich	Research Chemist	LB, NHLBI
Others:	Edward DeMoll	Staff Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
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Section on Enzymes

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

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

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

The term "nitrogen control" describes the phenomenon whereby a limitation of the ammonia supply during growth of bacteria results in an increase in the synthesis of ammonia-assimilatory enzymes, some amino acid binding proteins, and some amino acid catabolic enzymes. Genetic studies with enterobacteria reveal that regulation via nitrogen availability is under the control of three regulatory loci: glnF, glnG, and glnL. Both positive and negative controls operate at the transcriptional level. GlnF codes for a specific sigma factor, whereas the product of glnL appears to mediate the interconversion of the glnG product, NR1, from repressor to positive activator in response to nutritional conditions. However, neither the biochemistry of NR1 activation nor the process by which the level of intracellular ammonia signals this interconversion is understood at present.

For this reason, a study of the physiological parameters of the nitrogen control response was made in E. coli K12 using the level of glutamine synthetase (GS) as a measure of regulation. It was determined that addition of some D-amino acids to cells growing in medium containing excess ammonium nitrogen elicited an increase in the level of synthesis of GS. Because the rate of increase effected by a combination of D-glu, D-thr, D-lys and gly is equivalent to that which occurs when ammonia is exhausted, it was reasoned that these amino acids might participate, either directly or indirectly, in the generation of the specific metabolic signal for the nitrogen control response.

When the distribution of the amino nitrogen from these amino acids, added to cells cultured in media containing isotopic nitrogen was examined by mass spectrometry, an increase in serine biosynthesis was observed. It was subsequently demonstrated that the increase in GS level elicited by the D-amino acids is dependent on the activity of serine hydroxymethyltransferase (SHMT). Further studies with inhibitors and mutants suggest that SHMT is involved in generating the putative metabolic signal which ultimately may be an early purine intermediate.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00224-09 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Calcium-regulated Protein Kinases and Phosphatases

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:	Marina Lanciotti	Visiting Fellow (appointment ends July 1986)	LB, NHLBI
	Aile Zhang	Visiting Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*

Jitendra Patel, Biological Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.7	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) The calmodulin-dependent protein phosphatase requires divalent metal ions such as Ni(II), Mn(II), Mg(II), etc. for expression of full catalytic activity. Two Ni(II) ions can bind to the phosphatase. The first Ni(II) ion binding leads to dramatic activation, whereas the second Ni(II) ion binding results in deactivation. The mechanism of Ni(II)-deactivation has been studied in detail, both experimental and theoretically. It involves an initial loose binding step (dissociation constant ~ 21 mM) and a subsequent conformational rearrangement (rate constant = 0.078 per min). Binding of Ca(II) to the B subunit is vital to activation of the catalytic A subunit by the first Ni(II) ion. The Ni(II) ion interaction with the phosphatse can be described by an overall mechanism in which the two Ni(II) ions combine with the enzyme in an ordered manner.

(2) Phosphorylation of the calmodulin-dependent protein phosphatase by protein kinase C has been investigated in greater detail. Two moles of phosphate are incorporated per mole of phosphatase. One of the phosphoryl groups is dephosphorylated by a new Ca(II)-inhibited phosphatase. The phosphorylation and dephosphorylation of the enzyme appears to be tied to the release and sequestration of Ca(II) ion in vivo.

(3) The Ca(II)-inhibited protein phosphatase has been purified ~ 1500-fold. The purity is estimated at ~ 60%, and the MW = 40,000.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00225-09 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mixed-Function 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:	Michel Chevalier	Visiting Fellow	LB, NHLBI
	Javier Cervera	Guest Worker	LB, NHLBI
	A. Jennifer Rivett	Visiting Fellow	LB, NHLBI

COOPERATING UNITS *(if any)* Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts; Instituto de Investigaciones Citólogicas de la Caja de Ahorros de Valencia, Valencia, Spain; Department of Biochemistry, University of Oregon, Eugene, Oregon.

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	2.9	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.)*

Many enzymes are now known to be subject to a covalent modification mediated by mixed function oxidation. This modification may have physiologic and pathologic significance in diverse processes. These include the regulation of protein turnover, accumulation of modified proteins during aging, killing of pathogens by host defense mechanisms, limitation of autolysis, pulmonary damage by smoking and air pollutants, and in oxygen toxicity.

Multiple oxidative modifications may be introduced into a protein. Glutamine synthetase was subjected to varying times of exposure to mixed function oxidation to provide samples of graded oxidation. Amino acid analysis revealed loss of two histidine residues. No other changes in amino acid composition were detected. The enzyme lost both catalytic activity and a divalent metal binding site upon oxidation of the first histidine residue. This form of the enzyme was not susceptible to proteolytic degradation by several purified proteases. Oxidation of the second histidine residue rendered the enzyme susceptible to degradation. Studies of the surface hydrophobicity of glutamine synthetase revealed that oxidative modification modulates that hydrophobicity. Initial oxidation converts the protein to a more hydrophilic species which is not a substrate for a purified protease. Additional oxidation generates a more hydrophobic form which is a substrate. Studies with a transition-state analog demonstrated that occupancy of the active site blocks oxidative modification and prevents the changes normally induced by oxidation. Occupancy of the active site provides a mechanism by which cellular metabolites may regulate mixed function oxidation of specific proteins.

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PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00237-07 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Toxicity and Transport of Bilirubin

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:	Paul E. Stobie	Guest Worker	LB, NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Neurosciences
National Institute on Aging

Small Animal Section
Veterinary Resources Branch

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	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.)*

Hyperbilirubinemia is likely the most frequently diagnosed and treated condition in the newborn. Treatment is aimed at preventing the entry of bilirubin into the brain because of the risk of permanent neurologic damage (kernicterus). The mode by which bilirubin enters the brain, its metabolic fate after entry, and the biochemical basis of its toxicity are unknown. The congenitally jaundiced rat (Gunn strain) lacks glucuronyl transferase and develops unconjugated hyperbilirubinemia in the neonatal period. It provides a model for human neonatal jaundice. However, the genetic background of Gunn mutants varies from laboratory to laboratory. The Gunn strain has thus been transferred into two genetically defined backgrounds, RHA and ACI. We studied the two strains from birth through adolescence, following growth, survival, serum albumin, serum bilirubin, hematocrit, and liver glucuronyl transferase activities. Both strains are jaundiced in the neonatal period, with serum bilirubin levels peaking about two weeks of age. Survival of jaundiced (homozygotes) and non-jaundiced (heterozygotes) pups were essentially the same in both strains. However, survival of the ACI rats was low while that of the RHA was high. Thus, the RHA strain of congenitally jaundiced rats provides a useful model for the study of neonatal jaundice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 HL 00239-07 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Glutamine Synthetase in E. coli and S. cerevisiae

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

PI:	Sue Goo Rhee	Research Chemist	LB, NHLBI
Others:	Kang Hwa Kim	Visiting Fellow	LB, NHLBI
	Heung Soo Son	Guest Worker	LB, NHLBI
	Ki Young Lee	Guest Worker	LB, NHLBI
	Earl R. Stadtman	Chief, Laboratory of Biochemistry	LB, NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Metabolic Regulation

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.0	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.)*

(1) The bicyclic cascade regulation of glutamine synthetase (GS) in E. coli involves 4 protein components, GS, regulatory protein, adenylyltransferase, and uridylyltransferase. Using polyclonal antibodies derived against them, their intracellular concentrations were measured.

(2) The bicyclic cascade was reconstituted by mixing the 4 proteins in accordance with the ratio determined in vivo. The state of adenylylation of GS and the state of uridylylation of regulatory protein were measured at various concentrations of glutamine and α-ketoglutarate. Then the sensitivity indexes with respect to glutamine and α-ketoglutarate were obtained.

(3) S. cerevisiae contains 2 forms of GS, active and inactive. Several lines of evidence (molecular weight, antibody cross-reactivity, peptide analyses) indicate that they are the same gene products.

(4) Kinetic parameters of active and inactive forms of yeast GS were measured.

(5) The active form of GS was characterized in detail. Amino acid sequence of several peptides including acetylated N-terminal peptides were established. Sulfhydryl contents, sedimentation coefficients optical properties were also measured.

(6) Strong subunit interaction in the octameric yeast GS was revealed.

(7) Yeast extracts contain a protein which can provide protection against the oxidative inactivation of several enzymes including GS. This noble protein was purified to homogeneity and its capacity to protect against various oxidative modification systems was measured.

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PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00241-07 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Detection and Regulation of Phosphotyrosine Modification in Cellular Proteins

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

PI: Todd M. Martensen Guest Worker LB, NHLBI

COOPERATING UNITS *(if any)*

Department of Biological Chemistry, Johns Hopkins University, Baltimore, MD
Laboratory of Vision Research, NEI

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Enzymes

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.8	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.)*

Posttranslational phosphorylation of protein tyrosine residues in cells has been investigated by chemical and immunological methods to identify and characterize the catalysts and their substrates. Tyrosine phosphate (Tyr-P) residues are resistant to alkaline conditions (1 N NaOH, 65°C) which destroy most Ser-P and Thr-P residues. A straightforward procedure to assay the base resistant [32-P]protein phosphoryl groups in in vitro labeled cells was developed by electroblotting SDS PAGE separated proteins to nylon blotting paper which can be incubated in base. This procedure is rapid and technically superior to treatment of gels. This technique was used to characterize the base resistant [32-P]phosphoproteins of several retrovirus transformed cell lines.

Immunodecoration of proteins containing Tyr-P on electroblots is possible by incubating the electroblot of SDS gels with sheep antibodies which bind Tyr-P. The region of the blot with bound antibodies is detected with affinity purified anti•sheep IgG conjugated with horseradish peroxidase. The procedure was tested with authentic proteins containing Tyr-P or Ser-P residues and appears to be specific.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00255-03 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Metabolism of Purine and Pyrimidines by Methanococcus vannielii.

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

PI: L. Edward DeMoll, III 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.)*

Purine and pyrimidine metabolizing pathways were discovered to be present in Methanococcus vannielii. The pathways are constituitive, but amplifiable, and active to an extent such that guanine, uric acid, xanthine, hypoxanthine, uridine, or thymine, but not adenine or cytosine, can serve as sole nitrogen source for the organism. The interconversion of purines was examined, and I determined that guanine nucleotides are rapidly dephosphorylated to guanosine. Guanosine is then metabolized to the free base by purine nucleoside phosphorylase. The free base is then rapidly deaminated to xanthine. Uric acid is reduced to xanthine by xanthine dehydrogenase, which I have partially purified. Hypoxanthine is oxidized to xanthine by the organism by an unknown enzymatic activity, possibly coupled to a hydrogenase. Xanthine is degraded by a series of reactions that resemble those described for clostridia. All of the reactions investigated are oxygen sensitive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00256-03 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Enzyme Inactivation in Red Cells During Aging

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

PI:	Bong-whan Ahn	Visiting Fellow	LB, NHLBI
Others:	E.R. Stadtman	Chief	LB, NHLBI
	C.N. Oliver	Staff Fellow	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

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 oxidative modification of proteins and enzyme inactivation during protein turnover and aging in an effort to understand the possible physiological role of this process. Oxidative inactivation of enzymes mediated by mixed-function oxidation systems is accompanied by the formation of protein carbonyl derivatives. We have used this property and developed several assays to detect and quantitate the levels of oxidized protein in tissue extract preparations from young and old animals. It is likely that these studies will permit us to identify and isolate oxidatively modified proteins from biological systems.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00258-02 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Diol Dehydratase and Diol Metabolism in Clostridium glycolicum

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

PI: Maris G. N. Hartmanis Visiting Fellow LB, NHLBI

COOPERATING UNITS *(if any)*

Dr. Hideo Kon, Laboratory of Chemical Physics, NIADDK, NIH, Bethesda, Maryland.

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.)*

The oxygen sensitive and membrane bound diol dehydratase from Clostridium glycolicum was solubilized from its matrix by sonication of crude membrane preparations anaerobically in 0.1 M CHES buffer, pH 8.5 or 9.0, containing 2 mM dithiothreitol. Treatment with organic solvents, a variety of ionic and nonionic detergents, high ionic strength, or phospholipase A did not solubilize any diol dehydratase activity. Addition of 30% dimethylsulfoxide and 0.15 mg/ml of lysophosphatidylcholine to the CHES buffer before sonication markedly increased recovery of enzyme activity. Up to 45% of the activity could be recovered after centrifugation for 1 h at 105,000 x g. This solubilization method was also shown to work for the membrane bound formate dehydrogenase from E. coli. More than 95% of the activity was recovered in the supernatant after sonication and centrifugation.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00259-02 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Kinetics and Regulation of Biochemical Reactions at the Cell Membrane

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

PI: R. Dean Astumian, Staff Fellow, Laboratory of Biochemistry, NHLBI

Others: P. Boon Chock, Chief, Section on Metabolic Regulation, LB, NHLBI

COOPERATING UNITS *(if any)* T. Y. Tsong, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland
H. V. Westerhoff, Section on Theoretical Molecular Biology, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, Maryland

LAB/BRANCH

Laboratory of Biochemistry

SECTION

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 spece provided.)*

1. In continuing our work on the theoretical description of interfacial reaction dynamics we have reformulated the expression for the diffusion controlled dissociation rate constant such that it is applicable to cases where the dissociating moieties have significantly disparate sizes (e.g., ligand dissociating from a cell surface). Our correction is equivalent to mathematically acknowledging that the reactants have finite sizes and are not mutually interpenatrable. We have also shown analytically the equivalence of the branching method and the classical kinetic formulation for evaluating diffusion controlled reactions.

2. We have continued our development of the theory of interactions between transmembrane proteins and the membrane electric potential. The equations and theory developed allow us to calculate the influence of changes in the electric field on the function of membrane proteins.

If the membrane potential is caused to oscillate, or to randomly fluctuate in a manner uncorrelated to the enzyme state in the region of fluctuation, an enzyme can transduce energy from the modulated potential and convert it to stored chemical energy (e.g., ATP synthesis or the formation of an ion gradient). This of great theoretical importance in interpreting experiments in which ATP synthesis is observed even when there is apparently "insufficient" thermodynamic driving force contained in the proton electrochemical gradient.

Considerations along these lines have resulted in the development of a very simple physical model for energy transduction. While this model certainly does not represent an accurate description of any one actual enzyme, its simplicity makes it an outstanding tool for understanding one physical mechanism by which an enzyme could couple two chemical reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00260-01 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Butyrate Kinase from Clostridium acetobutylicum

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

PI: Maris G. N. Hartmanis Visiting 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.)*

A butyrate kinase from Clostridium acetobutylicum has been purified 50-fold to homogeneity in a five-step procedure with a 31% yield. The purification involved ammonium sulfate fractionation, two hydrophobic interaction chromatography steps, affinity chromatography, and gel filtration. The isoelectric point, the molecular weights of the native and denatured enzyme, the pH optimum, the substrate specificity, and the amino acid composition of the enzyme have been determined. Antibodies to butyrate kinase are currently being prepared in a rabbit. These will be used to study the expression of the enzyme as a function of fermentation time.

GPO 914-918

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00261-01 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

CO Dehydrogenase and Acetoclastic Methanogenesis in Methanosarcina barkeri

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

PI: David A. Grahame Postdoctoral Guest Research Worker LB, NHLBI

COOPERATING UNITS *(if any)*

Supported by grant to T.C. Stadtman from Gas Research Institute of Chicago.

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.)*

A study has been conducted on carbon monoxide dehydrogenase (CODH) from acetate-grown cells of Methanosarcina barkeri. Under conditions of high ionic strength the enzyme exists in an aggregated state along with a discrete set of other proteins (total mol. wt. approx. 3,000,000). Dissociation of the aggregate occurred when the ionic strength was decreased by dialysis. The disaggregated form of CODH retained full activity and exhibited a molecular weight of approximately 161,000. An efficient purification procedure was developed which produced high yields of pure CODH. The method consisted of the following steps which were carried out under strictly anaerobic conditions in the NIH Anaerobic Laboratory: 1. Isolation of the CODH aggregate by gel-filtration and subsequent dissociation; 2. Chromatography on Phenyl Sepharose; and 3. Hydroxylapatite chromatography. CODH appeared to be less hydrophobic than any of the other protein components of the aggregate. It bound tightly to hydroxylapatite and, thus, may have a relatively large number of exposed carboxylic acid residues. Pure CODH is composed of two subunits of molecular weights 93,800 and 20,400. Among various compounds tested, oxygen and cyanide are potent inactivators. Glyoxaldehyde inactivation occurred only during enzymatic turnover, which suggests that a reactive group is formed, or exposed, on an enzyme intermediate in catalysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT Z01 HL 00262-01 LB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Oxidative Modification in Cellular Protein Turnover and Aging

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

PI:	Pamela E. Starke	Staff Fellow	LB, NHLBI
Others:	E. R. Stadtman	Chief, Laboratory of Biochemistry	LB, NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Biochemistry

SECTION
Section on Enzymes

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.85	0.75	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.)*

During the normal aging process, cellular enzymes accumulate as catalytically inactive or less active forms. The mechanism(s) by which these enzymes became altered, as yet unknown, may involve the oxidative modification of critical amino acid residues. In order to define the mechanism(s) responsible for these modifications, we have developed an in vitro model system to investigate the relationship between enzyme modification by mixed-function oxidase (MFO) systems and the accumulation of altered enzymes during aging. To this end, the level of carbonyl groups, known to be generated in MFO systems, have been determined in crude extracts of freshly isolated hepatocyte cultures derived from rats of different ages. Preliminary studies have shown an increase in carbonyl content in hepatocytes isolated from old rats and in hepatocytes from rates of all ages cultured in the presence of an acute oxidative stress. The oxidative modification of proteins will also be investigated by determining carbonyl content in hepatocyte cultures derived from rats exposed to vigorous exercise, or dietary regimens known to effect longevity. Additionally, effects of factors which activate or inhibit MFO-protein oxidation in these cells will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00263-01 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Regulation of Phosphatidylinositol-specific Phospholipase C

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

PI:	Sue Goo Rhee	Research Chemist	LB, NHLBI
Others:	Key Seung Cho	Guest Worker	LB, NHLBI
	Kee Young Lee	Visiting Fellow	LB, NHLBI
	Sung Ho Ryu	Visiting Fellow	LB, NHLBI
	Pann-Ghill Suh	Visiting Fellow	LB, NHLBI
	P. Boon Chock	Chief, Section on Metabolic Regulation	LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Section on Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	4.1	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.)*

Phosphatidylinositol-specific phospholipase C (PLC) plays a crucial role in initiating the surface receptor mediated signal transduction by generating two second messenger molecules, diacylglycerol and inositol 1,4,5-triphosphate. We resolved two forms of bovine brain enzyme, PLC-I and PLC-II, on a HPLC-DEAE column and purified PLC-II to homogeneity. Upon analysis of PLC-II on SDS-PAGE, a single band of MW = 145,000 was observed. When the same sample was subjected to native gradient polyacrylamide gel, four bands, one major band of MW = 200,000 and three minor bands with molecular weights corresponding to different oligomeric states of the 200K Da protein, were visible. Western blot experiments using anti-PLC-II antibody indicated that PLC-I might be derived from PLC-II by proteolytic cleavage. Multiple forms of brain PLC enzymes described in the literature might be, therefore, attributed to the oligomerization and proteolysis of PLC-II. PLC-I and PLC-II hydrolyzed both phosphatidylinositol and phosphatidylinositol 4,5-diphosphate. Both activities were stimulated by Ca(II). However, the presence of Ca(II) was not an absolute requirement for the hydrolysis of phosphatidylinositol 4,5-diphosphate while phosphatidylinositol hydrolysis at neutral pH required Ca(II).

Protein kinase C phosphorylates PLC-II in a Ca(II) and phospholipid-dependent manner. The physiological meaning of this phosphorylation is not known yet.

130

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 00264-01 LB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Sperm Internal pH

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

PI: Robert W. Schackmann Guest Worker LB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry

SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.3	1.0	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.)*

Regulation of intracellular calcium concentration is vitally important to a wide range of cell functions. This project is designed to measure rapid changes in intracellular pH and intracellular calcium in sperm of the sea urchin Strongylocentrotus purpuratus. Changes in these parameters accompany modifications of the sperm which ready it for fusion with the egg to initiate development of a new organism. I have applied optically detected fluorescent dyes to systematically measure intracellular pH (with carboxyfluoresceins) or intracellular calcium (with fura-2 or indo-1) to investigate: 1) physiological effects of a peptide from the egg which causes chemotaxis of the sperm, and 2) effects of a complex egg coat which causes the sperm acrosome reaction. The acrosome reaction includes exocytosis of the sperm acrosomal granule and is essential for fertilization. 1) Our results show that the chemoattractant peptide speract causes a rapid increase in intracellular pH that allows for a transient rise in intracellular calcium. Inhibition of the increase in pH inhibits the calcium entry. The increase in calcium is important because sperm chemotaxis does not occur in the absence of external calcium. 2) The morphological changes of the sperm acrosome reaction also follow increases in intracellular pH and calcium. The increase in intracellular calcium is larger than that induced by the chemoattractant and is not transient. Inhibitors which block the acrosome reaction partially inhibit both pH and calcium increases. These studies form a basis for identification of biochemical components of the sperm plasma membrane regulating movements of calcium and hydrogen ions. Initial studies with monoclonal antibodies implicate a sperm plasma membrane protein of 210 kDa as important to the regulation of sperm calcium metabolism.

ANNUAL REPORT OF THE CARDIOLOGY BRANCH

National Heart, Lung, and Blood Institute

October 1, 1985 through September 30, 1986

The experimental interests of the Cardiology Branch focus on 1) elucidating the mechanisms responsible for dynamic alterations in coronary vascular resistance; 2) defininining the pathophysiology and treatment of coronary artery disease, angina pectoris, and hypertrophic cardiomyopathy; 3) identifying the determinants of irrevesible heart failure and defining optimal time for operating on patients with valvular heart disease. In the past year we have also been studying the role of angiogenesis in the pathophysiology and treatment of ischemic heart disease.

DYNAMIC CORONARY VASOCONSTRICTION AS A CAUSE OF MYOCARDIAL ISCHEMIA

Traditionally, angina pectoris has been considered to result from a fixed stenosis limiting blood flow to the myocardium. The recognition of vasospastic angina (Prinzmetal, or variant angina) focused attention on the fact that the coronary arteries can spontaneously constrict and that this constriction can be severe enough to precipitate myocardial ischemia. However, the clinical syndrome of vasospastic angina was limited to spasm-induced total or near-total occlusion of the large epicardial coronary arteries. Over the past three years we have explored the possibility that dynamic coronary vasoconstriction may not only involve large epicardial coronary vessels, but also the small intramural coronary arteries.

Ischemia caused by small coronary artery vasoconstriction in pts with angina: We previously demonstrated that about two-thirds 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, an abnormality exacerbated by ergonovine. The chest pain was also associated with diminished myocardial lactate consumption and abnormalities in LV function. We concluded that these pts have true myocardial ischemia, that this is due to a reduced capacity of the small coronary arteries to vasodilate in response to increases in myocardial O_2 demand, and that drugs with vasoconstrictor potential can further compromise vasodilator reserve. We also demonstrated that the reduced vasodilator reserve occurred not only in response to metabolic stimuli (pacing-induced increase in myocardial O_2), but also to a diminished absolute capacity of the coronary vessels to dilate. This was demonstrated by analyzing data derived during the administration of the potent coronary arteriolar vasodilator, dipyridamole. Further analyses of these data suggested that the flow limitation is due to narrowing of the small pre-arteriolar coronary arteries, rather than of the arterioles, per se.

Evidence of a diffuse disorder of smooth muscle: Several other intriguing findings have evolved from the original studies. Abnormal esophageal tone was found in 19 of 32 pts with abnormal coronary tone. To determine whether this indicated a syndrome characterized by generalized increase in smooth muscle tone, we studied the vasodilator reserve of another vascular bed -- the forearm resistance vessels. We subjected the forearm to an ischemic stress

(by inflating a blood pressure cuff to supra-systolic pressures) and measured (by plethysmographic techniques) reactive hyperemia following release of ischemia. Peak flows were reduced in pts with abnormal coronary vasodilator reserve compared to an age and sex matched control group, and vascular resistance after 5 minutes of ischemia was considerably higher (3.9 ± 1.0 in pts, 2.3 ± 0.4 in controls; $p<0.001$). These findings generated two important and novel hypotheses. First, they suggest that pts with angina due to abnormal small coronary arteries have a more generalized defect involving an increase in smooth muscle tone. Second, they suggest that a common etiologic link might exist between three diseases: 1) the syndrome of angina due to increased small coronary artery tone; 2) HCM; 3) hypertension.

HYPERTROPHIC CARDIOMYOPATHY

HCM is a disease currently thought to be a primary cardiomyopathy, and is characterized by myocardial hypertrophy, myocardial diastolic dysfunction, and myocardial ischemia occuring in the absence of disease of the large coronary arteries.

Progression of hypertrophy in HCM: Myocardial hypertrophy is one of the primary abnormalities in HCM. To determine whether this occurs throughout the course of the disease and is an important determinant in changing symptoms, serial studies were performed in pts with HCM at various ages. The results demonstrated that progressive hypertrophy occurred frequently in children with HCM, being particularly marked during their adolescent growth spurt. Several pts were found to develop hypertrophy de novo during this period, having had normal echocardiographic studies prior to age 10. Despite the high frequency of progression in hypertrophy in children, no progression in LV wall thickness was observed in 65 pts with HCM 23-50 years of age over a mean follow-up of 4 years. However, 14% of the pts demonstrated a substantial decrease in wall thickness, usually associated with mild left ventricular dilatation, LV wall thinning, decreased ejection fraction, and severe symptoms.

Causes of myocardial ischemia: Last year we reported that over 80% of pts dying with HCM have anatomically abnormal intramural coronary arteries (IMCA). IMCAs had markedly thickened intima and media, and many evidenced severe lumenal narrowing. Moreover, IMAs were identified frequently in prominent areas of replacement fibrosis, suggesting a role of small vessel disease in the pathophysiology of ischemic injury. We subsequently found that ergonovine reduced the peak coronary flow response to cardiac pacing, and increased coronary resistance. Since no large vessel vasospasm occurred, these findings were compatible with the concept that the small intramural coronary arteries were susceptible to the vasoconstrictor effects of ergonovine.

To further characterize the ischemia presumed to be present in pts with HCM, we employed thallium-201 single photon emission computed tomography to evaluate myocardial perfusion. Exercise-induced reversible regional perfusion defects were identified in 9 of the 18 pts; irreversible perfusion defects occurred in 6, and 5 of these pts had reduced resting LV ejection fraction (less than .47). These studies demonstrated that reversible regional perfusion

defects suggestive of ischemia, and fixed defects suggestive of myocardial scar frequently occur in HCM pts. They also suggested that myocardial ischemia not only contributes to symptoms in HCM but may also result in fibrosis and transmural scar, and thereby to LV dysfunction. To determine the relative prevalence of perfusion defects among asymptomatic pts with HCM, we assessed LV perfusion in 17 asymtomatic and 54 symptomatic pts. Surprisingly, 53% of the asymptomatic pts had reversible perfusion defects, a prevalence similar to that observed in symptomatic pts (57%). Fixed defects were found in 1 (6%) of the asymptomatic pts versus 15 (28%) of the symptomatic pts. Likewise, whereas only 1 (6%) of the asymptomatic pts had a depressed EF, 14 (26%) of the symptomatic pts did. Although the prognostic implications of these findings are not certain, these data suggest that asymptomatic pts frequently experience silent myocardial ischemia. The data derived from the older, symptomatic pts further suggest that the asymptomatic pts with normal EF and reversible perfusion defects may be at risk of developing angina, myocardial scar and depressed EF.

Functional abnormalities of the small coronary arteries of HCM pts resemble those of the coronary tone pts. To determine whether HCM pts also have a more generalized disorder of vascular smooth muscle vasodilator reserve, we employed plethysmography to study the hyperemic response to forearm ischemia. As with the coronary tone pts, after 5 minutes of ischemia the HCM pts had lower peak flow responses following ischemia, and higher minimal vascular resistance (3.37 ± 0.96 for pts versus 2.26 ± 0.34 for controls; $p<0.005$). Our still preliminary, but intriguing findings, therefore suggest that pts with HCM may also have a generalized disorder of smooth muscle tone affecting the vasodilator reserve of both the peripheral and the myocardial arteries.

Diastolic dysfunction in HCM: LV relaxation and diastolic filling are impaired in many pts with HCM. We employed radionuclide angiography to investigate the influence of regional heterogeneity on these global diastolic abnormalities. Regional function was assessed by subdividing the LV region of interest into 20 segments from which regional time activity curves were derived. Regional variation in timing between minimum volume and peak filling rate was used as a measure of diastolic asynchrony. We found that HCM pts had asynchronous and nonuniform regional diastolic function, abnormalities that might contribute importantly to the severity of impaired LV diastolic filling. Moreover, when the studies were repeated after 1 to 2 weeks of oral verapamil, global diastolic function improved and the improvement was associated with more uniform regional diastolic performance. Although several explanations can be offered to account for the verapamil effects, it is possible that one of the primary actions leading to the improved synchrony and diastolic function is a reduction in calcium cellular influx, brought about by the calcium channel blocking actions of verapamil.

Electrophysiologic abnormalities in HCM: Although ventricular tachycardia (VT) occurs on ambulatory monitoring in about 20% of HCM pts, of whom 25% die over a 3 year period, further identification of high risk subgroups has not

been achieved. To determine the role of programmed electrical stimulation (PES) in both defining factors contributing to major clinical events and directing therapy, we studied 29 HCM pts with cardiac arrest, syncope, near-syncope, or asymptomatic nonsustained VT. PES identified abnormalities in 52% of pts. Pts with near-syncope or asymptomatic nonsustained VT did not have inducible VT. However, a potential contributing abnormality was found in 73% of pts with syncope, and inducible VT was found in 80% of cardiac arrest survivors. These data indicate: 1) asymptomatic nonsustained VT and near-syncope may represent lower risk subgroups, and 2) PES frequently identifies potential mechanisms of syncope or cardiac arrest, and therefore may be useful in directing therapeutic strategies.

NEW APPROACHES TO THE TREATMENT OF REFRACTORY ANGINA PECTORIS

Approximately 2-3 years ago we recognized that one of the most important problems in cardiology is how to improve blood flow to the heart of pts whose own coronary arteries were extensively diseased by severe atherosclerosis, and who had chronic refractory ischemic symptoms no longer responsive to pharmacologic therapy or amenable to coronary bypass surgery. We began to think of alternative approaches of treating these pts, focussing on possibilities that might lead to increased myocardial blood flow.

Intravascular Ablative Techniques in the Treatment of Cardiovascular Disease: For the past three years a multidisciplinary research group coordinated through NHLBI and the Cardiology Branch has investigated the feasibility of new technologies in an attempt to expand the range of patient candidates with coronary and peripheral vascular disease who would be amenable to percutaneous intravascular remodeling procedures. The major portion of Cardiology Branch investigations involves the use of lasers which are transmitted through optical fibers incorporated within catheter delivery systems for the purpose of atheroma ablation of intravascular target site lesions. More recently, we have become interested in investigating the feasibility of electrical thermal angioplasty as an alternative technique for plaque removal. Our multiphase approach includes initial in vitro tissue interaction studies, atheroma "photochemistry" experiments, small and large animal models of atherosclerosis for in vivo testing, prototype delivery system catheter fabrication, and finally, human clinical trials which would be initiated in patients with peripheral vascular disease and later extended to patients with coronary artery disease.

Tissue interaction studies were performed on fresh human cadaver coronary arteries that were longitudinally incised and exposed in air or in a wet field (saline and whole blood) to different energy sources. Tissue effects were analyzed using a uniform methodology incorporating analysis of gress morphology, light microscopy, quantitative occular micrometry, surface thermography utilizing infrared photography, and fast-reactive thermocouples placed on the adventitia to assess transmural temperature changes. Energy

sources included several different lasers (CO_2, Nd:YAG, Argon, Excimers, and Er:YAG) and an electrical thermal tip which is rapidly heated by a high voltage arc.

In vitro tissue experiments clearly demonstrated that superficial ablation without associated thermal tissue injury can be optimized with a combination of proper wavelength selection (ultraviolet or infrared), and specific lasing transmitted via either commercially available or prototype fiberoptics. In contrast, the electrical hot tip catheter results in effective tissue ablation with moderate surrounding thermal injury; it may be an important device for recanalization of obstructed larger peripheral vessels. Preliminary animal investigations have included a rabbit and swine model of atherosclerosis, both of which combine atherogenic diet and peripheral vessel balloon denudation to produce focal and severe atherosclerotic lesions. These ongoing animal studies involve both acute and chronic investigations of laser and electrical angioplasty techniques.

Initial results indicate that electrical thermal angioplasty is effective in a rabbit model of atherosclerosis; recanalization of long totally obstructed vessel segments was achieved. Excimer laser angioplasty in the same rabbit model was also effective in ablating atheroma, but resulted in frequent vessel wall perforation (principally caused by mechanical factors associated with the rigidity of currently available fiberoptics). Clearly, additional experiments are required to develop prototype catheters that will safely deliver laser energy.

In an attempt to engineer an acceptable clinical delivery system, a major investigative thrust has been directed towards analysis of target lesions to differentiate normal from non-normal tissue. Work has been done in the area of tissue absorption spectroscopy, quantitative and videl surface fluorescence studies, and chemical photosensitization of atheroma. Utilizing a microscope spectrofluorimeter and video enhanced fluoro-microscopy, atherosclerotic plaque can be identified and differentiated visually from normal tissue. Analysis of surface fluorescence emissions through the same fiberoptic delivering laser energy would permit the fabrication of "smart" catheters which would allow an interpretable feedback signal to activate a laser (or other energy source) and monitor plaque removal. Additional work has been done with intravascular angioscopy as an adjunct to laser angioplasty. This technique requires a bloodless field, coaxial positioning of the angioscope, adequate illumination, and high quality endoscopic and video equipment. It is unlikely that such methodologies will be incorporated within a multifunctional catheter design that is small enough to permit intravascular target visualization in addition to atheroma removal.

Our ongoing efforts in areas of basic science and in vivo testing are moving rapidly to a stage that will include human clinical investigations in patients with peripheral vascular disease before the end of this year.

Myocardial neovascularization by angiogenic factors: Oncologic research has determined that increases in certain solid tumor cell populations must be preceded by an increase in new capillaries that converge upon the tumor and supply it with blood. This hypothesis implies that angiogenesis is a rate-limiting step common to most solid neoplasms. It also led to studies seeking to identify those factors responsible for neovascularization (and therefore tumor expansion), with the ultimate hope of developing sustances that would inhibit angiogenesis (and thus tumor growth). We were intrigued by the thought that we might employ an analogous but opposite approach: to use angiogenic factors to promote rather than inhibit blood vessel growth in ischemic myocardium. We have therefore initiated studies to determine whether it would be possible to potentiate angiogenesis in ischemic myocardium. The first phase is taking place in our Experimental Physiology and Pharmacology Laboratory. Our initial studies are designed to determine whether we can promote neovascularization in ischemic situations, and if we can, whether it can prevent or reduce the consequences of ischemia. We have developed a small animal model of ischemia to test different angiogenic approaches and, in parallel, have developed a dog model of ischemia, which may have direct clinical applicability. We are also exploring certain biochemical questions that are linked to furthering our understanding of angiogenesis in the heart. Preliminary studies in rats indicate that heparin, which facilitates the effects of tumor angiogenic factor on cell proliferation and migration in vitro, lowers mortality and diminishes the size of the infarcted zone in rats with acute coronary ligation. The model is based on four days of isoproterenol administration, which we believe imposes an ischemic stress to the myocardium. Half of the animals are treated with heparin, half with saline. The heparin and isoproterenol are discontinued 24 hours prior to coronary ligation, at which time no residual effects of the isoproterenol or heparin are apparent. Similar beneficial effects were found with the tetrasaccharide heparin fragment, which is devoid of anticoagulant activity. We are in the process of confirming these results as well as assessing whether the beneficial effects of heparin are due to a facilitation of ischemia-induced neovascularization.

The large animal approach we are currently testing is based on the magnitude of intracoronary collateralization that develops following implantation of the internal mammary artery (IMA) to ischemic regions of the LV. This operation has been applied to pts in the past (Vineberg operation) but the total flow the IMA is capable of delivering is generally insufficient to importantly influence clinical outcome. We are currently implanting IMA grafts into the anterior wall of dogs, which are randomly assigned to receive continuous administration into the IMA of either heparin or normal saline. The area of ventricle into which the IMA graft is placed is rendered ischemic over a 2-4 week period by positioning amaroid constrictors around the LAD coronary artery. Animals are studied 8 weeks postoperatively to determine ischemic myocardial flow at baseline and during maximal vasodilator stimulation, gross anatomic distribution of vascular anastamoses, and histologically determined density of the myocardial microvasculature.

Our biochemical studies have focused on the question as to whether or not growth factors are present in the normal and ischemic heart. Preliminary results suggest that ischemia induces the synthesis of fibroblast growth factor (FGF) extremely rapidly. Preliminary attempts to isolate FGF from normal and ischemic myocardium are encouraging, with apparent isolation of active growth factors eluted from a heparin-sepharose column by 1.0 and 1.5 molar saline, exactly the extraction characteristics of acidic and basic fibroblast growth factors. Our substances appear mitogenic to 3T3 cells.

CORONARY ARTERY DISEASE

Prognostic implications of "silent" versus symptomatic ischemia: In mildly symptomatic pts with CAD, exercise induced ischemia identifies pts with a high likelihood of left main or 3 vessel disease at risk of death during medical therapy. To determine if development of angina during exercise provides added prognostic data we studied 131 consecutive CAD pts with mild or no symptoms by exercise ECG and radionuclide angiography. Pts with angina (54% of all pts) had a greater prevalence of left main or 3 VD (59 versus 25%) and a greater decrease in EF with exercise than pts without angina. All deaths occurred in the subgroup with both a decreased EF and abnormal ST segment responses. Both decreased EF and abnormal ST segment response occurred in 61% of angina pts but in only 27% of pts without angina. However, the likelihood of left main, 3 VD or death in pts with both decreased EF and abnormal ST segment response was similar, regardless of the presence or absence of angina. Thus, mildly symptomatic pts developing angina have a greater prevalence of potentially lethal coronary anatomy than pts without angina; however, in pts with similar coronary anatomy, the prognosis in pts with "silent" versus symptomatic ischemia during exercise testing appears the same.

Determinants of ventricular arrhythmias in mildly symptomatic pts with CAD: To determine the relationship between ventricular arrhythmias and prognostic factors in CAD we studied 131 minimally symptomatic pts by RNA and ambulatory ECG recording. We found that high grade ventricular arrhythmias in mildly symptomatic CAD pts are related to both extent of CAD and severity of regional and global LV dysfunction. Moreover, high grade ventricular arrhythmias were most prevalent in pts with resting LV dysfunction who developed further reversible LV dysfunction during exercise (reduction in EF), factors indicating poor long-term prognosis during nonsurgical therapy.

VALVULAR HEART DISEASE

Value of regurgitant volume to end-diastolic volume ratio: Preoperative LV systolic function is an important determinant of survival and functional results following aortic valve replacement for chronic aortic regurgitation. However, many pts with pre-op LV dysfunction manifest improved LV function after AVR and have an excellent prognosis. We examined the hypothesis that the magnitude of the regurgitant volume (RV) relative to end-diastolic volume (EDV) may predict post-op outcome in such pts. In patients with subnormal pre-op LV EF, the pre-op RV EDV ratio did distinguish between groups at high and at low risk of death and/or post-op heart failure. It would appear that this index may provide additional prognostic information regarding survival and clinical outcome in aortic regurgitation pts.

Post-op changes in LV function in AR: In most pts with AR, valve replacement results in reduced LV dilatation and increased EF. To determine the relation between serial changes in dilatation and changes in EF, we studied 50 AR pts by echo and radionuclide angiography before, 6-8 months after, and 3-7 years after AVR. At 6-8 months, LV diastolic dimension decreased relative to pre-op (75±7 to 56±9 mm) and EF increased (45±10 to 51±17). From this early study to the late post-op study, diastolic dimension did not change, but EF increased further (to 57±20%). Increased EF from early to late study occurred only in subgroups with an initial increase in EF between the pre-op and early post-op value, in pts with normal pre-op rest EF, and in pts with subnormal pre-op EF in whom duration of LV dysfunction was brief (less than 14 months prior to AVR). In other pts with subnormal pre-op EF, EF did not change from pre-op to early or from early to late follow-up. Extent of change in EF correlated with change in diastolic dimension. Thus, post-op reduction in LV dilatation is an important determinant of early and late increase in EF after AVR. Moreover, late improvement in EF occurs commonly in pts with an early increase in EF, but is unlikely in pts with no change in EF during the first 6 months following AVR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04067-03 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Detrimental effect of ergonovine in hypertrophic cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Patients with hypertrophic cardiomyopathy frequently experience chest pain that occurs with variable threshold of onset and is often prolonged in duration. The study was designed to evaluate the effect of a pharmacologic vasoconstrictor agent, ergonovine, on the coronary vasculature of patients with hypertrophic cardiomyopathy. Twenty-four patients with hypertrophic cardiomyopathy and a history of angina pectoris despite normal epicardial coronary arteries underwent a study of coronary flow, and myocardial function and metabolism. During pacing to an average heart rate of 133, 18 of the 24 patients experienced their typical chest pain. During pacing after the administration of ergonovine, 22 of 24 patients experienced chest pain. Despite a significantly higher blood pressure following ergonovine administration, the coronary flow at an average pacing rate of 138 beats/min was significantly lower than during pacing to a similar heart rate prior to administration of ergonovine. There was no epicardial coronary artery narrowing during coronary angiography after ergonovine administration. Thus, peak coronary flow decreases with ergonovine in patients with hypertrophic cardiomyopathy, probably due to vasoconstriction of a maximally dilated microvascular bed, or vasoconstriction of prearteriolar small coronary arteries. Small vessel coronary vasoconstriction may explain many of the atypical features of angina pectoris in patients with hypertrophic cardiomyopathy, causing pain at rest or during variable levels of effort.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-HL-04094-02-CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
John E. Brush, Jr., M.D.	Senior Staff Fellow	CB NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis

INSTITUTE AND LOCATION
NHLBI NIH, Bethesda, Md 20892

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

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

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

We have previously demonstrated limitation in coronary flow reserve of the coronary microcirculation to be a frequent mechanism of myocardial ischemia and angina pectoris in patients with angiographically normal epicardial coronary arteries. We have further found that limited coronary flow reserve can be demonstrated during rapid atrial pacing, especially after ergonovine administration, associated with angina pectoris and metabolic and hemodynamic evidence of myocardial ischemia. Because ergonovine administration increases coronary resistance without discernible changes in the epicardial coronary arteries, our hypothesis is that ergonovine is inducing vasoconstriction of the coronary microcirculation, resulting in limited flow reserve to stress. Because pacing does not allow assessment of total transmural coronary flow reserve, a potent coronary arteriolar vasodilator, dipyridamole, was used to investigate peak transmural flow reserve in patients with anginal pain despite normal epicardial coronary arteries. Twenty-five patients were identified as having limited flow reserve during the stress of rapid atrial pacing following administration of ergonovine and an additional 15 patients were felt not to have evidence of coronary vasoconstriction after ergonovine administration. After administration of dipyridamole 0.5 to 0.75 mg/kg intravenously, the lowest absolute levels to which coronary resistance fell and the maximum absolute levels to which great cardiac vein flow rose were impaired in the 25 patients with ergonovine-induced flow limitation compared to the 15 patients without limitation after ergonovine administration. These studies suggest that patients with anginal chest pain despite normal epicardial coronary arteries may have exaggerated coronary responses to vasoconstrictor stimuli, which can result in myocardial ischemia during stress, as well as attenuated responses to coronary vasodilator stimuli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-04095-02-CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanisms of myocardial ischemia in hypertrophic cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Cynthia M. Tracy, M.D.	Senior Medical Staff Fellow	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
John E. Brush, Jr., M.D.	Senior Staff Fellow	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have previously demonstrated that myocardial ischemia can be induced in patients with hypertrophic cardiomyopathy by rapid atrial pacing, precipitating symptoms of chest pain and shortness of breath identical to symptoms described by history. In order to elucidate mechanisms of myocardial ischemia in patients with hypertrophic cardiomyopathy and to assess whether the presence of obstruction in left ventricular outflow mattered in the pathogenesis of ischemia, 50 patients with hypertrophic cardiomyopahty and normal epicardial coronary arteries underwent invasive study of coronary and myocardial hemodynamics in the basal state and during the stress of pacing. The 23 patients with basal obstruction (mean left ventricular outflow gradient 77$\pm$33mmHg) had significantly lower coronary resistance and higher basal coronary flow than the 27 patients without basal obstruction. During the stress of pacing, myocardial oxygen consumption and blood flow were significantly higher in patients with obstruction compared to patients without outflow obstruction. At a heart rate of 130, when most patients were experiencing chest pain, peak flow was significantly higher in patients with obstruction, with myocardial ischemia occurring at a significantly lower flow and higher coronary resistance and lower myocardial oxygen consumption in the patients without obstruction. This study suggests that the elevated left ventricular systolic pressures associated with left ventricular outflow obstruction significantly increases myocardial oxygen demands and results in rapid exhaustion of coronary flow reserve during stress. In patients without basal obstruction, exhaustion of flow reserve at a lower peak flow suggests significant impairment in coronary flow delivery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04096-02 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effect of surgical relief of obstruction in hypertrophic cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Charles L. McIntosh, M.D.	Senior Surgeon	SU	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Surgical relief of left ventricular obstruction by left ventricular myotomy/myectomy or mitral valve replacement is a therapeutic option in patients with hypertrophic cardiomyopathy who are severely symptomatic and refractory to medical management. To determine the effects of surgical relief of left ventricular outflow obstruction in patients with hypertrophic cardiomyopathy, 8 patients were studied at rest and during atrial pacing before and after operation (septal myectomy in 4 and mitral valve replacement in 4). Coronary flow to the anterior left ventricle and septum, the site of maximum hypertrophy in these patients, was assessed by thermodilution. In all eight patients there was successful relief of resting left ventricular outflow tract gradient from a preoperative gradient of 78±36 to 3±5 mmHg postperatively. Surgical relief of left ventricular outflow tract obstruction significantly reduced left ventricular systolic pressure, coronary flow, and myocardial oxygen consumption at rest and during pacing, lowered left ventricular end-diastolic pressure following pacing, and improved anginal threshold and metabolic evidence of ischemia. These results demonstrate the importance of left ventricular outflow tract gradients in hypertrophic cardiomyopathy as well as the mechanism of improved effort tolerance after surgical relief of obstruction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04109-02 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Promotion of angiogenesis by heparin in the canine heart

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

Ellis F. Unger, M.D.	Medical Staff Fellow	CB	NHLBI
Stepehn 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, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.5	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.)*

One of the major problems in cardiology today is how to more effectively treat individuals with coronary artery disease who have symptoms refractory to conventional therapy, including antianginal drugs and coronary artery bypass surgery. One potential approach we are currently investigating is implantation of the internal mammary artery (IMA) into ischemic regions of the left ventricle. This operation has been performed on patients in the past, but the blood flow through the IMA has been found to be generally insufficient, incapable of delivering enough blood flow to importantly influence symptoms. Heparin, a commonly utilized anticoagulant, has been found to play a major role in the process of angiogenesis, the formation of new blood vessels, in vitro. This experiment is designed to assess the ability of heparin to potentiate the growth of vascular connections derived from the IMA when implanted in ischemic myocardium in a canine model. Foxhounds will undergo IMA implantation into the anterior wall of the left ventricle (Vineberg Procedure). Animals will randomly be assigned to receive continuous administration into the IMA of either heparin or normal saline (control group). The area of the left ventricle in which the IMA graft is placed will be rendered ischemic over a two to three week period by positioning an ameroid constrictor around the left anterior descending coronary artery. Animals will be studied eight weeks postoperatively to determine both the baseline myocardial blood flow and the maximum capacity for myocardial blood flow (vasodilator reserve) in the ischemic zone. The gross and microscopic distribution of vascular anastomoses as well as the density of capillaries within the ischemic area will subsequently be determined with a digital video analyzer and comparisons will be made between the heparin and control groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04110-02 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Electrical stimulation in patients with HCM at risk for sudden death

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

Cynthia M. Tracy, M.D.	Senior Medical Staff Fellow	CB NHLBI
Judith Winkler, R.N.	Registered Nurse	CB NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Eben E. Tucker, M.D.	Chief, Consultative Services	CB NHLBI
Richard O. Cannon, III,M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Albert Del Negro	Director Electrophysiology Dept.	Georgetown Univ.
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

Georgetown University, Washington, D.C.

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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.)*

Ventricular tachycardia occurs on ambulatory monitoring in 19% of patients with hypertrophic cardiomyopathy (HCM), of whom 8% die yearly. However, high risk subgroups have not been identified. To determine the role of programmed electrical stimulation in defining contributing factors to major clinical events and in directing therapy, we studied 29 HCM patients with cardiac arrest, syncope, near-syncope, or asymptomatic nonsustained ventricular tachycardia. Programmed electrical stimulation identified abnormalities in 15 patients (52%), including inducible ventricular tachycardia in 6 patients, atrial ventricular node disease in 5, bypass tracts in 2 and supraventricular tachycardia in 3. Patients with near syncope or asymptomatic nonsustained ventricular tachycardia did not have inducible tachycardia. However, in 73% of patients with syncope a potential contributing abnormality was found, and 80% of cardiac arrest survivors had inducible ventricular tachycardia. These data indicate: 1) asymptomatic nonsustained ventricular tachycardia and near syncope may represent lower risk subgroups and 2) programmed electrical stimulation frequently identifies potential mechanisms of syncope or cardiac arrest, and therefore may be useful in directing therapeutic strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-04111-02-CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Coronary flow reserve in idiopathic dilated cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Sebastian Palmeri, M.D.	Head, Consultative Services	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

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.)*

In the majority of patients with dilated cardiomyopathy the etiology is unknown. Many patients with dilated cardiomyopathy complain of anginal-type pain despite angiographically normal epicardial coronary arteries. To examine whether abnormalities in coronary flow exist in dilated cardiomyopathy, 26 patients with dilated cardiomyopathy and normal epicardial coronary arteries, 12 of whom had frequent chest pain by history, underwent measurement of great cardiac vein flow and myocardial metabolism at rest and during pacing to a heart rate of 150. During pacing following administration of ergonovine, all 12 patients with a history of chest pain experienced their typical pain. Compared to patients without chest pain, their coronary flow was lower and coronary resistance higher, with increased myocardial oxygen extraction suggestive of myocardial ischemia. Additionally, there was a greater increase in left ventricular filling pressures in this group. There was no significant change in EKG or epicardial coronary luminal diameter by angiography. Administration of dipyridamole 0.5 to 0.75 mg intravenously to 20 patients demonstrated that those 7 patients with a history of angina pectoris also had impairment in transmural coronary flow reserve compared to the 13 patients without chest pain. Thus, patients with dilated cardiomyopathy and chest pain by history may have limited coronary vasodilator reserve, expecially after vasoconstrictor stimulus. Whether this contributes to myocardial damage in dilated cardiomyopathy or is an epiphenomenon of an unrelated etiology, remains to be determined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04112 01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Quantitation of Calcium Channels in Human Myocardium

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

Frederic L. Sax, M.D.	Medical Staff Fellow	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI
Charles McIntosh, M.D.	Senior Surgeon	SB NHLBI
William Roberts, M.D.	Chief, Pathology Svc.	PA NHLBI

COOPERATING UNITS *(if any)*

Cardiac Surgery, Pathology, NIH
Johns Hopkins University

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI 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.)*

Clinical evidence points to the possibility that patients with hypertrophic cardiomyopathy may have disordered regulation of cytosolic calcium. One hypothesis is that these patients might have an increased number of calcium channels so that for a given signal, they have increased calcium influx. Such an increased number of calcium channels has recently been reported in the Syrian hamster model of cardiomyopathy. To study calcium channel density in human myocardium we have been using right atrial appendages isolated during cardiac surgery on patients with or without HCM. In a very few patients that have been studied as of this time, there may be some patients with HCM who have a greater number of calcium channels than a non-HCM heart disease control population. Studies are ongoing to confirm this observation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04113-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Platelet Calcium Levels in Hypertrophic Cardiomyopathy

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

Frederic L. Sax, M.D.	Medical Staff Fellow	CB NHLBI
Michael A. Beaven, Ph.D.	Senior Investigator	CP NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Chemical Pharmacology

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI 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.)*

There is circumstantial clinical evidence to suggest that patients with hypertrophic cardiomyopathy (HCM) have disordered regulation of intra-cellular calcium. The hypercontractile myocardium with poor diastolic relaxation could be explained, for example, by increased cytosolic Ca+2 in the cardiac myocyte. Given that some forms of HCM are genetically transmitted, we postulated that a disorder of cytosolic calcium metabolism might be present in other, non-cardiac tissue. To study this, we isolated platelets from the plasma of patients with HCM and normals and measured intra-cellular calcium levels using the fluorescent indicator QuinII. Our preliminary results (on only a small number of patients and controls) indicates that resting Ca+2 levels are the same in these populations. When the cells are stimulated with vasopressin -- which causes both intra-cellular calcium mobilizaton and Ca+2 influx -- some patients appear to have a blunted response to this stimulant. Whether this is an epiphenomenon still remains to be determined, but if these results are substantiaed, they point to wide-spread Ca+2 dysregulation. The mechanism of this could be determined and might give us an important clue as to the etiology of this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04114-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Forearm Flow in Patients with Angina and Normal Coronary Arteries

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

Frederic L. Sax, M.D.	Medical Staff-Fellow	CB NHLBI
Richard O. Cannon, III, M.D.	Senior Investigator	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.3	.15	.15

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Many patients with anginal chest pain and normal epicardial coronary arteries show abnormal coronary flow response to electrical pacing, the vasoconstrictor, ergonerine and the vasdilator, dipyridamole. These stimuli, in fact, often reproduce their chest pain. The studies elliciting this data suggest these patients have dysregulation of vascular smooth muscle tone, and, in particular, decreased vasodilator reserve. To examine if these phenomena represent a more generalized abnormality of vascular smooth muscle function, we studied another vascular bed by studying blood flow to skeletal muscle in the forearm. We used the non-invasive technique of strain-gauge plethysmography and studied vasodilator capacity by subjecting the forearm to ischemia (an upper arm cuff inflated to supra-systolic pressures) of increased lengths of duration. Compared to an approximately age and sex matched control population, the patients with myocardial "tone" abnormalities had blunted peak flows at all durations of ischemic time (1 min, 3 min, 5 min). Their minimal vascular resistance (mean blood pressure divided flow) was also higher than controls. This suggests a decreased vasodilator capacity of the forearm musculature and points to a more generalized disorder of smooth muscle function in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04115-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Forearm Flow in Patients with Hypertrophic Cardiomyopathy

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

Frederic L. Sax, M.D.	Medical Staff Fellow	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION

INSTITUTE AND LOCATION
NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.25	.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.)*

Patients with hypertrophic cardiomyopathy (HCM) exhibit abnormalities in coronary flow response to stress stimuli such as pacing and ergonovine, and the vasodilator, dipyridamole. These stimuli often ellicit anginal chest pain in such patients. It has therefore been suggessted that patients with HCM have decreased small coronary vasodilator reserve. To determine if this phenomenon applied to other vasular beds, we studied the forearm vasodilator capacity using ischemia (occlusion of the circulation) as the vasodilator stimulus. We found that patients with HCM in fact do have decreased vasodilator capacity in their forearm vasculature compared to normals. This is manifested by a decreased peak flow and increased vascular resistance at various durations of ischemia, compared to normals. This suggests that patients with HCM may have an abnormality of smooth muscle regulation that affects both the mycoardial and peripheral vasculature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04116-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Atherosclerotic plaque identification using surface fluorescence

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

David Y. Lu, M.D.	Medical Staff Fellow	CB	NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Paul D. Smith, Ph.D.	Senior Research Fellow	BEIB	DRS
Robert S. Balaban, Ph.D.	Senior Investigator	KE	NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Kidney and Electrolyte Metabolism
Biomedical Engineering and Instrumentation Branch

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Laser angioplasty is currently being investigated as a possible technique to recanalize obstructed blood vessels. To reduce the high incidence of vessel wall perforation associated with this technique, a new method of in vivo plaque identification is needed to direct and control the laser energy so as to obtain precise and selective plaque ablation with minimal injury to the surrounding tissues. The purpose of this study is to see if it is possible to accurately differentiate normal from atherosclerotic regions using surface fluorescence signals from arterial lumen surfaces.

6 fresh human aortic segments with varying degree of atherosclerosis were analyzed for its surface fluorescence characteristics using a custom designed microfluorospectrometer. Using blue excitation (450-490nm), the surface fluorescence spectra showed a statistically significant difference in fluorescence intensity at 540nm comparing normal to diseased regions. However, there was no difference in the spectral shape of the different regions analyzed. A video enhanced fluorescence image of the arterial surface also demonstrated that the atheroma can easily be distinguished with high contrast and excellent resolution.

It appears that the fluorescence signal comes from the elastic fibers in the media, and that the intervening atheroma either prevents the excitation or filters out the fluorescence signal from the elastic fibers underneath. Therefore, it is possible to identify atherosclerotic plaques using quantitative and video surface fluorescence, and this may provide the feedback signal to activate a laser source for selective plaque removal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04117-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Electrical thermal angioplasty

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

David Y. Lu, M.D.	Medical Staff Fellow	CB	NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Robert L. Bowman, M.D.	Chief, Technical Development	CB	NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Technical Development

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

Recently it has been shown that a laser heated metallic tip can effectively recanalize obstructed human peripheral vessels with a low incidence of vessel wall perforation. This technique has the potential of being applied to recanalize obstructed human coronary arteries. However, a simpler, less expensive, and portable energy source for thermal angioplasty would be desirable. As such, a new electrical thermal tip catheter (ETC) has been designed and fabricated.

The operating principle of the ETC is based on establishing an electric arc between a central electrode and the inside of an enclosing metallic tip. The ETC (3F and 5F) can be rapidly heated (<1 sec) and boil water in a saline bath at <1 watt. Human atherosclerotic lesions can be effectively ablated, in vitro, with 9 watts or more. There is some associated zone of thermal injury. Preliminary in vitro studies in an atherosclerotic rabbit model showed that obstructed iliofemoral vessels (9 vessels) with significant lesions (7 vessels) can be recanalized with low incidence of vessel wall perforation (1 vessel), and with minimal histologic thermal injury.

The initial results are promising. Further studies are planned to test the safety and the reliability of the ETC, and to assess the long term patency rate of recanalized vessels prior to any in vivo human trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04118-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Lidoflazine in patients with HCM refactory to standard medical therapy

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

Cynthia M. Tracy, M.D.	Senior Medical Staff Fellow	CB	NHLBI
Richard O. Cannon, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
John E. Brush, Jr., M.D.	Senior Staff Fellow	CB	NHLBI
S. Ward Casscells, III, M.D.	Senior Staff Fellow	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB	NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

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.)*

Many patients with hypertrophic cardiomyopathy have severe symptoms in spite of medical therapy with beta adrenergic blocking agents and/or calcium channel blocking agents. Recently we have been investigating the use of amiodarone, a benzofuran derivative with potent hemodynamic and antiarrhythmic properties in this same subgroup of patients and have noted an improvement in cardiac symptoms and an increase in exercise capacity. However, there remains a subgroup of patients who are intolerant of amiodarone or who do not improve on amiodarone and continue to have marked symptomatology. In response to a compelling clinical need in this this subgroup of refractory patients, we felt it appropriate to explore other potential pharmacologic modalities. We have hypothesized that the functional and structural abnormalities in HCM are related to a primary membrane disorder leading to increased cytosolic calcium levels as a result of altered calcium fluxes involving both the myocardium and the vascular smooth muscle of the small intramural coronary arteries. Lidoflazine has been shown to be a potent calcium entry blocker, and has a cellular protective effect against calcium overload in vascular smooth smooth muscle and cardiac muscle during ischemia, preventing ischemic contracti- and myonecrosis. These properties of the drug afford an ideal mechanism for testing the above hypotheses, as well as offering a potentially important therapeutic alternative. The study will consist of 3 phases. The first phase to assess the clinical efficacy of the drug; the second to characterize the hemodynamic/metabolic correlates of the drug that may determine its efficacy; and the third to compare in a double blind fashion lidoflazine versus standard therapy. We have enrolled thus far 3 patients in phase I, two of whom have had symptomatic and exercise improvements.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04119-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Prognostic Implications of "Silent" vs Symptomatic Myocardial Ischemia

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
Stephen L. Bacharach, Ph.D.	Phycicist	NM CC
Michael V. Green, M.S.	Chief, Imaging Physics Section	NM CC
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

Department of Nuclear Medicine, CC

LAB/BRANCH

Cardiology Branch

SECTION

Nuclear Cardiology

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

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.)*

Exercise-induced ischemia in mildly symptematic patients with coronary artery disease identifies a subgroup with a high likelihood of left main disease, 3-vessel disease, and sudden death during subsequent therapy. Many such patients have no chest pain during exercise despite objective evidence of ischemia. To determine the prognostic implications of "silent" ischemia with exercise, we studied 131 consecutive patients with mild or no symptoms and with preserved resting left ventricular function by exercise ECG and radionuclide angiography. Patients developing angina with exercise had a greater prevalence and severity of reversible myocardial ischemia. However, those patients manifesting an ischemic response to stress had similar prevalence of left main or 3-vessel disease and a similar mortality rate during medical therapy whether or not chest pain developed with exercise. Thus, the prognosis in patients with "silent" vs symptomatic ischemia during exercise testing is similar.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04120-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Myocardial perfusion defects in patients with angina and normal coronary arteries

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

Patrick T. O'Gara, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB	NHLBI
Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
Barbara A. Damske	Staff Nurse	NM	CC
Stephen L. Bacharach, Ph.D.	Physicist	NM	CC
Steven M. Larsen, M.D.	Chief, Nuclear Medicine Dept.	NM	CC
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

Nuclear Medicine Dept., CC

LAB/BRANCH

Cardiology Branch

SECTION

Nuclear Cardiology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Many patients with anginal chest pain, despite angiographically normal coronary arteries, display limitations of coronary blood flow under a variety of conditions. Previous work has established that many of these patients are unable to augment their coronary blood flow normally in response to certain stimuli. They frequently develop chest pain under these conditions in association with both hemodynamic and metabolic evidence for myocardial ischemia. It appears that their reduced ability to increase coronary blood flow in response to stress is a dynamic abnormality of coronary arteries too small to be visualized during angiography. The current protocol was designed to determine the location, extent and severity of such coronary flow abnormalities using Thallium-201 emission computed tomography. Accordingly, 13 patients with previously documented abnormal coronary vasomotor tone underwent Thallium-201 perfusion imaging following an infusion of dipyridamole. Despite the provocation of chest pain and hemodynamic alterations in the majority of these patients, only one patient demonstrated a perfusion defect compatible with regional myocardial ischemia. We have concluded from these studies that dipyridamole Thallium-201 perfusion imaging is an insensitive technique for the visualization and characterization of abnormalities of myocardial blood flow in patients with chest pain and normal coronary arteries. Such negative results may reflect the fact that the regional flow disparities in such patients are of too small a magnitude to be detected with this current technology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04121-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of myocardial perfusion in subjects without cardiovascular disease

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

Patrick T. O'Gara, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB	NHLBI
Barbara Damske	Staff Nurse	NM	CC
Arthur VanLingen	Guest Researcher	NM	CC
Stephen L. Bacharach,Ph.D.	Physicist	NM	CC
Steven M. Larsen, M.D.	Chief, Nuclear Medicine Department	NM	CC

COOPERATING UNITS *(if any)*
Nuclear Medicine Dept., CC

LAB/BRANCH
Cardiology Branch

SECTION
Nuclear Cardiology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md

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

To provide a better understanding of normal myocardial perfusion and to establish a data base against which to compare the results of studies in patient populations, we evaluated 51 healthy volunteers using exercise Thallium-201 emission-computed tomography. Cardiac disease was excluded in these volunteers on the basis of a normal history, physical examination, echocardiogram, chest radiograph and resting and exercise electrocardiograms. All subjects were able to exercise to the point of limiting fatigue or shortness of breath. At peak exercise, Thallium-201 was injected by peripheral vein and exercise was continued for an additional minute to allow for adequate circulation of the isotope. Rotational tomographic imaging was begun within 10 minutes of exercise and again after a three hour delay. Anatomically comparable tomographic slices from each of the three major planes of the heart were then analyzed for the distribution and intensity of the isotope. A quantitative analysis program was developed based on a radial distribution method. Tomographic slices were divided into 32 sectors, each of which spanned 11.25 degrees. Determinations of both maximal and total sector activity were made and the results normalized to the maximal value for the entire heart. Wash out profiles were then constructed representing the change in activity between the initial and delayed studies. These programs have allowed us to easily construct normal data bases for both sexes. Patients with heart disease can be compared quickly with the values derived from the normal volunteers and the results of any Thallium perfusion study can be expressed objectively and quantitatively without the risk of observer bias. These methods will serve to automate and validate future interpretations for any patient population.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04122-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Verapamil effects on myocardial perfusion in hypertrophic cardiomyopathy

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

Patrick T. O'Gara, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB	NHLBI
James Udelson, M.D.	Medical Staff Fellow	CB	NHLBI
Barbara Damske	Staff Nurse	CB	NHLBI
Arthur VanLingen	Guest Researcher	NM	CC
Stephen L. Bacharach, Ph.D.	Physicist	NM	CC
Steven M. Larsen, M.D.	Chief, Nuclear Medicine Department	NM	CC
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*
Nuclear Medicine Dept., CC

LAB/BRANCH
Cardiology Branch

SECTION
Nuclear Cardiology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

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

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

Myocardial Thallium-201 perfusion defects, suggestive of ischemia, are commonly observed among patients with hypertrohic cardiomyopathy, regardless of symptomatic state. Medical therapy is usually reserved for those patients with limiting symptoms, but may be indicated even in asymptomatic patients if such therapy is found to improve or normalize the indices of silent ischemia often observed in such patients. The current protocol is designed to assess the effects of verapamil on abnormalities of myocardial perfusion and left ventricular diastolic function in asymptomatic and minimally symptomatic patients with hypertrophic cardiomyopathy. The results of this study could alter the clinical management of this very large patient population.

Beginning in July 1986, we plan to enroll 20 patients in a randomized trial, comparing verapamil with placebo in the treatment of asymptomatic or minimall asymptomatic patients with hypertrophic cardiomyopathy. Following two weeks of drug therapy, patients will undergo Thallium-201 emission-computed tomography in conjunction with maximal treadmill exercise. Qualitative and quantitative assessments of myocardial perfusion will then be made. The patients will also undergo resting radionuclide cineangiography to evaluate both systolic and diastolic left ventricular function. Following a one week washout period, the patients will then cross over to alternate therapy (either placebo or verapamil) and repeat radionuclide investigations will occur two weeks later. The cardiovascular and systemic effects of either drug will be carefully monitored by close supervision during the course of the study. Should verapamil prove efficacious in improving or normalizing the abnormalities found among these patients, we will then consider a more long-term randomized trial to assess the effects of medical therapy in a much larger patient group.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04123-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Myocardial perfusion abnormalities in patients with hypertrophic cardiomyopathy

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

Patrick T. O'Gara, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Sec.	CB	NHLBI
Barbara Damske	Staff Nurse	NM	CC
Barry J. Maron, M.D.	Senior Investigator	CB	NHLBI
Stephen L. Bacharach, Ph.D.	Physicist	NM	CC
Steven M. Larsen, M.D.	Chief, Nuclear Medicine Dept.	NM	CC
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB	NHLBI

COOPERATING UNITS *(if any)*

Nuclear Medicine Department, CC

LAB/BRANCH

Cardiology Branch

SECTION

Nuclear Cardiology Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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.)*

Several lines of evidence indicate that myocardial ischemia, due to alterations in blood flow, may play a central role in the national history of hypertrophic cardiomyopathy. Myocardial imaging with the isotope Thallium-201 offers a non-invasive means of assessing blood flow. We undertook the present study to determine the relative prevalence of perfusion abnormalities across a wide spectrum of patients with hypertrohic cardiomyopathy. Accordingly, 72 patients ranging in age from 12 to 69 years underwent Thallium-201 emission computed tomography (ECT) in conjunction with treadmill exercise. Fifteen of the patients had resting depression of left ventricular function as manifested by a reduced ejection fraction. Fourteen of these 15 patients demonstrated fixed or only partially reversible perfusion abnormalities consistent with underlying areas of myocardial fibrosis and/or severe ischemia. Of the remaining 57 patients with normal or hyperdynamic left ventricular function, 48% demonstrated perfusion abnormalities predominantly of the reversible type. These latter defects are consistent with dynamic, stress-induced ischemia.

These results extend and confirm previous observations concerning Thallium perfusion defects in patients with hypertrohic cardiomyopathy. The fixed or only partially versible defects seen in patients with resting left ventricular dysfunction most likely do represent areas of underlying scar and hence provide an explanation for the associated impairment in contractile function. The reversible defects observed in the other patient subgroup reflect a more dynamic process, which, if allowed to continue, may eventuate in either the progression of symptoms, the development of an arrhythmic complication, or the gradual replacement of myocardium by a process of necrosis and infarction leading eventually to left ventricular dysfunction and congestive heart failure.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04124-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Regurgitant volume to end-diastolic volume ratio in aortic regurgitation

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

Patrick T. O'Gara, M.D.	Guest Researcher	CB	NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB	NHLBI
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Barry J. Maron, M.D.	Senior Investigator	CB	NHLBI
Stephen L. Bacharach, Ph.D.	Physicist	NM	CC
Michael V. Green, M.S.	Chief, Imaging Physics Section	NM	CC

COOPERATING UNITS *(if any)*

Nuclear Medicine Dept., CC

LAB/BRANCH

Cardiology Branch

SECTION

Nuclear Cardiology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Preoperative left ventricular systolic function is an important determinant of prognosis following aortic valve replacement for isolated chronic aortic regurgitation. Although patients with subnormal function are at greater risk for death or heart failure, many such patients enjoy an excellent outcome postoperatively. A means of objectively assigning risk among patients with depressed systolic function is desirable. In the current study, we examined the prognostic value of the left ventricular regurgitant volume to end-diastolic volume ratio, an index which provides information concerning both the magnitude of the imposed volume load, that is, the regurgitant volume, as well as the left ventricular response to this load, the end-diastolic volume.

We evaluated the results of aortic valve replacement in 59 patients with isolated severe chronic aortic regurgitation undergoing operation between February 1975 and August 1983. Several indices of preoperative left ventricular function were identified which were significantly associated with subsequent cardiac death and heart failure. These included both the left ventricular ejection fraction and the regurgitant volume to end-diastolic volume ratio (RV/EDV). Overall, survival was significantly reduced in patients with left ventricular dysfunction (EF<0.45). Among such patients, an RV/EDV ratio $\leq$0.25 was associated with a greater risk of death or heart failure. This observation suggests that the LV enlargement seen in these patients exceeds that which could be attributable to the regurgitant volume load and may reflect some degree of irreversible dysfunction placing them at higher risk. Therefore, the regurgitant volume of end-diastolic volume ratio may help assign risk among patients with comparable degrees of left ventricular dysfunction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04125-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Arterial surface fluorescence becomes normal after laser atheroma ablation

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

Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
David Y. Lu, M.D.	Medical Staff Fellow	CB NHLBI
Paul D. Smith, Ph.D.	Senior Research Fellow	BEIB DRS
Robert F. Bonner, Ph.D.	Senior Research Fellow	BEIB DRS
Robert S. Balaban, Ph.D.	Senior Investigator	KE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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.)*

Previous experiments in our laboratories have indicated that quantitative and video analysis of surface fluorescence from atherosclerotic necropsy specimens provids a means for sensitive differentiation of normal and atherosclerotic segments. These data indicate that surface fluorescence emissions are reduced from atherosclerotic zones and this changes can be displayed quantitatively as well as visually utilizing video enhanced images employing a custom design microscope spectrofluorimeter. The present investigation was designed to analyze surface fluorescence changes associated with laser-induced atheroma ablation. Both argon and excimer lasers were employed and plaque was removed from necropsy human specimens of aorta containing varying degrees of surface atheroma. We found that plaque removal was associated with increased fluorescence intensity and that residual intima thickness after laser ablation was strongly associated with fluorescence intensity. Moreover, transmural analysis of plaque failed to identify important plaque-related fluorochromes which might be responsible for the observed fluorescence emissions. We concluded that laser atheroma ablation returns surface fluorescence to normal and that plaque appears to behave as an absorbing internal filter decreasing autofluorescence from elastic fibers in the underlying media. Thus, surface fluorescence intensity may be a useful technique to monitor plaque removal during laser angioplasty.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04126-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

In vivo Excimer laser angioplasty: Design criteria and preliminary animal results

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

Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Paul D. Smith, Ph.D.	Senior Research Fellow	BEIB DRS
David Y. Lu, M.D.	Medical Staff Fellow	CB NHLBI
Joseph T. Dodd, M.D.	Medical Staff Fellow	SU NHLBI
Robert F. Bonner, Ph.D.	Senior Research Fellow	BEIB DRS

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Over the past 18 months laser tissue interaction studies using Excimer lasers (both Xenon Chloride-308nm and Krypton Fluoride-248nm) have been performed to define specific ablative thresholds and ablative efficiency, as well as the mechanisms of tissue ablation for human normal and atherosclerotic specimens. However, before clinical trials can be considered, the efficiency of transmitting fibers and efficacy of Excimer laser angioplasty in animal models must be carefully determined. Thus, a XeCl Excimer laser (40 nsec pulses) was delivered through commercial 600u silicon fibers in saline and whole blood wet fields. The ablative threshold and efficiency was similar to previous experiments without fibers in air using both normal sheep aorta and human necropsy specimens. The depth of ablation was linearly related to energy density and fiber damage occurred at fluences 5-6 times greater than the ablative threshold. Thus, a narrow operating margin between ablative threshold and fiber damage was defined mandating fiber-target contact to ensure predictable tissue ablation. Thereafter, Excimer laser angioplasty was attempted in New Zealand white rabbits which were fed a 2% cholesterol diet followed by endothelial balloon barotrauma to induce focal severe atherosclerosis in the iliofemoral arterial system bilaterally. Attempted recanalization of stenosed or occluded iliac arteries resulted in angiographic perforations in every animal. The histology of excised vessels was without significant thermal injury and perforations were due largely to fiber stiffness and mechanical factors. Therefore, we have concluded that XeCl Excimer lasers can be transmitted by fibers at energy densities sufficient to cause precise tissue ablation without significant thermal tissue injury, but Excimer laser angioplasty in an atherosclerotic rabbit model was associated with frequent vascular perforations emphasizing the need for more flexible delivery systems.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04127-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Abnormal esophageal motility in patients with limited coronary flow reserve

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diag.	CB NHLBI
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COOPERATING UNITS *(if any)*

National Naval Medical Center, Bethesda, Md.

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Explanations for chest pain in patients with chest pain, despite angiographically normal coronary arteries include abnormal coronary flow reserve and esophageal motility disorders. To ascertain the frequency of cardiac versus esophageal functional abnormalities in such patients, 43 patients with chest pain despite normal epicardial coronary arteries underwent measurement of coronary resistance during pacing at a heart rate of 150, heart rate of 150 after ergonovine, 0.5-0.3 mg intravenously and after dipyridamole 0.5-0.75 mg/kg intravenously. Those patients who had dynamic limitation in flow reserve to ergonovine and limited flow reserve after dipyridamole had a higher prevalence of esophageal motility disorders than those patients who had no vasoconstrictor response to ergonovine and had a normal flow reserve after dipyridamole. The high prevalence of abnormal esophageal motility in patients with dynamic limitation in coronary flow reserve suggests that this syndrome may be part of a generalized abnormality of smooth muscle rectivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04128-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Left ventricular pulsus alternans in hypertrophic cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
William H. Schenke	Cardiovascular Technician	CB NHLBI
Robert O. Bonow, M.D.	Head, Nuclear Cardiology Section	CB NHLBI
Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Left ventricular pulsus alternans, arhythmic beat to beat variation in left ventricular systolic pressure has long been considered a sign of myocardial disease, commonly occurring in the setting of increased afterload such as aortic stenosis and hypertension, expecially in the setting of myocardial failure. We noted the occurrence of left ventricular pulsus alternans, arrhythmic beat to beat variation left ventricular systolic pressure and outflow gradient in 35 of 200 consecutive patients with hypertrophic cardiomyopathy undergoing hemodynamic studies and in sinus rhythm. All patients with left ventricular pulses alternans had severe outflow gradients at rest or during provocation. No patient without obstruction at rest or provocation demonstrated left ventricular pulsus alternans. Eight patients with severe resting outflow obstruction and left ventricular pulsus alternans underwent ventricular septal myotomy/myectomy; all had successful abolition of basal outflow gradient and none demonstrated left ventricular pulsus alternans during post-operative hemodynamic study. Thus, left ventricular pulsus alternans is commonly seen in patients with hypertrophic cardiomyopathy with severe left ventricular outflow gradients, and may represent inadequate left ventricular contractile function in the presence of high left ventricular systolic pressures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04129-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Amiodarone therapy in patients with HCM and refractory cardiac symptoms

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

Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnosis	CB NHLBI
Robert O. Bonow, M.D.	Chief, Nuclear Cardiology Section	CB NHLBI
Cynthia M. Tracy, M.D.	Senior Medical Staff Fellow	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION
Cardiovascular Diagnosis

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

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

An ongoing clinical trial was formulated to assess the therapeutic efficacy of high dose oral amiodarone in patients with hypertrophic cardiomyopathy and refractory cardiac symptoms compared with standard pharmacologic agents and to characterize hemodynamic and left ventricular functional responses associated with amiodarone to help define mechanisms of action. Thusfar, 40 patients with refractory cardiac symptoms on standard medical therapies have been studied as inpatients on the Cardiology Service and have been seen as outpatients at 2 months, 6 months, and 1 year after instituting oral amiodarone treatment. After stopping medications for several days in the hospital, high dose oral amiodarone resulted in beneficial clinical responses (defined as at least a 50% improvement in exercise performance compared with no medication values and a decrease in cardiac symptoms by at least one functional class) in 68% of the patient group. During chronic lower dose outpatient amiodarone treatment, improvement was generally maintained (63% clinical responders); some patients manifested further symptom benefit, and in others symptoms were worse compared with higher dose acute treatment. As might be expected in this group of severely symptomatic patients unresponsive to standard pharmacologic therapy, many patients failed to experience maintained clinical benefit. Nonetheless, 45% of the total patient cohort are still receiving amiodarone for an average follow-up of 14 months and have continued to describe persistent improvement in symptoms. One half of all patients with left ventricular outflow tract obstruction who were deemed operative candidates prior to initiating amiodarone have experienced sufficient symptomatic improvement so that surgery could be deferred. From this ongoing clinical trial we conclude that high dose oral amiodarone improves exercise capacity and reduces cardiac symptoms in the majority of patients with hypertrophic cardiomyopathy who have been refractory to standard medical therapy and long-term clinical folow-up indicates a modest diminution in clinical efficacy although almost one half of all patients have continued treatment after the first year with continued symptomatic improvement.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04130-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

A new Erbium laser and infrared fiber system for laser angioplasty

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

Martin B. Leon, M.D.	Co-Director Cardiovascular Diagnsosi	CB	NHLBI
Robert F. Bonner, Ph.D.	Senior Research Fellow	BEIB	DRS
Paul D. Smith, Ph.D.	Senior Research Fellow	BEIB	DRS

COOPERATING UNITS *(if any)*

Naval Research Laboratory

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

As an extension of previous experiments attempting to characterize the optimal laser source and transmitting optical fiber for intravascular precise microablative surgery, we have been working with Drs. Leon Esterowitz and Daniel Tran from the Naval Research Laboratory utilizing a prototype Erbium (Er):YAG laser with developmental zirconium fluoride fibers in necropsy human and animal tissues. The Er:YAG laser operates in the infrared (2.9 nm) and all of the energy is absorbed within a 10 micron zone of tissue, due to the very high absorption coefficient of water at this wavelength. Studies in air and through fibers in a wet field demonstrated histologic effects resulting in precise triangular crater formation without significant surrounding thermal tissue injury similar to previous work done with Excimer lasers in our laboratories. The ablative threshold for tissue using the Er:YAG laser and optical fiber system was comparable to XeF (248mm) whereas the fiber damage threshold was greater than 400 mJ/mm2. In addition, this laser-fiber combination was capable of easily ablating calcified tissue (including bone), albeit at much higher ablative thresholds and lower ablative efficiencies. We believe that this new infrared laser-fiber system is highly suited for intravascular work and offers several advantages compared with Excimer lasers. These include 1) a solid state more compact reliable system design, 2) no ultraviolet radiation or gas hazards, 3) similar precise histology effects, and 4) delivery in vivo through smaller, more flexible fibers with a more favorable overall energy density operating range.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04131-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Supraventricular tachycardia and syncope in hypertrophic cardiomyopathy

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

Richard O. Cannon, III, M.D.	Co-Director Cardiovascular Diagnosis	CB	NHLBI
William H. Schenke	Cardiovascular Technician	CB	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Cardiovascular Diagnosis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Syncope is a frequent symptom in patients with hypertrophic cardiomyopathy, and may be caused by ventricular tachycardia in many patients, due to a fall in cardiac output. Supraventricular tachycardia is generally well tolerated by patients with normal left ventricles, and rarely results in syncope. To examine the effect of supraventricular tachycardia in hypertrophic cardiomyopathy, rapid atrial pacing at a heart rate of 150 was performed with measurement of mean blood pressure in 25 patients with hypertrophic cardiomyopathy and 21 patients with normal left ventricles. Coronary flow was estimated in the great cardiac vein in 12 patients with hypertrophic cardiomyopathy and 8 patients with normal left ventricles, all with normal epicardial coronary arteries. In comparison to patients with normal left ventricles, patients with hypertrophic cardiomyopathy demonstrated a marked fall in systemic blood pressure during rapid atrial pacing. This may relate to a fall in preload due to shortened diastolic filling period and loss of coordinated atrial systole in a noncompliant ventricle operating on an ascending limb of the Starling curve, resulting in a fall in stroke work. The fall in blood pressure also resulted in a marked fall in coronary flow, which may cause myocardial ischemia and left ventricular dysfunction. Thus, supraventricular tachycardia, simulated by rapid atrial pacing, may account for presyncope and syncope in many patients with hypertrophic cardiomyopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04132-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cardiac angiogenesis

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

S. W. Casscells III, M.D.	Senior Staff Fellow	CB NHLBI
Ellis Unger, M.D.	Senior Staff Fellow	CB NHLBI
Edith Speir, B.S.	Chemist	CB NHLBI
Sidney Yoon, M.D.	Guest Worker	CB NHLBI
Ben Calvo, M.D.	Guest Worker	CB NHLBI
Ed Yang, B.S.	Guest Worker	CB NHLBI
Cedriz Sheffield, M.D.	Guest Worker	CB NHLBI

COOPERATING UNITS *(if any)* Dept. Surgical Research, Children's Hospital, Harvard Med. School

LAB/BRANCH

Cardiology Branch

SECTION

Experimental Physiology and Pharmacology

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.)*

Despite recent improvements in treatment, coronary artery disease remains the number one cause of mortality in the United States. The Cardiology Branch sees more and more patients who can no longer be helped by surgery or drugs in current use. Although there is as yet no direct evidence of angiogenesis (new blood vessel growth) occurring in the human heart, our attempt has been to try to understand and to enhance this process. We have followed the lead of cancer researchers who have purified and cloned a family of proteins which cause angiogenesis in vivo and cause migration and mitosis of endothelial cells and fibroblasts in vitro. They have also found that heparin (which is used to prevent blood clotting in heart patients) enhances angiogenesis caused by tumors but is not angiogenic by itself. A non-anticoagulant fraction of heparin has the same effects.

We devised a model of ischemia in the rat, in which we have shown that treatment with heparin or a non-anticoagulant fragment of heparin prior to coronary ligation results in a smaller myocardial infarction and lower mortality. Heparin has multiple actions, but our evidence to-date suggests an angiogenic mechanism of action. Autoradiographic and quantitative histologic studies are nearly completed and should answer this question definitively. We have also shown that subcutaneous injections of purified fibroblast and endothelial growth factor causes a marked increase in DNA synthesis of vascular cells in normal rats. Electron microscopy is being performed to determine if these are predominantly endothelial cells, fibroblasts, or arterial smooth muscle cells.

We have also extracted and purified a protein of approximately 17,000 molecular weight from normal dog myocardium which so far appears very similar to the angiogenic proteins extracted from tumors. We are currently trying to determine by radioimmunoassay if there is an increase in this factor in response to ischemia.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04133-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effect of Normal Aging on Left Ventricular Function

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
Stephen L. Bacharach, Ph.D.	Physicist	NM CC
Michael V. Green, M.S.	Chief, Imaging Physics Section	NM CC
Dino F. Vitale, M.D.	Guest Worker	NM CC
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Richard O. Cannon, III, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS *(if any)*

Department of Nuclear Medicine, CC

LAB/BRANCH

Cardiology Branch

SECTION

Neclear Cardiology

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Cardiovascular function is altered as a process of aging. To assess the effect of age on left ventricular function, we studied 66 normal volunteers (age 21-77) by radionuclide angiography. All subjects had normal physical exams, blood pressures, electrocardiograms, and echocardiograms. The resting ejection fraction did not vary with age, but the increase in ejection fraction during maximal supine exercise declined linearly as a function of age. Although systolic function was unrelated to age, left ventricular diastolic filling declined with age, which was associated with an age-related increase in regional left ventricular diastolic asynchrony. Thus, aging does not affect left ventricular systolic function at rest but significantly influences left ventricular diastolic function at rest as well as the ejection fraction response during maximum exercise.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04134-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Left Ventricular Function After Valve Replacement for Aortic Regurgitation

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
Joseph T. Dodd, M.D.	Medical Staff Fellow	SU NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Patrick T. O'Gara, M.D.	Guest Worker	CB NHLBI
Charles L. McIntosh, M.D.	Senior Surgeon	SU NHLBI
Richard E. Clark, M.D.	Chief, Cardiac Surgery Branch	SU NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

Surgery Branch, NHLBI

LAB/BRANCH

Cardiology

SECTION

Nuclear Cardiology

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In the majority of patients with aortic regurgitation, valve replacement results in reduction in left ventricular dilatation and improvement in ejection fraction. To determine the relation between serial changes in left ventricular diastolic dimension and simultaneous changes in ejection fraction, we studied 50 patients by both echocardiography and radionuclide angiography before operation and early (6-8 months) and late (3-7 years) after operation. During both early and late postoperative evaluations, changes in diastolic size correlated significantly with changes in ejection fraction ($p<.001$). Late improvement in ejection fraction after the early study occurred only in those patients manifesting an increase in ejection fraction between the preoperative and early postoperative studies. These improvements occurred predominantly in patients with normal preoperative ejection fraction or patients with subnormal ejection fraction but with preserved exercise tolerance and only a brief duration (<14 months) of preoperative left ventricular dysfunction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 04135-01 CB

PERIOD COVERED: October 1, 1985 to September 30, 1986

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

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

Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Paolo Spirito, M.D.	Guest Researcher	
Yvonne Wesley	Echo Technician	
Javier Arce, M.D.	Medical Technician	

COOPERATING UNITS (if any)
None

LAB/BRANCH
Cardiology

SECTION
Echo

INSTITUTE AND LOCATION
NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	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.)

To determine whether magnitude and distribution of left ventricular hypertrophy is largely established at birth in patients with hypertrophic cardiomyopathy, or may substantially increase during the first years of life, 39 children with family history or morphologic evidence of hypertrophic cardiomyopathy were studied serially with echocardiography. Patients were initially investigated at ages 4-15 years (mean 11) and most recently at 9-20 years (mean 16). Over 2.5-6.8 year (mean 4) follow-up, 17 patients showed marked increase in magnitude and extent of pre-existent left ventricular hypertrophy, and 5 others demonstrated evolution from a morphologically normal appearing heart to substantial hypertrophy. In these 22 patients, increases in left ventricular wall thickness were striking (6-23mm; 101± 62% change), greatly exceeded that expected to occur as a consequence of normal growth (13 ± 10%; $p < 0.001$) and were not associated with symptomatic deterioration or secondary to subaortic obstruction.

These findings demonstrated that left ventricular hypertrophy may spontaneously develop or progress substantially in patients with hypertrophic cardiomyopathy during childhood when body growth is considerable. Since echocardiographic studies may be normal during childhood before the morphologic features of hypertrophic cardiomyopathy develop, echocardiographic screening of youthful relatives of patients with hypertrophic cardiomyopathy cannot always definitively exclude this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04136-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Absence of Progression of Hypertrophy in Adults with Hypertrophic Cardiomyopathy

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

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

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	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.)*

Development of progression of left ventricular hypertrophy has recently been described in children with hypertrophic cardiomyopathy. To determine whether similar changes in magnitude and distribution of left ventricular hypertrophy may also occur in adult patients with this disease, serial assessment of left ventricular wall thickness was obtained with M-mode and two-dimensional echocardiography in 65 patients with hypertrophic cardiomyopathy who were 23 to 50 years of age. Follow-up period was 3 to 6 years (mean 4). None of the study patients showed a substantial increase ($\geq$ 5 mm) in left ventricular wall thickness. However, nine (14%) of the study patients demonstrated a substantial decrease of 5-9 mm in lef ventricular wall thickness; wall thinning most commonly involved the anterior ventricular septum (7 patients), but was also identified in the posterior septum (6 patients), the lateral free wall (2 patients), and the posterior free wall (1 patient). In the nine patients who showed wall thinning, left ventricular end-diastolic diameter increased significantly (from 44 $\pm$ 6 mm to 51 $\pm$ 6 mm; $p < 0.001$); however, in seven of the nine patients, absolute cavity size remained within normal limits ($\leq$ 52 mm) at the most recent evaluation. Eight of the nine patients with left ventricular wall thinning and relative cavity enlargement were severely symptomatic and one was mildly symptomatic. In conclusion, substantial progression of left ventricular hypertrophy was not identified in any of our adult patients with hypertrophic cardiomyopathy. Hence, if this phenomenon occurs in adults with hypertrophic cardiomyopathy, it is probably rare. Conversely, an important minority of adult patients with hypertrophic cardiomyopathy may show progressive left ventricular wall thinning and relative cavity enlargement which are usually associated with poor clinical prognosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04137-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Functional Limitation in Hypertrophic Cardiomyopathy and Only Mild Hypertrophy

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

Paolo Spirito, M.D.	Guest Researcher	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Robert O. Bonow, M.D.	Senior Investigator	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.9	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.)*

Ten patients with nonobstructive hypertrophic cardiomyopathy and only mild localized left ventricular hypertrophy who had severe symptoms of cardiac failure are described. During a mean follow-up period of five years, six of these 10 patients showed a substantial increase in left ventricular internal dimension (6 to 15 mm, mean 10), as assessed with M-mode echocardiography, although absolute left ventricular cavity size remained within normal limits in five of the six; four patients demonstrated substantial septal thinning (5 to 14 mm, mean 8). left ventricular diastolic function, assessed by radionuclide angiography in nine patients, was impaired in eight who showed decreased peak filling rate (< 2.5 end-diastolic-volume/sec) and prolonged time to peak rate of filling (≥ 180 msec). Furthermore, left ventricular systolic function, usually supernormal in patients with hypertrophic cardiomyopathy, was depressed (ejection fraction ≤ 45%) in six patients. Hence, we have identified a subset of patients with nonobstructive hypertrophic cardiomyopathy and only mild localized left ventricular hypertrophy who experienced severe cardiac symptoms. The majority of these patients showed both systolic and diastolic left ventricular dysfunction in the presence of a progressive increase in left ventricular internal dimension (but without absolute left ventricular dilatation) and/or ventricular septal thinning. Such patients may represent an important component of the natural history of hypertrophic cardiomyopathy which has not been previously fully appreciated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04138-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Influence of Age on Left Ventricular Diastolic Function

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

Paolo Spirito, M.D.	Guest Worker	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	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.)

The present investigation was performed to assess the influence of age on Doppler variables of left ventricular diastolic function. Six Doppler diastolic indexes were analyzed in 86 normal volunteers ranging in age from 20 to 74 years (mean 36). All 6 indexes showed a linear relationship to age. Duration of isovolumic relaxation, duration of the early diastolic flow-velocity peak and maximal late diastolic (atrial) flow-velocity increased with age ($r = 0.41$, $r = 0.42$, and $r = 0.63$, respectively; $p < 0.01$ to $p < 0.001$). Conversely, maximal early diastolic flow-velocity, the rate of decrease (descent) of flow-velocity in early diastole, and the ratio between maximal early and late diastolic flow-velocities decreased with age ($r = -0.40$, $r = -0.42$, and $r = -0.66$, respectively; $p < 0.001$). Comparison of Doppler indexes of diastolic function among different age groups (20 to 29 years, 30 to 49 years, and 50 to 74 years) also demonstrated an influence of age on these diastolic variables. Isovolumic relaxation was significantly prolonged in older subjects compared to either the intermediate ($p < 0.05$) or the younger age groups ($p < 0.001$). In addition, both the rate of decrease of flow-velocity in early diastole and the ratio between maximal early and late diastolic flow-velocities were reduced in older compared to younger subjects ($p < 0.001$). In conclusion, the isovolumic relaxation phase and the early and late filling phases of diastole, as assessed by Doppler echocardiography, appear to be importantly affected by aging. Specifically, in older subjects, the duration of isovolumic relaxation is prolonged and the rate of early diastolic filling velocity is reduced; as an apparent compensation, the relative contribution of atrial systole to overall left ventricular filling is increased. These diastolic alterations qualitatively resemble those observed in patients with cardiac diseases associated with left ventricular diastolic impairment; hence, the effects of age should be taken into consideration in formulating "normal" limits for left ventricular diastolic function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04139-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Progressive Ventricular Wall Thinning and Cavity Dilatation in HCM

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

Paolo Spirito, M.D.	Guest Worker	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Robert O. Bonow, M.D.	Head, Nuclear Cardiology	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	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 present investigation was undertaken to assess the prevalence of these morphologic and functional alterations in a large population of patients with hypertrophic cardiomyopathy and to determine their role in the natural history of this disease. Of 217 consecutive patients with hypertrophic cardiomyopathy, the majority of whom was severely symptomatic, 197 (91%) had left ventricular ejection fraction $\geq$ 50% and 20 (9%) had ejection fraction < 50%, as assessed with radionuclide angiography. Changes in left ventricular wall thickness and cavity dimension were evaluated with serial M-mode and two-dimenstional echocardiography, over an average follow-up of 3.6 years, in 67 of the 217 patients (54 patients with ejection fraction $\geq$ 50% and 13 with ejection fraction < 50%). A substantial decrease ($\geq$ 5 mm) in left ventricular wall thickness, as assessed with two-dimensional echocardiography, was identified in 8 (62%) of the 13 patients with depressed ejection fraction, but only in 2 (4%) of the 54 patients with ejection fraction $\geq$ 50% ($p < 0.001$). Left ventricular cavity dimension, as assessed with M-mode echocardiography, increased signiticantly in the 13 patients with depressed ejection fraction (from 44 $\pm$ 5 to 49 $\pm$ 7 mm; $p < 0.005$); however, absolute cavity size remained within normal limits ($\leq$ 52 mm) in 10 of these 13 patients. Clinical course was poor in each of the patients who showed wall thinning and systolic dysfunction; all had severe symptoms, including 4 who died of congestive cardiac failure, and 1 who underwent cardiac transplantation. In conclusion, our findings show that left ventricular systolic impairment, associated with progressive left ventricular wall thinning and cavity enlargement (although absolute cavity size usually remains within normal limits), are present in an important minority (almost 10%) of patients with hypertrophic cardiomyopathy and severe symptoms.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04140-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Left ventricular filling by Doppler in hypertrophic cardiomyopathy

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

Barry J. Maron, M.D.	Head, Echocardiography Lab	CB NHLBI
Paolo Spirito, M.D.	Guest Worker	CB NHLBI
Javier Arce, M.D.	Medical Technician	CB NHLBI
Robert O. Bonow, M.D.	Head, Nuclear Cardiology	CB NHLBI
Yvonne Wesley	Medical Technician	CB NHLBI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.5	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.)

There are few truly non-invasive tests available to measure left ventricular diastolic function in patients with cardiac disease. In this study we utilized pulsed Doppler to assess transmitral flow-velocity patterns and characterize left ventricular filling and relaxation in 109 patients with hypertrophic cardiomyopathy and 86 normal controls. All Doppler indexes of diastolic function in patients with hypertrophic cardiomyopathy differed significantly from those in 86 control subjects without heart disease of similar ages ($p < 0.001$): duration of isovolumic relaxation (94.2 ± 24 versus 77.6 ± 12 ms) and duration of the early diastolic peak of flow-velocity (245 ± 54 versus 220 ± 28 ms) were both prolonged and the rate of decrease (descent) of flow-velocity in early diastole diastolic filling velocity was reduced (3.4 ± 1.4 versus 4.9 ± 1.4 m/sec2); as an apparent compensation for impaired relaxation and early diastolic filling, the atrial contribution to left ventricular filling was increased, as shown by the reduced ratio between the heights of the early and late (atrial) peaks of flow-velocity (1.5 ± 0.8 versus 2.1 ± 0.9). The vast majority of patients with hypertrophic cardiomyopathy showed evidence of imparied left ventricular diastolic function based on alterations in the Doppler waveform (-- of 109 patients, or 77%). Diastolic dysfunction was identified with similar frequency in patients without left ventricular outflow obstruction (78%) or with obstruction (70%), as well as in asymptomatic (73%) or symptomatic patients (82%). These findings demonstrate that pulsed Doppler echocardiography may be used to quantitatively assess left ventricular function and that impairment in left ventricular filling and relaxation are common and clinically important abnormalities in a population of patients with hypertrophic cardiomyopathy.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04141-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Variations in Flow-Velocity Waveforms in the Normal Human Aorta

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

Eric K. Louie, M.D.	Guest Worker	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Kimberly J. Green, M.S.	Echo Technician	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.7	0.5	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)*

To characterize the contour and duration of aortic flow-velocity waveforms in the normal human, the ascending aorta of 23 subejcts without evidence of cardiovascular disease was interrogated systematically with pulsed Doppler echocardiography. In 16 of the 23 subjects, measurements throughout the ascending aorta showed flow-velocity waveforms of similar contour and duration characterized by flow-velocity peaking in early to mid-systole with most flow-velocity (60 ± 4%) occurring in the first one-half of the available systolic ejection period, and then gradually decreasing to zero baseline coincident with aortic valve closure. In the other 7 subjects, aortic flow-velocity waveforms recorded at the majority of sampling sites also revealed a normal flow-velocity pattern; however, in eachof these subjects, 1 to 3 sites which displayed a distinct alteration from the normal pattern were also identified. The waveforms recorded from thee latter sites were characterized by flow-velocity peaking earlier in systole and decelerating to zero baseline approximately 100 msec before aortic valve closure; consequently, a particularly large fraction of flow-velocity (88 ± 9%) occurred in the first one-half of the systolic ejection period. These apparently shortened waveforms were always detected at sites near the medial aortic wall and often at or near the junction of the ascending and transverse aorta. Hence, aortic flow-velocity waveforms with altered contour and duration (resembling those recorded in patients with obstructive hypertrophic cardiomyopathy) were infrequently identified by pulsed Doppler echocardiography in subjects with normal hearts and were not characteristic of the overall aortic flow-velocity pattern in any of these subjects. The origin of these waveforms is uncertain, although it is likely that they reflect local aberrations in aortic flow-velocity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04142-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Relation Between LV Hypertrophy and Ventricular Tachycardia in HCM

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

Paolo Spirito, M.D.	Guest Researcher	CB NHLBI
Rita M. Watson, M.D.	Dept. of Medicine	Columbia University
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Cardiology Branch

SECTION
Echo

INSTITUTE AND LOCATION
NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	55

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This study was undertaken to determine whether marked left ventricular hypertrophy may predispose to the occurrence of ventricular tachycardia in patients with hypertrophic cardiomyopathy. Extent of left ventricular hypertrophy was assessed, using two-dimensional echocardiography, in a group of 30 patients with hypertrophic cardiomyopathy in whom ventricular tachycardia had been documented on 24-hour ambulatory ECG monitoring, and compared to 61 patients with hypertrophic cardiomyopathy who had normal ambulatory electrocardiograms. Severe hypertrophy, involving at least three of the four segments in which the left ventricle had been divided, was significantly more common in patients with documented ventricular tachycardia (16 of 30, 53%) than in those with normal ambulatory ECGs (13 of 61, 21%; $p < 0.002$). Conversely, mild hypertrophy, involving only one left ventricular segment, was significantly less common in patients with ventricular tachycardia (5 of 30, 17%) than in controls (32 of 61, 52%; $p < 0.001$). Moderate hypertrophy, invovling two of the four left ventricular segments, occurred about as frequently in patients with ventricular tachycardia (9 of 30, 30%) as in patients with normal ambulatory ECGs (16 of 61, 26%; $p > 0.05$). In addition, the left ventricular hypertrophy, was also significantly higher (thereby indicating a greater magnitude of hypertrophy) in patients with documented ventricular tachycardia (72 $\pm$ 17 mm) than in those with normal ambulatory ECG recordings (61 $\pm$ 14 mm; $p < 0.005$). In conclusion, our data show a strong association between magnitude of left ventricular hypertrophy and occurrence of ventricular tachycardia in patients with hypertrophic cardiomyopathy; these findings provide new insights into the components of the disease process associated with ventricular tachycardia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04143-01 CB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intramural Coronary Artery Disease in Hypertrophic Cardiomyopathy

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

Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
James K. Wolfson, M.S.	Guest Worker	CB NHLBI
Stephen E. Epstein, M.D.	Chief, Cardiology Branch	CB NHLBI
William C. Roberts, M.D.	Chief, Pathology Branch	PA NHLBI

COOPERATING UNITS *(if any)*
Pathology Branch

LAB/BRANCH
Cardiology Branch

SECTION
Echocardiography

INSTITUTE AND LOCATION
NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.9	0.8	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.)*

Many patients with hypertrophic cardiomyopathy have signs and symptoms of myocardial ischemia and dysfunction. To determine the prevalence and extent as well as the clinical relevance of abnormal intramural coronary arteries, a histologic analysis of left ventricular myocardium obtained at necropsy was performed in 48 patients with hypertrophic cardiomyopathy and in 68 controls. In hypertrophic cardiomyopathy, abnormal intramural coronary arteries were characterized by thickening of the vessel wall and a decrease in luminal size. The wall thickening was due to proliferation of medial and/or intimal components, particularly smooth muscle cells and collagen. Of the 48 patients with hypertrophic cardiomyopathy, 40 (83%) had abnormalities of intramural coronary arteries located in the ventricular septum (33 patients), anterior left ventricular free wall (20 patients) or posterior free wall (9 patients); an average of 3.0 $\pm$ 0.7 abnormal arteries were identified per tissue section. Altered intramural coronary arteries were also significantly more common in tissue sections having considerable myocardial fibrosis (31 of 42, 74%) than in those with no or mild fibrosis (31 of 102, 30%; $p < 0.001$). In contrast, only rare altered intramural coronary arteries were identified in 6 (9%) of the 68 control patients, and those arteries showed only mild thickening of the wall and minimal luminal narrowing (abnormal arteries per section, 0.1 $\pm$ 0.05; $p < 0.001$). Hence, abnormal intramural coronary arteries with markedly thickened walls and narrowed lumens are present in increased numbers in most patients with hypertrophic cardiomyopathy at necropsy and they may represent a congenital component of the underlying cardiomyopathic process.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04144-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Reproducibility of Doppler Echocardiographic Measurements of Diastolic Function

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

Paolo Spirito, M.D.	Guest Researcher	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI
Joel I. Verter, Ph.D.	Biostatistician	CB NHLBI

COOPERATING UNITS *(if any)*

Division of Epidemiology and Clinical Aplications, Biostatistics Research Branch,

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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 present investigation was undertaken in 12 normal subjects to determine the magnitude of technical and biologic variability of six previously validated Doppler indexes of diastolic function. Technical variability due to the reader was small for each of the six Doppler indexes. Variability due to the technician was larger than for the reader, and became sizeable when measurements obtained in a single subject were compared; however, variability due to the technician was negligible when mean differences between groups of subjects were analyzed, and none of these differences achieved statistical significance. Day-to-day variability (i.e., biologic variability) was larger than technical variability (reader and technician) for the great majority of the comparisons, both in the individual (28 of 30 comparisons) and group data analyses (27 of 30 comparisons). The two Doppler indexes assessing late diastolic events (i.e., maximal late diastolic [atrial] flow-velocity, and the ratio between maximal early and late flow-velocity) did show significant change on a day-to-day basis ($p < 0.05$). In conclusion, Doppler indexes that represent a measure of the relaxation and early filling phases of diastole showed good reproducibility for group data analysis. Hence, these Doppler indexes can be utilized to assess left ventricular diastolic function noninvasively in large groups of patients. However, extensively utilized indexes assessing the atrial contribution to ventricular filling, such as maximal late (atrial) diastolic flow-velocity and the ratio between maximal early and late flow-velocities, have a relatively large variability for both individual and group data analysis; therefore, conclusions based on these late diastolic indexes should be drawn with caution.

225

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04145-01 CB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Hypertrophic Cardiomyopathy With Extreme Increase in LV Wall Thickness

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

Eric K. Louie, M.D.	Guest Worker	CB NHLBI
Barry J. Maron, M.D.	Senior Investigator	CB NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cardiology Branch

SECTION

Echocardiography

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.6	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.)*

Clinical and morphologic features of 34 patients with hypertrophic cardiomyopathy and particularly marked left ventricular hypertrophy were analyzed. Only patients with a ventricular septal thickness of at least 35 mm (range to 52 mm) were selected for the study; 31 (90%) had a diffuse pattern of hypertrophy also involving substantial portions of the left ventricular free wall. Ten patients (29%) had hemodynamic or echocardiographic evidence of basal subaortic obstruction (average gradient, 63 mm Hg); however, the majority (24 [71%]) had no evidence of obstruction at rest, despite substantial hypertrophy of the basal anterior portions of septum and free wall. The clinical course was variable in 30 patients who were followed up for at least 1 year (mean 6 years). Although no patient died, nine (30%) have exhibited clinical deterioration, including two who spontaneously developed complete heart block and one who collapsed with ventricular fibrillation but survived. However, the clinical condition of the majority of patients (21 [70%]) remained unchanged or improved. At the most recent evaluation, 20 (67%) of the 30 patients were asymptomatic or only mildly symptomatic, including 7 who remained without symptoms throughout the period of follow-up. The subset of patients described in this report shows the most striking morphologic alterations that occur in hypertrophic cardiomyopathy. Although such extreme increases in left ventricular mass might intuitively suggest a unique clinical expression and course for these patients, the patients nevertheless demonstrated a variety of clinical manifestations; their natural history did not reflect a uniformly poor prognosis over the period of follow-up, and two-thirds of the patients had only mild or no symptoms at the most recent evaluation.

PHS 6040 (Rev. 1/84) GPO 914-918

Annual Report of the Laboratory of Cell Biology
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

The Laboratory of Cell Biology consists of four independent Sections conducting research in five different areas of biochemistry and cell biology. In this summary, just a few of the major advances of the past year will be discussed.

Actin Polymerization: Under the general leadership of Dr. Korn, research continues on the role of ATP hydrolysis and the effects of other proteins in the polymerization of actin, the major cytoskeletal protein of eucaryotic cells. Last year, a new model was proposed for the mechanism of hydrolysis of ATP that accompanies actin polymerization. According to this model, ATP hydrolysis occurs on the F-actin filament preferentially at an ATP-actin subunit adjacent to an ADP-actin subunit more internally placed in the filament. This model leads to the formation of a cap of ATP-actin subunits which is quite long at initial states of elongation and reduces to perhaps 2 or 3 subunits at steady state. The model explained quite well the different rates of actin filament elongation as a function of F-actin concentration, the different rates depending on whether the filament has no ATP cap, a short ATP cap or a very long ATP cap.

This year a second prediction of the model was tested. According to the model, the initial rate of ATP hydrolysis on F-actin should be very low at G-actin concentrations below the critical concentration (where mostly dissociation events occur), should equal the initial rate of elongation as the G-actin concentration is raised above the critical concentration and then should remain constant at V_{max} as the G-actin concentration is raised further although the rate of elongation continues to increase in proportion to the G-actin. Almost exactly this result was observed for the polymerization of Mg-actin, but rather than remaining constant above a certain G-actin concentration there was a very slight continued increase in the rate of ATP hydrolysis. The data could be fit by the model by assuming that the preferred site for ATP hydrolysis on F-actin has a rate constant of 18 s^{-1} while there is very slow random hydrolysis on the ATP cap with a rate constant of 0.001 s^{-1}. With Ca-actin a very different result was found with ATP hydrolysis lagging behind elongation at all G-actin concentrations apparently because there is no preferred hydrolysis site for ATP. Rather ATP hydrolysis on Ca-F-actin appears to occur randomly in the ATP cap.

The binding of divalent cations to the single high-affinity site on G-actin was re-investigated this year and found to be 3-4 orders of magnitude greater than previously reported by others. Ca^{2+} binds with a K_D of about 5 nM (making actin the strongest Ca^{2+}-binding protein known) and Mg^{2+} with a K_D of about 0.5 μM. In vivo, actin will all be Mg-actin.

Two new actin-binding proteins have been discovered in Acanthamoeba castellanii, bringing the total to about 15, not counting myosins. One of these proteins is a dimer of 12,500-dalton (apparently identical) subunits. From its effects on the initial rates of elongation and the final extent of polymerization of actin this protein (actobindin) seems to bind the G-actin

monomer (1 actobindin dimer/monomer) with a K_D of 5 μM. In this way it is very similar to profilin but it is a different protein in all respects (amino acid composition, relative affinities for muscle and amoeba actins, isoelectric point, etc.). The second new actin-binding protein is a hexamer of apparently identical 35,000-dalton subunits which cross-links F-actin in an ATP-sensitive reaction. The gel formed between this protein and F-actin is disrupted by physiological concentrations of ATP. This is not only the first hexameric cross-linking protein but is also the first cross-linking protein known to be sensitive to ATP.

Non-Muscle Myosins: The emphasis of this work continues to be the three myosin isoenzymes of Acanthamoeba castellanii. Under the direction of Dr. Hammer, the sequence of the myosin II heavy chain gene has been completed and the sequence of the leader sequence of the non-translated mRNA shown to be identical to the sequence of the genomic DNA, proving that this is a transcribed gene. The coding sequence is interrupted by only 3 introns near the 5' end, two of which are in identical positions as in several muscle genes. The deduced amino acid sequence of the head has about 60% similarity to muscle myosins and the tail, which has little direct sequence analogy to any other myosin, has the same periodicity of hydrophobic and charged amino acids as most other myosins predicting formation of an alpha helical coiled-coil. The tail is shorter than that of muscle myosin, however, (about 90 nm vs 160 nm) and the coiled-coil structure is interrupted about 2/3 down the rod by a proline and polar residues that may provide a hinge region. A bend in the tail at about this position is seen in electron micrographs taken by Dr. Bowers. This hinge structure may be important in regulation of myosin II activity by phosphorylation that occurs at the tip of the tail distal to the hinge.

The gene for myosin IB heavy chain has been 95% sequenced. It contains at least 21 introns and spans at least 6 kilobases although it codes for a protein of only 125,000 daltons. The deduced amino acid sequence for the N-terminal 90,000 daltons is 55% similar to the corresponding regions of myosin II and muscle heavy chains. But where other myosin heavy chains have a coiled-coil rod-like tail, the C-terminal end of myosin IB has a very unusual composition, the last 200 amino acids of which contain 22% glycine, 22% proline and 10% alanine. These sequence data agree with other data showing that myosin IB (and IA) has the enzymatic and actin-binding properties of other myosins (properties attributed to the N-terminal head) but lack the ability to form dimers and filaments (properties of the coiled-coil helical tail that is missing from myosin I).

Perfectly consistent evidence about the structure of myosin I isoenzymes has come from protein chemical studies on myosin IA. The heavy chain of this isoenzyme was cleaved by chymotrypsin into a 100-000-dalton N-terminal segment that was still associated with the undegraded light chain and 30,000-dalton C-terminal fragment. The C-terminal peptide contained 20% proline and 30% glycine. Both fragments bound to actin: the 30,000-dalton peptide bound with the same K_D of less than 0.5 μM in the presence and absence of MgATP but the 100,000-dalton peptide bound to actin with a K_D of 0.5 μM in the presence of MgATP and less than 0.01 μM in its absence. The 100,000-dalton peptide also contained the regulatory phosphorylatable serine and had actin-activated ATPase activity with normal hyperbolic kinetics as a function of actin concentration.
All of these data fit with our previous model that native myosins IA and IB

contain two actin-binding sites - a high-affinity site that is ATP insensitive and not associated with the catalytic site and an ATP-sensitive binding site that is associated with catalysis and can function in a typical myosin cross-bridge cycle. In this way myosin I can function to move one actin filament relative to another without the necessity to form bipolar filaments as for conventional myosins.

Biochemistry of Muscle Contraction: The cross-bridge cycle of muscle contraction proposed by Dr. Eisenberg and Dr. Greene invokes two alternate conformations of the myosin cross-bridge: a state that binds strongly to actin and a state that binds weakly to actin and rapidly equilibrates between attached and detached states. The latter state is associated with ATP hydrolysis. The model includes a slow conformational change between two different states of actomyosin·ADP·P_i, subsequent to the hydrolysis of actomyosin·ATP, as the rate-limiting step in the catalytic cycle. An alternate model proposes that the hydrolysis of ATP is itself the rate-limiting step. In principle, these models can be distinguished by measuring the extent of ^{18}O exchange between ^{18}O-labeled water and ATP during the catalytic cycle. If ATP hydrolysis is slow and Pi release is fast there will be relatively little ^{18}O exchange whereas if ATP hydrolysis if fast and the slow step is the conversion of one form of actomyosin·ADP·Pi to another ^{18}O exchange could be extensive. With skeletal muscle acto-subfragment-1, experiments last year showed less exchange than was compatible with the Eisenberg-Greene model which meant either that the model was wrong or that there was limited rotation of the Pi on the actomyosin for some unknown reason. This year, experiments with cardiac acto-subfragment-1 have provided unequivocal results of extensive exchange of ^{18}O which is consistent only with a model that has a rapid hydrolysis of actomyosin·ATP with a subsequent slow step as in the Eisenberg-Greene model.

Previously, it was observed that AMP-PNP and PPi bind weakly to myosin cross-bridges in muscle fibers just as they do to acto-subfragment-1 *in vitro*. Now it has been found that ADP binds very strongly to cross-bridges *in vivo* just as it does to acto-subfragment-1 *in vitro*. Similarly, cross-bridges separated from actin filaments *in vivo* (by stretching the muscle fiber) bind strongly to AMPPNP and PPi just as does free subfragment-1 *in vitro*. These studies provide additional evidence that the study of acto-subfragment-1 *in vitro* provide information that is applicable to the situation of the muscle fiber *in vivo*.

The Eisenberg-Greene model predicts that cross-bridges in their high-affinity conformation (with no bound nucleotide or bound ADP) will bind to F-actin at a 45° angle while cross-bridges in their low-affinity state (with bound ATP, ADP·Pi, or analogous AMPPNP and PPi) will bind at a 90° angle. This is difficult to show because most of the myosin in the low-affinity state will be dissociated from the actin filament. Last year, Dr. Greene developed a new experimental model in which the conformation of covalently cross-linked acto-S-1 was examined by negative staining electron microscopy. In the absence of nucleotide or presence of ADP, the 45° angle was seen and in the presence of ATP a disordered state was observed. This year, experiments with PPi confirmed the results with ATP. But AMPPNP did not give rise to the disordered state as would have been expected. This may be related to the fact that in free solution the dissociation rate constant

for S-1 from F-actin is much smaller in the presence of AMPPNP than in the presence of PPi.

Drs. Greene and Eisenberg have also addressed the question of the regulation of actomyosin ATPase by tropomyosin-troponin. Their model proposes that regulated actin (actin-tropomyosin-troponin complex) can exist in a "turned on" and a "turned off" form. The former fully activates myosin subfragment-1 ATPase and the other shows little activation. The model further predicts that subfragment-1·ATP in the absence of Ca^{2+}, should not turn on regulated actin because it binds equally well to the turned on and turned off forms. To test this unequivocally, it was first necessary to determine the activity of maximally turned on acto-S-1. This was done by using the enzymatically inactive NEM-subfragment-1 to turn on regulated actin in the presence of Ca^{2+}. When compared to this rate, it was found that subfragment-1 does not turn on regulated actin significantly in the absence of Ca^{2+}. Different effects are seen in the presence of Ca^{2+} which partially shifts regulated actin to the turned on state. Then, as little as a 2-fold preferential binding of subfragment-1 to the turned on form would lead to a cooperative shift of the regulated actin to the turned on form. This was demonstrated experimentally.

Structure, Assembly and Function of Microtubules: MAP-2 is a 270,000 dalton protein from brain dendritic processes that copolymerizes with tubulin. Dr. Flavins' group has used a multiphosphorylated form of MAP-2 to show that it can be a substrate for cAMP and CAM II kinases in vivo as well as for C kinase as shown last year. A brain phosphatase specific for MAP-2 has been purified to homogeneity and shown to consist of 3 subunits.

A brain carboxypeptidase that removes specifically the C-terminal tyrosine from the α-chain of tubulin has been purified about 200-fold but is still far from homogeneous. In a related project, the state of tyrosination of tubulin from the several compartments of the protozoan Crithidia fasciculata has been studied. Tubulin accounts for about 15% of the cytoplasmic, flagellar and pellicular protein pools. The organism also contains a tyrosinating enzyme that is specific for Crithidia tubulin and can tyrosinate tubulin from each of the 3 cell compartments in vitro. As for brain, the exact nature of the non-substrate flagellar tubulin is not known.

Pellicular tubulin may be associated with a major 42,000-dalton protein that seems not to be actin. Cytoplasmic tubulin from Crithidia contains a number of potential microtubule-associated proteins including, from preliminary data, heat-labile proteins of 56,000 and 39,000 daltons and heat-stable proteins of 125,000, 43,000, 33,000 and 27,000 daltons.

Bioenergetics: Dr. Hendler had previously observed that the binding of CO to cytochrome a3 did not raise its Em contrary to what would be expected for the binding of a ligand to the reduced member of a redox couple. This was rationalized by assuming that protons are second ligands that bind more strongly to the oxidized member of the CO-liganded couple and thus balance the effect of the CO. This proposal has been further developed by an extensive theoretical analysis of cooperativity of a three-liganded system (electron, proton and CO, in this case) which showed how multi-liganded cooperative interactions could be utilized for a redox-driven Bohr-type proton pump. This analysis is now being extended to systems capable of

multi-redox site interactions. For example, cytochrome oxidase contains potentially as many as 13 redox centers in a single molecular species, and the Em of any one of these redox centers might depend on how many and which other redox centers were reduced.

Membrane Flow: Dr. Bowers has continued her studies on the inter-relationships between plasma membrane and endocytic membranes in the amoeba Acanthamoeba castellanii. Cells were fed increasing amounts of radioactive yeast and the rates of digestion of the yeast and of the appearance of hydrolases in the phagosomes were measured. The evidence was clear that large loads saturated the digestive capacity and that fewer phagosomes contained hydrolases when large quantities of yeast were phagocytosed. Moreover, later-formed phagosomes did not acquire hydrolases when large loads of yeast resulted in the earlier-formed phagosomes acquiring all of the available hydrolases. This indicates that there is very little fusion of phagosomes and, therefore, little randomization of vesicle membrane or contents at this level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00401-20 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

COOPERATING UNITS *(if any)*
Hans V. Westerhoff, LMB, NIADDK; Barry Bunow, Civilized Software, Bethesda, MD; Britton Chance and Ali Naqui, Dept. Biochem. and Biophys. U. of PA, Phila, PA.

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:
0.6	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.)*

Many theoretical issues were raised by our newer findings on the spectral and thermodynamic characterization of cytochrome aa3. Our finding that CO did not raise the Em of cytochrome a3 is contrary to theory for a case where a ligand binds more strongly to the reduced member of a redox couple. We proposed that protons are second ligands that bind more strongly to the oxidized member of the CO-liganded couple. A theoretical paper published early this year using other considerations reached the opposite conclusion. In an intensive theoretical examination of cooperativity for a system involving three ligands (electron, proton, and CO), we were able to explain how the later results do not rule out cooperative interactions involving protons and how multi-ligand interactions could be effectively utilized for a redox-driven Bohr-type proton pump. Other of our newer findings posed additional theoretical questions. We concluded that the redox potential of cytochrome a3 was under the control of the redox state of other centers in the molecule. We found that lowering the voltage first oxidized cytochrome a3 and then reduced it. Existing theory does not account for these phenomena. Cytochrome oxidase can theoretically possess from 4 to 13 redox centers. The Em of any center may be cooperatively affected by any combination of electrons in the other centers. We have started a quantitative theoretical consideration of the expected effects of redox interactions in a variety of possible models based on cytochrome oxidase. Preliminary results indicate that redox cooperativity can account for our newer results. A research collaboration was initiated with the laboratory of Britton Chance. Our newer intensive analytical techniques, in equilibrium studies, revealed specific spectral components and interactions. We hope to use the same analytical technique in kinetic studies coupled with our newer characterizations of the components to study the mechanism of cytochrome oxidase activity. Preliminary results do show the same kinds of spectra we had encountered in our work.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00409-16 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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

PI:	Evan Eisenberg	Section Head	LCB, NHLBI
Others:	John A. Evans	Staff Fellow	LCB, NHLBI
	Lois E. Greene	Research Chemist	LCB, NHLBI
	José Biosca	Visiting Fellow	LCB, NHLBI
	Susan Smith	Visiting Associate	LCB, NHLBI

COOPERATING UNITS *(if any)*
Leonard Stein, State University of New York, Stony Brook, NY

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:
3.5	3.5	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.)*

We have proposed a cross-bridge model of muscle contraction in which, during each cycle of ATP hydrolysis, the myosin cross-bridge alternates between a conformation in which the cross-bridge binds strongly to actin, and a conformation in which the cross-bridge binds weakly to actin and is in rapid equilibrium between attached and detached cross-bridge states. It is while the cross-bridge is in the latter conformation that ATP hydrolysis and a separate rate-limiting occur. In the present study we have tested several predictions of this model both in vitro and in single skinned rabbit muscle fibers. First, we have measured O-18 exchange using both myosin subfragment-1 (S-1) from skeletal muscle and from cardiac muscle. Earlier results with skeletal muscle acto-S-1 suggested that bound Pi may not have complete freedom of rotation of the active site of S-1 which, in turn may limit the rate of O-18 exchange. Our results with cardiac S-1 strongly support this conclusion. Furthermore, they demonstrate that there must be a separate ATP hydrolysis step and rate limiting step during the cardiac acto-S-1 ATPase cycle just as we previously proposed for the skeletal muscle system. We have also studied the binding of AMP-PNP, PPi, and ADP to skinned muscle fibers and to myofibrils. In both cases, just as we previously observed with acto-S-1, the AMP-PNP and PPi bind weakly but ADP binds strongly. These data support the assumption of our model that the organization of actin and myosin into filament arrays does not affect the binding of nucleotide to the cross-bridge actin complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00413-10 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanism of the regulation of the actomyosin ATPase

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

PI:	Lois E. Greene	Research Chemist	LCB, NHLBI
Others:	David L. Williams, Jr.	Staff Fellow	LCB, NHLBI
	Evan Eisenberg	Section Head	LCB, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Section on Cellular Physiology

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	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.)*

In our model of muscle regulation, regulated actin can exist in either the turned on form, which fully activates the myosin S-1 ATPase activity, or the turned off form, which shows very little activation. The lack of activation by the turned off form is postulated to be due to troponin-tropomyosin inhibiting the release of Pi in the acto·S-1 ATPase cycle, rather than by blocking the binding of S-1·ATP (and S-1·ADP·Pi) to actin, as was suggested by the steric blocking model. We tested several aspects of our model. First our model predicts that S-1·ATP and the S-1·ATP analog, pPDM·S-1, should not turn on the regulated acto·S-1 ATPase activity in the absence of Ca-2+. In agreement with our model, we found that compared to the maximal turned on rate, neither S-1·ATP nor pPDM·S-1 significantly turns on the regulated acto·S-1 ATPase activity. Second, our model predicts that these S-1 species should significantly turn on the regulated acto·S-1 ATPase activity in the presence of Ca-2+ provided that S-1·ATP and pPDM·S-1 bind slightly stronger to the turned on form than to the turned off form of regulated actin. We find that under conditions in which pPDM·S-1 binds extensively to regulated actin, it does fully turn on the regulated acto·S-1 ATPase activity in the presence of Ca-2+. These data are consistent with our original model in which the equilibrium between the turned on and turned off forms of regulated actin is partially shifted towards the turned on form by Ca-2+. It does, however, rule out our alternate model in which regulated actin can exist in a continuum of forms, but under any given conditions, only one of these forms are in existence. Lastly our model predicts that in Ca-2+, the thin filament is only partially turned on, while it is necessary to have rigor bridges bound to the thin filament to completely turn it on. In agreement with this prediction, we found that the ATPase activity of regulated acto·S-1 was much less than the fully turned on rate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00418-06 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Kinetic studies of proton and electron flows during respiration

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

PI: Richard W. Hendler	Section Head	LCB, NHLBI
Surinder K. Vig	Visiting Associate	LCB, NHLBI

COOPERATING UNITS *(if any)*

Richard I. Shrager, Mathematician, LAS, DCRT; Barry Bunow, Civilized Software, Bethesda, MD; Baltazar Raynafare, Research Associate and Albert Lehninger, Johns Hopkins University, Jeffrey Froehlich, Medical Officer, NIA, NIH.

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:
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.)*

We have been able to establish that the method of obtaining zero time H+/O ratios based on extrapolation of data obtained after 0.8 s back to zero gives undependable results. This is because although both [H+] vs t data, and [O] vs t data can be fit by single exponentials, neither is truly single exponential in character. This finding might help resolve some of the long standing controversy as to whether the zero-time value obtained by extrapolation procedures is 6.0 or 8.0 when succinate is the substrate. A final group of experiments and controls on the direct measurement of H+/O ratios accompanying cytochrome c oxidation at site III of the respiratory chain were analyzed by computer. These results substantiate earlier findings of a burst of H+/O ratios within the first 300 ms of a respiratory pulse. Preliminary experiments have shown that light scattering measurements can be dynamically recorded with a light pipe and photocell during a respiratory pulse, using mitochondria. These changes are related to mitochondrial size and energy state and will be the basis for a new system using rapid changes in external ion probe concentrations to determine $\Delta\Psi$ and ΔpH during a pulse in mitochondrial respiration. Steps were taken to develop a system using caged H+ and O2 compounds to cause a step change in [H+] and [O2] in stirred solutions. This will enable the determination of electrode relaxation times *in situ* in the same stirred vessels used for respiratory pulse experiments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00419-06 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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	NHLBI, LCB
Others: Tom Olszewski	Biologist	NHLBI, LCB

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 long-range objectives of these studies are to understand mechansims involved in the exchange of membrane between the plasma membrane and internal membrane systems. We use the small soil ameba, Acanthamoeba, as a model system. Acanthamoeba feeds solely by endocytosis, resulting in a high volume of membrane internalization and recycling. The current experimental question was whether newly internalized endocytic membrane is randomized with the existing intracellular pool of vacuolar membrane by internal fusions. Radioactive yeast were fed to amebas and the rate of digestion determined as a function of yeast load. The results showed a "saturation" of digestive capacity by large loads and suggested that hydrolases were limiting. Cytochemical studies showed that fewer phagosomes acquire hydrolases in heavily loaded cells. Examination of the cells in the electron microscope showed virtually no evidence of phagosome-phagosome fusion within 60 min. We demonstrated that later-formed phagosomes are less likely to obtain hydrolases by feeding amebas varying loads of yeast followed by latex beads. The latex bead phagosomes were isolated and their hydrolase specific activity determined as a function of yeast load. When the yeast load was heavy, hydrolase content of the later-formed bead phagosomes was much reduced. Thus using the hydrolase content of phagosomes as an internal marker, we find no morphological or biochemical evidence for fusions of newly formed phagosomes. We conclude that the membrane entering the cell through endocytosis does not randomize by rapid internal fusions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00501-13 LCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

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:	Peter K. Lambooy	Staff Fellow	LCB/NHLBI
	Marie-France Carlier	Laboratoire d'Enzymologie, CNRS	
	Dominique Pantaloni	Laboratoire d'Enzymologie, CNRS	
	Martine Coué	College de France	

COOPERATING UNITS *(if any)*

Laboratoire d'Enzymologie, Gif-sur-Yvette, France and College de France, Paris, France

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1.4	1.4	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.)*

The model for the polymerization of ATP-actin proposed last year states that ATP hydrolysis occurs on the F-actin subsequent to the addition of the actin subunit. As a consequence of the fact that hydrolysis on F-actin is generally slower than the addition reaction, there exists a cap of ATP-actin subunits, even at steady state, and the length of the cap will vary with the G-actin concentration above the critical concentration. The model proposes that the hydrolysis of ATP occurs preferentially, if not exclusively, at the interface between the ATP cap and the ADP core, i.e. on an ATP-actin subunit adjacent to a more interior ADP-actin subunit. This vectorial or zipper hydrolysis predicts that the initial rates of ATP hydrolysis and elongation as a function of G-actin concentration should be the same near the critical concentration but, as the G-actin concentration increases, the rate of ATP hydrolysis will become constant while the rate of elongation will continue to increase. This prediction has been verified for the polymerization of Mg-actin with an hydrolysis rate constant for the preferred site of 18 s/-1 and an additional very slow random hydrolysis in the ATP cap with a rate constant of 0.001 s/-1. With Ca-actin, however, ATP hydrolysis occurs randomly in the ATP cap and is always slower than the rate of elongation so that a long ATP cap builds up in proportion to the G-actin concentration. G-actin binds Ca-2+ with a K-D of 5 nM (304 orders of magnitude tighter than previously thought) and Mg-2+ with a K-D of 0.5 μM. Ca-actin elongates at the same rate as Mg-actin but nucleates much more slowly. A new dimeric G-actin monomer-binding protein and a hexameric, ATP-sensitive F-actin crosslinking protein have been discovered in Acanthamoeba castellanii.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00503-14 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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	Head, Section on Organelle Biochemistry	LCB, NHLBI
Others:	Sulie Chang	Staff Fellow	LCB, NHLBI
	Charles Patterson	Staff Fellow	LCB, NHLBI
	Gregory Bramblett	Research Assistant	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:
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.)*

Our major focus is now on the microtubule cytoskeleton of a Trypanosomatid. Crithidia fasciculata presents many unusual features, perhaps most conspicuously a paraflagellar rod linked to doublets 4 to 7, and a regularly spaced subpellicular corset of microtubules linked to each other and to the plasma membrane. To elucidate the structure and regulation of these links, we have begun to characterize microtubule-associated proteins. We find a 42-kDa polypeptide in isolated pellicles, which does not appear to bind an antibody which recognizes actin in other protozoa. Taxal MAPs from cytoplasmic tubulin include 4 prominent heat-stable polypeptides (27-125 kDa) and at least 5 minor high molecular weight compounds.

Second we have resumed the study of tubulin modification by reversible C-terminal tyrosine addition in brain, and extended this also to Crithidia. The brain carboxypeptidase that releases tyrosine has been purified 2000-fold using an FPLC pH gradient column. Despite the presence of a specific tyrosine adding enzyme in Crithidia, cytoplasmic and pellicular tubulins have been isolated devoid of C-terminal tyrosine. Flagellar tubulin, however, is partially tyrosinated, and is further distinguished by the presence of some "non-substrate" species, as in mammalian tubulins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00506-11 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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: Joseph P. Albanesi, Staff Fellow/Guest Worker, LCB/NHLBI; Mark Atkinson, Visiting Associate, LCB/NHLBI; Hanna Brzeska, Visiting Fellow, LCB/NHLBI; Hisao Fujisaki, Visiting Associate, LCB/NHLBI; Thomas Lynch, Staff Fellow, LCB/NHLBI Ray Scharff, Chemist, LCB/NHLBI; Toshiyuki Yamakado, Visiting Fellow, LCB/NHLBI

None

COOPERATING UNITS *(if any)*

Laboratory of Cell Biology

LAB/BRANCH
Cellular Biochemistry and Ultrastructure

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

INSTITUTE AND LOCATION
7 7 0

TOTAL MAN-YEARS:	PROFESSIONAL:	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.)*

Acanthamoeba myosins IA and IB are unusual in that they consist of only a single, relatively small, heavy chain and one light chain and are unable to form the bipolar filaments typical of other myosins and thought to be necessary for their function. Yet myosins IA and IB have very high actin-activated ATPase activities and are able to crosslink actin filaments into gels and cause superprecipitation that is dependent on ATP hydrolysis. From the unusual triphasic pattern (activation, inhibition and then reactivation) of their ATPase activities as a function of actin concentration, and their ability to cross-link F-actin, we proposed that myosins IA and IB contain two actin binding sites - one insensitive to the presence of ATP and unrelated to ATPase activity and an ATP-sensitive binding site that is associated with catalytic activity. This hypothesis has received several kinds of direct experimental support. (1) Myosin bridges of about 7 nm, the hydrodynamic diameter of single molecules, have been directly visualized in negatively stained electron microscopic images of actomyosin IB complexes. (2) Cross-linking actin filaments by enzymatically inert cross-linking proteins or shortening the filaments by addition of gelsolin had the predicted results of facilitating and inhibiting, respectively, the cooperative interaction of myosin I molecules on the actin filaments. (3) The myosin IA heavy chain has been proteolytically cleaved into two peptides of 30,000 and 100,000 daltons. The C-terminal 30,000-dalton peptide binds to actin (but does not cross-link filaments, with the same low Kd in the presence or absence of ATP and has no catalytic activity. The 100,000-dalton peptide has the associated light chain and the regulatory phosphorylation site. It binds to F-actin much more tightly in the absence than in the presence of ATP and, when phosphorylated, has full actin-activated ATPase activity but not the cooperative kinetics of the native molecule. The 30,000-dalton peptide contains 20% proline and 30% glycine.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00510-05 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Conformational State of the Acto·S-1 Complex

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

PI: Lois E. Greene	Research Chemist	LCB, NHLBI
Others: Roger Craig	Asst. Professor	Univ. of Mass.

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Section on Cellular Physiology

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.)*

The cross-bridge model of Eisenberg and Greene proposes that the cross-bridge cycle of muscle is driven by the myosin cross-bridge alternating between two major conformations which differ markedly in their strength of binding to actin and in their overall structure. In the conformation, which occurs in the absence of nucleotide or the presence of ADP, myosin binds very tightly to actin at a 45° angle. In the other conformation, which occurs when ATP or ADP·Pi is bound to myosin, myosin binds very weakly to actin at an angle postulated to be 90°. These two conformations also differ in that the regulatory complex, troponin-tropomyosin, can greatly weaken the binding of the strong-binding conformation of myosin·S-1 to actin, but has almost no effect on the binding of the weak-binding conformation of S-1. We previously found that the structure of acto·S-1 is very different in the presence and absence of of ATP, in agreement with our model. The structure of acto·S-1 was examined by negative staining using cross-linked actin·S-1, which enables actin to remain bound to S-1 at the low concentrations of protein needed for electron microscopy. In the present study, we examined the structure of cross-linked actin·S-1 in the presence of different ATP analogs to determine whether these analogs cause the conformation of acto·S-1 to resemble that obtained in ATP. We found that even though the ATP analogs, AMP-PNP and PPi, dissociate acto·S-1 to a similar extent, they, surprisingly, do not cause the same structural changes in acto·S-1. Cross-linked actin·S-1 in the presence of AMP-PNP appears quite rigor-like whereas the structure of cross-linked actin·S-1 in the presence of PPi is ATP-like. We also examined the structure of pPDM modified S-1 when cross-linked to actin. Biochemical studies have shown that pPDM-modified S-1 resembles S-1·ATP in its interaction with actin both in the presence and absence of troponin-tropomyosin. In support of these biochemical studies, the structure of cross-linked actin·pPDM-modified S-1 in the absence of ATP resembles that of cross-linked actin·S-1 in the presence of ATP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-00514-03 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Structure and Sequence of Non-Muscle Myosin Genes

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

PI: John A. Hammer, III	Senior Staff Fellow	LCB, NHLBI
Goeh Jung	Visiting Fellow	LCB, NHLBI
Edward D. Korn	Chief	LCB, NHLBI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Cellular Biochemistry and Ultrastructure

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this project is to isolate the genes encoding the non-muscle myosins of Acanthamoeba and to use the genes as tools to investigate myosin structure/function relationships and the in vivo functions of these cytoplasmic myosins. This project is part of the general effort in the Laboratory of Cell Biology to understand the organization and function of the cytoskeleton, using as a model system the soil amoeba Acanthamoeba. Acanthamoeba expresses simultaneously at least three distinct myosin enzymes, myosin IA, myosin IB and myosin II. Using molecular cloning techniques, we have isolated and purified a myosin II heavy chain gene and a myosin IB heavy chain gene. This study will provide, for the first time, the complete amino acid sequence of a non-muscle myosin. While non-muscle and muscle myosins share many common features, non-muscle myosins do possess unique structural, enzymatic, and regulatory properties. The amoeba myosin sequence data will be of great value in furthering our understanding of the unique structural and functional aspects of the amoeba myosins, and hopefully provide insight into the properties of cytoplasmic myosins in general.

The significance of this work is that by using the tools of molecular biology we can approach the study of these myosins in novel ways which are not possible using the classical techniques of protein chemistry. For example, we can use the genes to (1) make single determinant antibodies to synthetic peptides as probes of myosin function, (2) assign functional sites in the 1° sequence in combination with the amino acid composition of chemically crosslinked peptides, (3) search for cytoplasmic myosin genes in higher eukaryotes, (4) alter the intracellular levels of myosin in the living cell as a way to study their roles in cell physiology, and (5) study structure/function relationships via site-directed mutagenesis of the gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-HL-00515-01 LCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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

PI:	Richard W. Hendler	Section Head	LCB, NHLBI
	Richard I. Shrager	Mathematician	LAS, DCRT
Others:	David Songco	Chief, Personal Work Station Office	DCRT
	Brian Collett	Biophysicist	LPB, NIADDK
	John E. Fletcher	Acting Chief	LAS, DCRT

COOPERATING UNITS *(if any)*
Alan M. Demerle, Chief, Computer Systems Lab, DCRT; Perry Plexico, Chief, Project Development Section, CSL, DCRT; Keith L. Gorlen, James S. Del Priore and James Sullivan, CSL, DCRT

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Membrane Enzymology

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.08	0.08	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 project to develop a minimal form of an MLAB-like program to run on relatively inexpensive microcomputers of the type IBM PC/AT or PC/XT was initiated by R.W. Hendler in March of this year. DCRT was receptive to the idea and the active participation of a number of experts from this branch was obtained. Independently, the Computer Systems Laboratory of DCRT contacted Dr. Hendler for a collaborative effort to accomplish a similar goal using a more powerful, but more expensive 32 bit microcomputer. Both efforts are proceeding simultaneously along parallel paths. Our group (using the 16 bit microcomputer) has already adapted two different software packages to solve some MLAB type problems on the 16 bit microcomputers. Some analytical tasks, however, will require a machine with 32 bit architecture. Rather than going directly to the more expensive 32 bit microcomputers, we will explore a less expensive alternative of modifying the 16 bit machine to a 32 bit capability by using a coprocessor board with accessories.

ANNUAL REPORT OF THE
LABORATORY OF CELLULAR METABOLISM
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

October 1, 1985 to September 30, 1986

Research in the Laboratory of Cellular Metabolism continues to be largely concentrated on the enzymes responsible for the synthesis and degradation of cAMP and cGMP through which many hormones, drugs, and other agents influence cellular function. Recently, work has been increasingly focussed on the hormone-sensitive adenylate cyclase. The objective of this effort is to elucidate the mechanisms for control of synthesis, assembly and operation of this ubiquitous regulatory system, which is basically analogous to certain other systems that serve in cell membranes to transduce stimuli from the environment through GTP-binding proteins to an internal effector signal. In addition, work is continuing on specific cyclic nucleotide phosphodiesterases and calmodulin-regulated proteins as well as on the ADP-ribosyltransferases of animal cells.

1. Adenylate Cyclase and the Role of GTP-binding Proteins in Signal Transduction.

Guanyl nucleotide-binding (G) proteins couple agonist interaction with cell surface receptors to an intracellular enzymatic response. In the adenylate cyclase system, inhibitory and stimulatory effects are mediated through G_i and G_s, respectively. In the visual excitation complex, the photon receptor rhodopsin is linked to its effector, cGMP phosphodiesterase, through transducin. Another G protein, G_o, of unknown function, is relatively abundant in brain. The G proteins are heterotrimers of α, β, and γ subunits; the α-subunits catalyze receptor-stimulated GTP hydrolysis. We are investigating structural, functional, and immunological relationships between G_s, G_i, G_o, transducin and other potential members of this family of regulatory proteins. With the goal of understanding at the molecular level the mechanism of action and control of synthesis of the G protein subunits, increasing effort is directed toward cloning the relevant genes.

A cDNA clone, λ609, isolated from a bovine retina library, provided by Dr. J. Nathans, using oligonucleotide probes complementary to reported sequences in two clones of the α subunits of transducin (T_α) was found to differ in sequence from reported T_α clones. In other studies we had obtained amino acid sequences of tryptic peptides from bovine brain $G_{o\alpha}$. These were identical to sequences deduced from the partial nucleotide sequence of λ609, thus establishing it as a $G_{o\alpha}$ alone. Nucleotide and deduced amino acid sequences of λ609 also revealed significant similarities to corresponding regions of bovine T_α, $G_{s\alpha}$, $G_{i\alpha}$, and rat brain $G_{o\alpha}$. λ609 codes for an amino acid sequence at the carboxy terminus which includes a cysteine residue at the position of the cysteine in T_α and $G_{i\alpha}$ that is ADP-ribosylated by pertussis toxin. Although $G_{s\alpha}$ is considered to be the major target for ADP-ribosylation by choleragen, the pertussis toxin substrates $G_{o\alpha}$ and $G_{i\alpha}$ can also be modified under certain conditions. λ609 encodes a sequence highly homologous to the region containing the arginine that is ADP-ribosylated by

choleragen in $G_{s\alpha}$ and T_α. Consistent with available information on the tissue distribution of $G_{o\alpha}$, Northern analysis of RNA from retina, liver, spleen, heart and brain revealed the highest levels of $G_{o\alpha}$ mRNA in brain.

To evaluate the interaction of $G_{o\alpha}$ from bovine brain with $\beta\gamma$ subunits and rhodopsin (as a model receptor), the purified proteins were reconstituted in phospholipid vesicles. The GTPase activity of $G_{o\alpha}$ was stimulated by photolyzed, but not dark, rhodopsin and was enhanced by bovine retinal $T_{\beta\gamma}$ as well as by rabbit liver $G_{\beta\gamma}$. $G_{o\alpha}$ in the presence of $G_{\beta\gamma}$ was a substrate for pertussis toxin-catalyzed ADP-ribosylation; modification was inhibited by photolyzed rhodopsin and enhanced by GDPβS. ADP-ribosylation of $G_{o\alpha}$ by pertussis toxin inhibited photolyzed rhodopsin-stimulated but not basal GTPase activity. Thus, in these functional assays, $G_{o\alpha}$ resembles T_α and $G_{i\alpha}$.

Two proteins that may be related to G protein α subunits have been detected immunologically. One of these, a protein of $\sim$ 30 kDa found in soluble fractions from bovine brain and liver, has been partially purified. It reacts with a monoclonal antibody against T_α that also reacts with $G_{i\alpha}$ but not $G_{o\alpha}$. The other, a larger soluble protein found in bovine brain, reacts with antiserum against a peptide (17 amino acids) containing a sequence common to all known G proteins. Further purification and characterization of these proteins is in progress.

The γ-subunit ($\sim$ 8 kDa) of transducin (T_γ) is purified as a complex with T_β. Unlike the β-subunits which are very similar in all G proteins, $G\gamma$ and $T\gamma$ differ in amino acid composition and immunoreactivity. The function of the γ subunit is unknown. It may serve as a membrane anchoring component and/or confer specificity on the $\beta\gamma$ complex. To begin to approach this question we have used a monoclonal antibody (2H3) against T_γ, which can immunoprecipitate the $T_{\beta\gamma}$ complex. The effects of 2H3 on GTP hydrolysis by transducin and ADP-ribosylation of T_α by pertussis toxin were evaluated. The GTPase activity of transducin is dependent on the presence of activated receptor (i.e., light-activated rhodopsin), whereas the preferred substrate for pertussis toxin action is thought to be the inactive $\alpha\beta\gamma$ complex in the absence of activated receptor. 2H3 was inhibitory to both these activities, consistent with the view that T_γ is important in formation of a functional $\alpha\beta\gamma$ complex production, interaction of which with rhodopsin is required for stimulation of GTPase activity.

Pertussis toxin is an oligomeric protein consisting of an enzymatically active subunit (S_1) linked to a binding pentamer (S_2S_4)(S_5)(S_3S_4). In addition to ADP-ribosylating $G_{i\alpha}$, $G_{o\alpha}$, and T_α it catalyzes the hydrolysis of NAD. The NAD glycohydrolase activity is dependent on the reduction of intrachain disulfide bonds in S_1. ADP-ribosylation of $G_{i\alpha}$ and T_α in some systems depends on detergent, phospholipid and/or ATP as well as thiol. Since NAD hydrolysis was stimulated by ATP, the effect of the nucleotide appeared to be directly on the toxin. To determine the loci of action of these effectors, we investigated the requirements for ADP-ribosyl-transferase and NAD glycohydrolase activity with both the holotoxin and its

S_1 subunit. It was concluded that whereas detergent and thiol activate the S_1 subunit, ATP is involved in activation of the holotoxin, not the isolated catalytic unit.

2. Cyclic Nucleotide Phosphodiesterases

In an extension of our earlier studies of the cGMP phosphodiesterase from bovine retinal rod outer segments we have found that several cGMP analogues differ in their effectiveness as inhibitors of cGMP binding and hydrolysis. These findings are consistent with the existence of distinct hydrolytic and non-hydrolytic cGMP binding sites that could play a role in regulation of enzyme activity. Antisera were developed against the retinal phosphodiesterase and against the so-called cGMP-stimulated phosphodieterase that we had purified from bovine liver. Neither antiserum cross-reacted with the other phosphodiesterase or with the calmodulin-sensitive phosphodiesterase purified from bovine brain. Earlier experiments with antisera against the calmodulin-activated enzyme had likewise failed to provide any evidence of immunological similarities between these phosphodiesterases.

We continue to use cultured 3T3-L1 adipocytes as well as isolated rat fat cells to investigate the mechanisms of insulin activation of the particulate cAMP phosphodiesterase and its role in the anti-lipolytic action of insulin. Phenyl-isopropyl adenosine (PIA), an analogue of adenosine that is antilipolytic, also increases particulate cAMP phosphodiesterase activity and this effect, like that of insulin, is prevented by prior treatment of the 3T3-L1 adipocytes with pertussis toxin. We have found that certain agents with insulin-like effects on glucose uptake, i.e., anti-insulin receptor antibodies and wheat germ agglutinin also activate the phosphodiesterase. Pertussis toxin inhibition of these effects suggests that they, as well as the effects of insulin and PIA, are mediated by a guanyl nucleotide-binding protein. For many years attempts to purify the insulin-activated phosphodiesterase have been largely unsuccessful. In collaborative studies, we have now succeeded in solubilizing the enzyme from rat adipose tissue (maintaining the activated state) with an alkyl poly-oxyethylene non-ionic detergent and achieving partial purification with good yield. Among a number of inhibitors tested, cilostamide and certain "cardiotonic" drugs (e.g., milrinone) were most potent. Preparation of an affinity matrix using one of these drugs may facilitate final purification. Based on the inhibitor effects it appears that the insulin-activated particulate phosphodiesterases of rat fat cells and 3T3-L1 adipocytes may be similar to a cAMP phosphodiesterase that has been described in soluble faction of heart, platelets, and other tissues.

3. Interaction of Calmodulin with Phosphodiesterase and Other Binding Proteins

We had reported that interaction of calmodulin with the calmodulin-sensitive phosphodiesterase occured at a Ca^{2+} concentration lower than that required for activation. However, in those studies the concentration of phosphodiesterase used to assay activity was of necessity two to three orders of magnitude lower that that required to monitor interaction. With a poorly hydrolyzed substrate N6-etheno cyclic AMP, we have now been able to assay activity and

interaction in identical samples. The regulatory properties of the enzyme with the alternative substrate were identical to those with cyclic AMP. The Ca^{2+}-dependence of interaction appeared non-cooperative with an apparent half-maximal concentration of 6 μM. Enzyme activation, however, required 3 to 4 times higher Ca^{2+} and appeared highly cooperative. These data, analagous to those obtained with the calmodulin-activated protein phosphatase calcineurin, support the hypothesis that interaction and activation are sequential Ca^{2+}-dependent events and that protein-protein interaction may lead to cooperativity in the activation by Ca^{2+}.

In our earlier studies with the phosphodiesterase, disulfide cross-linking to PDP-calmodulin (a reactive derivative) resulted in a complex that retained full activity even without added Ca^{2+}. Incubation of calcineurin with PDP-CaM led to virtually complete inhibition of the enzyme activity. The concentration dependence of inhibition was consistent with formation of a one-to-one complex. Activity of the isolated complex was stimulated by dimethylformamide and by Mn^{2+} and Ni^{2+} suggesting that calmodulin-dependent activity was specifically inhibited. Since calmodulin interaction with calcineurin is known to lead a time-dependent deactivation, it seems plausible that it is cross-linked to PDP-CaM in a conformation corresponding to the deactivated state. Activity of the complex was completely restored by addition of reductant, indicating that the inhibited form was maintained by intermolecular disulfide bonds. The cross-linked complex may be useful in determining the mechanism of enzyme deactivation.

Collaborative studies of localization and regulation of calmodulin-activated phosphodiesterase have been extended in the past year. The neurotoxin, 3-acetyl-pyridine (3 AP), selectively destroys certain nuclei (inferior olivary complex) which supply excitatory afferents to the dendrites of Purkinje cells. Brains from rats treated with 3 AP show loss of climbing fiber input to the Purkinje cells; the structure of the Purkinje and other cerebellar cells appears unaffected. In Purkinje cells, there was a virtually complete loss of phosphodiesterase immunoreactivity with no change in calcineurin. Phosphodiesterase-containing neurons in other regions (e.g., pyramidal cells in hippocampus and cerebral cortex) were apparently unaltered. There was > 70% reduction in phosphodiesterase content of whole cerebellar extracts; thus Purkinje cells represent by far, the major locus of phosphodiesterase in cerebellum. The selective reduction of phosphodiesterase after 3 AP treatment may indicate that its expression in these cells is under trans-synaptic regulation, i.e., linked to the presence of convergent excitatory input, consistent with the proposal that cyclic nucleotide hydrolysis plays a role in the integration of stimulatory signals.

We found that calcineurin is the major calmodulin-binding protein in murine spleen cells, with larger amounts in B than in T cells. Amounts in thymocytes and macrophages are comparable to that in T cells. In these cells, calmodulin-binding proteins of 150 and 200 kDa, which are probably calmodulin-binding cytoskeletal proteins, were also seen. The major calmodulin-binding protein in thymocytes, a peptide of 78-70 kDa, was present in 5-10 times the amount of

calcineurin; it did not react with antibodies against calcineurin. Differentiation of B or T cells by mitogens did not alter amounts of calcineurin, however, amounts of higher molecular weight calmodulin-binding proteins were increased. The relationship of these changes to cell activation is under study.

In preparation for investigation of mechanisms underlying developmental or regulatory changes in calmodulin-activated phosphodiesterase and calcineurin, isolation of cDNA clones for these proteins has been initiated. Immunoscreening of a rat brain cDNA library in the expression vector lamba gt11 yielded 10 phosphodiesterase and seven calcineurin clones. Most of the inserts are relatively small. Isolated inserts are being prepared for use as probes to obtain larger cDNAs.

4. ADP-ribosylation of Proteins in Animal Cells

The ADP-ribosyl transferase activities of choleragen and pertussis toxin have been extensively used to probe the function of guanyl nucleotide-binding proteins of the adenylate cyclase and other systems. In the past few years, we have identified a family of NAD:arginine ADP-ribosyltransferases in animal cells. Although they catalyze the same model reactions as does choleragen, which in cells rather specifically modifies G_s and T_α, their natural substrates are unknown. If the animal transferases play a regulatory role, there would likely be enzyme that remove the ADP-ribose moiety. Last year we were finally able to demonstrate such an ADP-ribosylarginine hydrolase activity in turkey erythrocytes. The products of the reaction, which is stimulated by Mg^{2+} and dithiothreitol, were identified as ADP-ribose and arginine. The enzyme has been partially purified and characterized. During purification, the hydrolase was separated from the transferases, establishing different enzymes are responsible for ADP-ribosylarginine synthesis and cleavage. The hydrolase is inhibited by 5 mM NaF or 200 mM NaCl whereas an NAD:arginine ADP-ribosyltransferases from erythrocytes, previously shown to be activated by NaCl, is not affected by NaF (or by Mg^{2+} and dithiothreitol), activators of the hydrolase.

The ADP-ribose moiety plays a critical role in substrate recognition by the hydrolase. ADP-ribosylarginine and ADP-ribosyl guanidine were clearly better substrates than phosphoribosylarginine or ribosylarginine; the latter were also poor inhibitors. ADP-ribose was a potent inhibitor, much better than ADP or AMP. Arginine, guanidine, and agmatine, an arginine analogue, did not significantly inhibit enzymatic activity. Thus, the primary recognition site for the hydrolase appears to be the ADP-ribose moiety. Animal tissues contain several enzymes that may degrade ADP-ribosylarginine. Phosphodiesterases generate phosphoribosylarginine and 5'-AMP; phosphatases degrade phosphoribosylarginine to ribosylarginine. As the products of these reactions are poor substrates for the hydrolase, their action could prevent cleavage of the ribosyl-(arginine) protein linkage by the hydrolase and regeneration of the (arginine) protein acceptor. There may be, however, other enzymes with different substrate specificity that can cleave the phosphodiesterase and the phosphodiesterase-phosphatase products.

Some NAD:arginine ADP-ribosyltransferases can utilize NADP as well as NAD. Transferase A from erythrocytes in the presence of NAD or NADP synthesizes ADP-ribosyl- or 2'-phospho-ADP-ribosylarginine, respectively. The erythrocyte ADP-ribosylarginine hydrolase cleaved ribosylarginine linkages in both products, although the V_{max} with the phosphorylated compound was significantly less. The hydrolase thus appears able to act on both types of transferase products. Our earlier studies established the stereospecificity of the reaction catalyzed by the NAD:arginine ADP-ribosyltransferases which utilize β-NAD generating an α-anomeric product. We have now shown that the ADP-ribosylarginine hydrolase preferentially cleaves the α-anomer, consistent with the stereospecific coupling of the transferase-hydrolase reactions. α-ADP-ribosylarginine formed in vitro undergoes nonenzymatic anomerization. ADP-ribosylation of a physiological protein acceptor, however, may result in an α-anomeric linkage that is stabilized by physical constraints imposed by the protein. Under these circumstances, anomerization would not influence the rate of release of the ADP-ribose moiety by the hydrolase and tighter regulatory control could result.

5. Regulation of cAMP and cGMP Metabolism in Intact Cells

As we have reported, bradykinin acts through B-2 type receptors on cultured human fibroblasts to release arachidonate and cyclooxygenase products that activate adenylate cyclase resulting in increased cell cAMP content. We recently found that several agents (pertussis toxin, choleragen, forskolin, 8 Br-cAMP, phosphodiesterase inhibitors) which increase cell cAMP content enhance the effects of bradykinin on prostaglandin formation and cAMP. Nitroprusside, a drug that, like bradykinin, causes vasodilatation and hypertension, also modified responses to bradykinin. Nitroprusside markedly increased fibroblast cGMP content and bradykinin, which alone caused only a slight increase, dramatically altered the time course of this effect. Similarly, cAMP responses to bradykinin plus nitroprusside differed quantitatively and temporally from the sum of the effects of each alone. Inhibition of cyclooxygenase influenced in different ways the cGMP responses to nitroprusside and bradykinin. Through interactions of this type, in vivo responses to drugs like nitroprusside may be influenced by levels of bradykinin, prostaglandins, or other endogenous mediators.

Atrial natriuretic factor (ANF), a peptide synthesized and secreted by atrial cardiocytes in response to increased atrial pressure, plays a major role in blood pressure and fluid homeostasis. Cyclic nucleotides have been implicated as second messengers mediating at least some of these effects. In cultured human fibroblasts, we found a single class of high-affinity ANF receptor sites. ANF caused a dose-dependent increase in cell cGMP content and decrease agonist-elevated cAMP. Pertussis toxin-catalyzed ADP-ribosylation of $G_{i\alpha}$ did not block the ANF-induced reduction of cAMP, which, therefore, probably does not depend on G_i. Several phosphodiesterase inhibitors did block the inhibitory action of ANF, from which we infer that it may result from the activation of a cAMP phosphodiesterase. The cGMP analogue, 8-Br cGMP, inhibited agonist-stimulated cAMP accumulation consistent with the possibility that ANF-induced elevation of cGMP activates a cAMP phosphodiesterase that decreases cell cAMP content.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00606-15 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Regulation of cAMP Content and Prostaglandin Production of Cultured Cells

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

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Others:	Joel Moss, M.D., Ph.D.	Head, Section on Molecular Mechanisms	CM, NHLBI
	Su-Chen Tsai, Ph.D.	Research Chemist	CM, NHLBI
	Jane Halpern, Ph.D.	Staff Fellow	CM, NHLBI

COOPERATING UNITS *(if any)*

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National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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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.)*

Previous work has shown that bradykinin (BK) interacts with B-2 type receptors in human fibroblasts and initiates a series of biochemical events resulting in increased phospholipase activity, release of arachidonate, and formation of prostaglandins. These prostaglandins, in turn, activate adenyl cyclase and increase cAMP content. The effects of BK on fibroblast cAMP content can be inhibited by the cyclooxygenase inhibitor indomethacin.

Several factors alter responsiveness to BK. A number of agents, all of which can increase cAMP by different mechanisms, enhanced effects of BK on both prostaglandin formation and cAMP content. Muscarinic agonists, via interaction with receptors presumably coupled to the guanyl nucleotide-binding protein Ni, also enhanced the effect of BK on prostaglandin formation and cAMP content.

Human fibroblasts were also utilized to study the interaction of BK and sodium nitroprusside (SNP). Incubation with SNP markedly increased cGMP content which reached a maximum in <30 sec and then declined. BK, which itself only slightly increased cGMP content, dramatically altered the time course of cGMP accumulation in response to SNP; in the presence of both BK and SNP, maximal increases in cGMP content were not attained until 90 sec. SNP, which itself had little or no effect on cAMP content and prostaglandins, enhanced responsiveness to BK. These interactions between SNP and BK were modified by cyclooxygenase products of arachidonate metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00614-09 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Interaction of Calmodulin with Phosphodiesterase and other Binding Proteins

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

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Others:	Martha Vaughan, M.D.	Chief, Laboratory of Cellular Metabolism	CM, NHLBI

COOPERATING UNITS *(if any)*

Pennsylvania State University (M.L. Billingsley, C.D. Balaban); Laboratory of Immunology, NIAID, NIH (M.V. Sitkovsky).

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SECTION

Metabolic Regulation

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

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

☐ (a1) Minors

☐ (a2) Interviews

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

A poorly hydrolyzed substrate, N6-etheno cyclic AMP, was used to assay high concentrations (0.2 - 0.6 μM) of cyclic nucleotide phosphodiesterase (PDE) permitting direct comparison of activity with changes in physical properties of the enzyme. Although the interaction constant for this substrate (2-3 mM) was 100-fold higher than that for cAMP, the regulatory properties of the enzyme were comparable (e.g., Ka for Mg2+, Ki for spermine, degree of stimulation by calmodulin (CaM). When the Ca2+-dependence of enzyme activation was compared with that for interaction with dansyl-calmodulin (D-CaM) using identical experimental samples, less Ca2+ was required for interaction than for stimulation of activity; this suggested sequential steps in the mechanism of PDE activation by CaM. Immunocytochemical studies in rat brain indicated that specific changes in the distribution of PDE in cerebellum occurred after pharmacologic lesions of the inferior olivary nucleus, while that of calcineurin (CN) did not. Since this treatment affects excitatory innervation of Purkinje cells, it is possible that such input pathways may modulate, transynaptically, the local expression of PDE. Using overlay procedures, CN has been identified as the predominant CaM-binding protein in isolated spleen cells and cultured PC-12 cells; smaller amounts of cytoskeletal CaM-binding proteins (caldesmon, spectrin) have also been found. In some instances, there were changes in the amounts of these proteins with differentiation, suggesting a role for Ca2+-dependent protein dephosphorylation and/or cytoskeletal modification during cellular activation. Expression vector immunoscreening procedures were optimized to permit isolation of putative cDNA clones for PDE and CN using a lambda GT-aa rat brain library. Lysogens of these clones were produced and the fusion proteins analyzed for immunoreactivity against affinity-purified CN and PDE antibodies, and against monoclonal anti-beta galactosidase antibody.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00622-09 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)*

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	Robert E. West, Jr., Ph.D.	Staff Fellow	CM, NHLBI
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	2.4	2.0

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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.)*

Hormonal control of adenylate cyclase is mediated by GTP-binding proteins, stimulation via Gs, inhibition via Gi. A similar GTP-binding protein, transducin couples the light receptor rhodopsin to a cGMP phosphodiesterase. Pertussis toxin, an etiologic agent in whooping cough, inactivates Gi and transducin by catalyzing the ADP-ribosylation of a critical cysteine. Pertussis-toxin catalyzed ADP-ribosylation of transducin, and NAD hydrolysis was stimulated by adenine nucleotide and either phospholipid or detergent. NAD hydrolysis was increased synergistically by ATP and detergents or phospholipids; the zwitterionic detergent CHAPS was more effective than the nonionic detergent Triton X-100 > lysophosphatidylcholine > phosphatidylcholine. In CHAPS, NAD hydrolysis was enhanced by ATP > ADP > AMP > adenosine; ATP was more effective than MgATP or the non-hydrolyzable analogue, adenyl-5'-yl-imidodiphosphate. GTP and guanyl-5'-yl-imidodiphosphate were less active than the corresponding adenine nucleotides. Activity in the presence of CHAPS and ATP was almost completely dependent on dithiothreitol. The isolated enzymatic (S1) component catalyzed the dithiothreitol-dependent hydrolysis of NAD; activity was enhanced by CHAPS but not ATP. The studies are consistent with the conclusion that adenine nucleotides, dithiothreitol, and CHAPS act on the toxin itself rather than on the substrate; adenine nucleotides appear to be involved in the activation of holotoxin but not the isolated catalytic unit.

In animal cells, ADP-ribosylation of proteins is a reversible process, catalyzed by synthetic and degradative enzymes known respectively as ADP-ribosyltransferases and ADP-ribosylarginine hydrolases. The specific substrates for a purified erythrocyte hydrolase were α-ADP-ribosylarginine and α-2'-phospho-ADP-ribosylarginine, products of the transferase reaction. The hydrolase and transferases possess a compatible sterospecificity and substrate specificity consistent with the conclusion that the two enzymatic activities may serve as opposing arms in an ADP-ribosylation cycle.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00627-08 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)*

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	Masatoshi Noda, Ph.D.	Visiting Fellow	CM, NHLBI
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National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.9	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.)*

Guanyl nucleotide-binding (G) proteins couple agonist interaction with cell surface receptors to an intracellular enzymatic response. In the adenylate cyclase system, inhibitory and stimulatory effects are mediated through the guanyl nucleotide-binding proteins, Gi and Gs, respectively. In the visual excitation complex, the photon receptor rhodopsin is linked to its effector, cGMP phosphodiesterase, through transducin. Bovine brain contains another G protein, Go. The G proteins are heterotrimers of α, β, and γ subunits; the α-subunits catalyze receptor-stimulated GTP hydrolysis. The interaction of Goα with the βγ subunits and rhodopsin reconstituted in phosphatidylcholine vesicles was examined. The GTPase activity of Goα purified from bovine brain was stimulated by photolyzed, but not dark, rhodopsin and was enhanced by bovine retinal Tβγ or by rabbit liver Gβγ. Goα in the presence of Gβγ is a substrate for pertussis toxin-catalyzed ADP-ribosylation; the modification was inhibited by photolyzed rhodopsin and enhanced by GDPβS. ADP-ribosylation of Goα by pertussis toxin inhibited photolyzed rhodopsin-stimulated but not basal GTPase activity. It would appear from this and prior studies that Goα is similar to Tα and Giα; all three exhibit photolyzed rhodopsin-stimulated GTPase activity, are pertussis toxin substrates, and functionally couple to Tβγ. Monoclonal and polyclonal antibodies against G protein subunits have been prepared and characterized. Some of these have effects on function and some have been useful for identification of G proteins in tissues.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00630-07 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Fatty Acids in Adrenoleukodystrophy; Studies on HMGCoA Reductase in Mammalian Cells

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

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COOPERATING UNITS *(if any)*

Department of Pediatrics, Medical College of Virginia (Dr. W.B. Rizzo), Molecular Disease Branch, NHLBI (Dr. Z.H. Beg).

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Metabolic Regulation

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

Experiments with human skin fibroblasts revealed a likely competition between the metabolism of normal very long chain fatty acids and phytanic acid. Studies *in vitro* and *in vivo* showed that exogenous oleic acid reduces the content of saturated VLFA while increasing the unsaturated ones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00634-06 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)

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	Martha Vaughan, M.D.	Chief, Laboratory of Cellular Metabolism	CM, NHLBI

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TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.9	1.4	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.)

Antibodies were produced in sheep and rabbits against the purified cGMP-stimulated cyclic nucleotide phosphodiesterase, and in sheep to the bovine rod outer segment cGMP PDE. No immunocrossreactivity was noted between these two PDEs and a calmodulin sensitive PDE from bovine brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00636-05 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Particulate PDE in Regulation of Lipolysis and Antilipolytic Action of Insulin

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

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Biochemical Physiology

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National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	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.)*

Following incubation with insulin, isomethylbutylxanthine (IBMX), and dexamethasone, confluent 3T3-L1 fibroblasts differentiate into cells with morphological and biochemical characteristics of mature rodent adipocytes. Differentiated 3T3-L1 adipocytes and isolated rat fat cells were utilized to investigate the activation of hormone-responsive, particulate cAMP phosphodiesterase (PDE) and the role played by this enzyme in the process of insulin-dependent regulation of lipolysis.

In intact 3T3-L1 adipocytes, the antilipolytic agents insulin and phenylisopropyladenosine (PIA) increase particulate cAMP PDE activity. Certain "insulin-like" agents such as wheat germ agglutinin (WGA) and anti-insulin-receptor antibodies increase hexose transport as well as particulate cAMP PDE activity. Effects of PIA, insulin and the "insulin-like" agents on particulate cAMP PDE were prevented in adipocytes exposed to pertussis toxin (PT) suggesting a role for guanyl nucleotide binding proteins in PDE regulation and/or insulin action.

With the goal of understanding the molecular regulation of the particulate cAMP PDE by insulin and other agents, lipolysis and particulate cAMP PDE are being studied under identical conditions, i.e., during activation of lipolysis by various combinations of adenosine deaminase and/or isoproterenol (plus/minus adenosine or PIA), and during inhibition of lipolysis by insulin.

The particulate cAMP PDE from rat adipose tissue has been solubilized with polyoxyethylene non-ionic detergents, partially purified and characterized in terms of inhibition by a number of phosphodiesterase inhibitors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00638-04 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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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	Suzanne Czarnecki, Ph.D.	Staff Fellow	CM, NHLBI
	Inez Serventi, Ph.D.	Staff Fellow	CM, NHLBI
	Krisa Van Meurs, M.D.	Guest Researcher	CM, NHLBI
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Metabolic Regulation

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.4	4.4	0.0

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

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

Guanyl nucleotide binding proteins (GNPs) are critical in the regulation of receptor mediated events. The stimulatory and inhibitory receptors of the adenylate cyclase system are coupled to the cyclase catalytic unit through two GNPs, Gs and Gi, which mediate stimulation and inhibition, respectively. In addition, rhodopsin, the photon receptor in retinas, is coupled to its target enzyme, a cGMP phosphodiesterase, through a GNP known as transducin (T). Go, a GNP which interacts functionally with rhogopsin and muscarinic receptors, does not appear to be involved in adenylate cyclase regulation. All of these GNPs exhibit structural and functional similarities and are heterotrimers of α, β, and γ subunits.

A cDNA clone, λ609, was isolated from a bovine retinal λgt10 library using oligonucleotide probes complementary to reported sequences in two clones of the α subunits of transducin (Tα). Sequences of several tryptic peptides from bovine brain Goα were identical to deduced amino acid sequences in λ609. Nucleotide and deduced amino acid sequences of λ609 also revealed significant similarities to corresponding regions of bovine Tα, Gsα, Giα, and rat brain Goα. λ609 encodes for an amino acid sequence highly homologous to the region surrounding the arginine residue that is ADP-ribosylated by choleragen in Tα as well as a sequence at the carboxy terminus which includes a cysteine residue at the position of the cysteine in Tα and Giα that is the substrate for ADP-ribosylation by pertussis toxin. Northern analysis revealed that, of several tissues examined, the levels of RNA coding for Goα are highest in the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00639-03 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Characterization of a Bovine Rod Outer Segment cGMP Phosphodiesterase

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

PI:	Vincent C. Manganiello, M.D., Ph.D.	Head, Section on Biochemical Physiology	CM, NHLBI
Others:	Joel Moss, M..D, Ph.D.	Head, Section on Molecular Mechanisms	CM, NHLBI
	Martha Vaughan, M.D.	Chief, Laboratory of Cellular Metabolism	CM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular Metabolism, NIH, NHLBI, Bethesda, MD

SECTION

Biochemical Physiology

INSTITUTE AND LOCATION

National Institutes of Health, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	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 cGMP phosphodiesterase (PDE) was extracted from bovine rod outer segments and purified by chromatography on AcA34. cGMP is the preferred substrate for this PDE. cGMP binding sites were studied using photolabelling techniques and direct binding studies. IBMX which inhibited cGMP hydrolysis in a competitive fashion did not interfere with [3H]cGMP binding or photolabelling with [32P]cGMP. cAMP was a very ineffective competitor for [3H]cGMP binding; only [32P]cGMP, not [32P]cAMP or 8-azido [32P]cAMP formed photoadducts with the ROS cGMP PDE. In general, several cAMP derivatives were not as effective as cGMP or 8-Br cGMP in inhibiting cGMP hydrolysis or competing for [3H]cGMP binding sites. 8-Chloropurine riboside cycle monophosphate was, however, more effective in inhibiting [3H]cGMP binding thin hydrolysis. These studies suggest that distinct sites with differing topography may be involved in binding and hydrolysis of cGMP by the ROS cGMP PDE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00641-02 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Studies on Muscarinic Receptors

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

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0.5	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.)*

High affinity specific binding of muscarinic ligands to adult human skin fibroblasts was investigated. The binding activity is being assayed in a number of cell lines. Interaction of bovine brain muscarinic receptors with transducin, or its subunits is being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00642-01 CM

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Atrial Natriuretic Factor Regulation of Cyclic Nucleotide Metabolism

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

PI:	Robert E. West, Jr., Ph.D.	Staff Fellow	CM, NHLBI
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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.)*

Atrial natriuretic factor (ANF) is a polypeptide hormone synthesized and secreted by atrial cardiocytes in response to increased atrial pressure; it plays a major role in blood pressure and fluid homeostasis. Cyclic nucleotides have been implicated as second messengers mediating at least some of these effects. Cultured human fibroblasts used as a model system have been found to have a single class of high-affinity ANF receptor sites. Treatment with ANF caused a dose-dependent increase in cGMP. ANF also mediated a reduction in agonist-stimulated cAMP levels without effects under basal conditions. Pertussis toxin-catalyzed ADP-ribosylation of the α subunit of Gi did not block the ANF-mediated reduction of cAMP levels: hence, Gi does not mediate ANF effects. The phosphodiesterase inhibitors IBMX, Ro 20-1724, and cilostamide did block the inhibitory action of ANF, from which we infer that the action of ANF may be mediated by the activation of a cAMP phosphodiesterase. The cGMP analogue, 8-Br cGMP, inhibited agonist-stimulated cAMP to a degree similar to that observed with ANF. The presence of 8-Br cGMP and ANF together resulted in no greater degree of inhibition. This suggests that the ANF-mediated reduction in agonist-stimulated cAMP may be caused by a cAMP phosphodiesterase, which was in turn activated by ANF-induced cGMP.

ANNUAL REPORT OF THE
LABORATORY OF CHEMICAL PHARMACOLOGY
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1985 through September 30, 1986

In recent years, this Laboratory has shifted its emphasis toward studies of possible mechanisms by which drugs, other foreign compounds and their metabolites may evoke various kinds of toxicities. A part of the Laboratory has focused its interest on mechanisms through which chemically reactive metabolites cause lesions in various target organs, but because of the central role that mast cells play in inflammatory and allergic reactions, the mechanisms by which antigens evoke the release of histamine and other substances from granules has also been a major focus of the Laboratory. In addition, the Laboratory has continued its efforts in identifying isozymes of cytochrome P-450 that catalyze the metabolism of foreign compounds and discovering factors that govern the relative rates of formation of metabolites by individual isozymes. It is also continuing to develop pharmacokinetic tools that elucidate parameters that may be useful in making comparisons between in vitro and in vivo experiments.

Mechanisms of Toxicity

Halothane. Halothane may be converted to chemically reactive metabolites either by reductive cleavage of a carbon-halogen bond to form a radical or by hydroxylation of the carbon-hydrogen bond followed spontaneously by dehydrohalogenation to form a trifluoroacetyl halide. Although an acute hepatotoxicity has been associated with the reductive pathway, there is evidence suggesting that the fulminant type of halothane hepatotoxicity observed in humans may be due to an immune reaction. During the past few years we have established that the administration of halothane to rats pretreated with phenobarbital results in the covalent binding of the trifluoroacetyl group to proteins localized predominantly in the endoplasmic reticulum of cells in the centrilobular region of liver. Subsequent work has revealed that the covalently bound trifluoroacetyl groups were associated with two microsomal proteins, having molecular weights of about 54 kD and 59 kD. The 54 kD protein was identified as an isozyme of cytochrome P-450. During the past year we have found that the administration of halothane to unpretreated rats also gives rise to a small amount of trifluoroacetyl adducts that are associated with three proteins having molecular weights of 59 kD, 76 kD and 92 kD. By passing solubilized microsomes from halothane treated rats through an affinity column containing an antibody against trifluoracetyl lysine groups and elution with trifluoroacetyl lysine, we have obtained sufficient amounts of the 59 kD protein to tentatively identify it as an isozyme of cytochrome P-450. The protein may thus be the largest cytochrome P-450 ever detected. Determination of the substrate and reaction specificities of the protein remains to be accomplished, but owing to the small amounts of the protein presented in liver microsomes, this will not be easily accomplished.

Whether the trifluoroacetyled cytochromes P-450 may serve as antigens or haptenic recognition sites that participate in the manifestation of halothane-induced hepatic injury remains conjecture. We have shown that some of the covalently bound metabolite is present on the surface of the

hepatocytes, a part of which appears to be associated with cytochrome P-450 within the plasma membrane, but a part of which is also due to endoplasmic reticulum from dead cells that contaminate the preparations.

Mechanisms of heme destruction. It has been established that some substances inactivate cytochrome P-450 by causing the destruction of heme. In the past, we have demonstrated that a portion of the heme decomposition products become covalently bound to the proteins of cytochrome P-450. During the past year we have discovered that covalent binding of heme decomposition products occurs not only with carbon tetrachloride, but also with allyl isopropylacetamide, norethindrone, halothane, chloramphenicol, hydralazine, phenylhydrazine and 3,5-bis(ethoxycarbonyl)-4-ethyl-2,6-dimethyl 1,4-dihydropyridine. Some of the heme decomposition products are soluble in water. HPLC of these products suggests that they are either tripyrroles or tetrapyrroles. The production of heme decomposition products and their covalent binding may occur through free radical mechanism. In accord with this view, irradiation of methyl-heme by cobalt 59 gamma radiolysis results in the formation of products that had HPLC characteristics similar to those obtained with the heme decomposition products produced by the toxicants.

Such reactions in living cells may cause alterations in the tertiary structure of cytochromes P-450 and thereby convert them to forms that are more easily hydrolyzed by proteases in cells. Such reactions may thus lead to decreases in protein bands associated with cytochromes P-450 *in vivo*.

Compounds that accelerate superoxide and hydrogen peroxide formation. Several foreign compounds are known to be reduced by various enzymes in cells to free radicals that undergo autooxidation to form superoxide anion, which in turn decomposes to hydrogen peroxide. In the presence of metallic ions, superoxide and hydrogen peroxide may also form hydroxyl radicals. Many workers have suggested oxygen species may cause cellular damage. But most of the evidence is based on cell free systems that lack the presence of "protective" enzymes, such as superoxide dismutase, glutathione peroxidase and catalase normally present in cells. It is not clear, therefore, whether toxicities caused by such redox cycling of foreign compounds within cells is due to the superoxide anion and hydrogen peroxide *per se*, to the free radical of the foreign compound, or to the cascade of events that occur as a result of the redox cycling, including changes in the redox potential of endogenous substances such as NADH, NADPH and glutathione.

In recent years, we have studied the mechanisms by which adriamycin and daunomycin causes damage to cardiomyocytes, but despite the many claims that the cardiomyopathy evoked by these drugs is caused by superoxide and hydrogen peroxide, we were unable to obtain any evidence that would confirm this view.

During the past year, we have shifted our focus to the study of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which causes a Parkinson-like syndrome in humans and primates. It is now believed that the mechanism by which toxicity occurs is through the conversion of MPTP by monoamine oxidase B to 1-methyl-4phenyl pyridinum ion (MPPP+), which undergoes redox cycling with the formation of superoxide and hydrogen peroxide. Although MPTP is not known to cause hepatotoxicity *in vivo*, we nevertheless believe that studies with

cultured hepatocytes might provide valuable clues to some aspects of the mechanism of toxicity. Accordingly, we have established that MPTP in rat hepatocytes is converted to MPP+ and causes cell death. Moreover, deprenyl, an inhibitor of monoamine oxidase B, inhibited the formation of MPP+ and delayed the onset of toxicity. We have further established that purified cytochrome P-450 reductase under aerobic conditions catalyzes the redox cycling of MPP+ with the formation of superoxide.

Mechanisms of Mast Cell Activation and Degranulation

The abundance of mast cells in blood vessels, heart and airways makes these sites vulnerable to the action of histamine and other diverse inflammatory mediators that are released from these cells through the action of IgE-directed antigens. Although the mast cell has become a primary model for studies of the mechanism of Ca^{2+}-dependent secretion, our interest in studying such a mechanism is based on the expectation that therapy directed towards suppression of secretion should be more effective, and probably more specific, than that based on antagonism of all mediators once they are released. An exciting development in the last few years is the recognition that a wide variety of receptors, which mediate Ca^{2+}-dependent responses, may be coupled through a GTP binding protein to phospholipase C. This enzyme catalyzes the rapid breakdown of membrane inositol phospholipids to yield inositol 1,4,5-trisphosphate ($I(1,4,5)P_3$) and other inositol phosphates as well as diacyl glycerol (DAG). $I(1,4,5)P_3$ has been shown to induce the release of Ca^{2+} ions from intracellular Ca^{2+} stores and thereby to promote transient increases in levels of Ca^{2+} in the cytosol, whereas DAG activates protein kinase C - a reaction that is dependent on the increase in cytosol Ca^{2+}. Both reactions are thought to provide synergistic signals within the cell. Beyond this little is known of the mechanism by which these signals are translated into the ultimate cellular response, although as with the adenylate cyclase-coupled systems the signals result in the phosphorylation of distinct proteins. It is now apparent from our work that antigen-induced secretion of histamine from mast cells and blood basophils is also associated with rapid breakdown of inositol phospholipids, an increase in cytosol Ca^{2+} and activation of kinase C.

Our original studies, which were initiated in the Department of Biochemistry, University of Cambridge (England), showed that antigen-induced secretion from a basophil tumor analog, the 2H3 cell, was associated with rapid hydrolysis of membrane inositol phospholipids and a 10 to 12 fold increase in concentration of cytosol Ca^{2+} ($[Ca^{2+}]_i$). The studies demonstrated also that the rise in $[Ca^{2+}]_i$ was an obligatory signal for secretion. In subsequent studies in our Laboratory (see last year's report), the stimulated hydrolysis of the inositol phospholipids was shown to be a direct consequence of aggregation of receptors for IgE on the plasma membrane. Furthermore, any enhancement (e.g. by addition of heavy water) or suppression (e.g. by raising or lowering temperature from 37° or addition of lipophilic agents) in the rate of hydrolysis, by whatever mechanism, resulted in analogous changes in the intensity of the Ca^{2+} signal. We obtained no evidence, however, that the Ca^{2+} signal was caused by release of $I(1,4,5)P_3$ and mobilization of intracellular Ca^{2+} ions, as others have demonstrated in a variety of permeabilized cells.

This year we have improved the resolution of our analytical procedures for the assay of inositol phosphates by use of high pressure liquid chromatography. These procedures revealed 6 inositol phosphate metabolites (mono-,bis-,tris-, tetra-, penta and hexaphosphate or IP_1, IP_2, IP_3,IP_4,IP_5 and IP_6) and multiple isomers of IP_1 and IP_2. The IP_3 fraction consisted of inositol (1,3,4) trisphosphate with barely detectable amounts of the (1,4,5) trisphosphate derivative. Of all these metabolites, IP_4 (tentatively identified as inositol (1,3,4,5) tetrakisphosphate) was best correlated with the Ca^{2+} signal. IP_6 and, to a lesser extent, IP_5 declined in levels during antigen stimulation.

The above findings are significant for two reasons. One is that 2H3 cells mobilize little or no intracellular Ca^{2+}. The Ca^{2+} signal is generated almost solely by influx of Ca^{2+} ions across the plasma membrane. The barely detectable amounts of inositol (1,4,5) trisphosphate might thus account for the inability of 2H3 cells to mobilize intracellular Ca^{2+} ions. The other is that the apparent correlation between the Ca^{2+} signal levels of IP_4 raises the possibility that this metabolite mediates the transfer of Ca^{2+} ions across the plasma membrane. This possibility will be tested directly by micropatch techniques with 2H3 cell plasma membranes when sufficient amounts of the metabolite have been collected. As indicated by studies with free cell extracts, the 2H3 cells contain a highly active kinase that rapidly converts inositol (1,4,5) trisphosphate to the tetrakisphosphate. The cells also contain the various phosphatases that degrade the tetrakisphosphate to inositol (1,3,4) trisphosphate, thence to lower phosphorylated derivatives and inositol.

The close association between aggregation of IgE receptors and the hydrolysis of the membrane inositol phospholipids was convincingly validated when IgE-receptor aggregates where disrupted through displacement of antigen (dinitrophenol conjugated with BSA) from receptor-bound IgE with a monovalent ligand (dinitrophenol lysine). This maneuver resulted in immediate abrogation of hydrolysis, the Ca^{2+} signal and degranulation. We have used these three responses to analyze other aspects of the degranulation process. For example, we find that the cells possess far more receptors and capacity for generation of intracellular signals (i.e. hydrolysis of inositol phospholipids and increase in $[Ca^{2+}]_i$) than are required for maximal secretory response. Consequently, when large concentrations of antigen are used "desensitization" of the cells is apparent from the decay in stimulatory signals well before the rate of histamine secretion declines. The stimulatory signals are highly dependent on intracellular ATP. They are, also, readily perturbed by lipophilic drugs and solvents or small changes in temperature. The requirement for synergistic signals is evident from studies with Ca^{2+} ionophores and activators of protein kinase C. Large increases in cytosol Ca^{2+} can be induced by low concentrations (< 100 nM) of ionophore (A23187) without causing secretion. Activators of protein kinase C, phorbol myristate acetate (PMA) and oleoylacetylglycerol, elicit neither stimulatory nor secretory responses, but together with low concentrations of the ionophores they induce secretion. High concentrations of the ionophore (200-1000 nM) cause secretion, but only as a consequence of breakdown of inositol phospholipids, which in this situation is secondary to the large increases in cytosol Ca^{2+}.

Analogous studies in different clones of the 2H3 cell and another mast cell line, the PT18 cell, have revealed marked differences between cells in the pattern of inositol phospholipid breakdown upon cell stimulation. The PT18 cell, when stimulated with antigen or oligomers, shows rapid rise in cytosolic Ca^{2+} concentration, even in the absence of external Ca^{2+} presumably by mobilizing Ca^{2+} from internal stores. Unlike the 2H3 cell, however, the PT18 cells produce detectable concentrations of $I(1,4,5)P_3$, which is consistent with the view that this IP_3 mediates the release of Ca^{++} from intracellular stores. Interestingly some clones of 2H3 cells show no stimulatory or secretory responses to antigen but possess a normal complement of IgE receptors and phospholipase C activity. Furthermore the cells can be activated by simultaneous exposure to ionophore and activators of protein kinase C. Unlike 2H3 cells, however, the defective clones cannot be stimulated by activators of the membrane GTP coupling proteins. Our suspicion that these clones lack such proteins will be investigated further with antibodies directed towards subunits of the G-proteins. Our ultimate goal would be to restore the responsiveness of the clones by gene-mediated transfer of the G-proteins.

Biochemistry and Kinetics of Drug Metabolism

Purification of isozymes of cytochrome P-450. During the past two years, we have isolated from liver microsomes of Sprague-Dawley rats, 16 different polypolypeptides that have spectral chracteristics of cytochrome P-450 (7 from untreated male rats, 4 from male and female rats treated with dexamethazone, 2 from rats treated with phenobarbital and 3 from rats treated with methylcholanthrene). Many of the isozymes have been previously isolated by others, but 5 appear to differ from those previously isolated by others (UT-12, Dex 1, Dex 2, Dex III and either P-450 C1 or P-450 C2). Polyclonal antibodies against several of the isozymes have been prepared. Based on their immunological properties, the isozymes were classified acording to 5 types: Type 1, UT-2 and UT-II, type 2, UT-3, UT-4, UT-5, Dex II and female Dex II; type 3, Dex I and Dex III; type 4, UT-7; type 5, UT-12. Dex III, which was purified according to its testosterone 6β-hydroxylase activity, appears to be unusually unstable. Thus, its substrate specificity remains unknown. Nevertheless, antibodies against it completely inhibited the formation of 6β-hydroxytestosterone in liver microsomes from untreated rats and from rats treated with dexamethazone.

The antibody against UT-2 was especially useful. In collaboration with Dr. Frank Gunzalez (NCI), we found that UT-2 was identical to P-450a previously isolated by the Roche group and were able to elucidate the complete amino acid sequence from UT-2-cDNA. The isozyme catalyzes the 7α-hydroxylation of testosterone. The messenger RNA of UT-2 can be induced by phenobarbital, dexamethazone, clofibrate and 3-methylcholanthrene.

A new metabolite of testosterone. During the course of purification of the various isoyzmes of cytochrome P-450, we noted that testosterone was converted to a metabolite having very unusual spectral characteristics. The metabolite has subsequently been identified as 17β-hydroxy-4,6-androstadiene-

3-one. Thus its formation is equivalent to a dehydrogenation, a kind of reaction that rarely is catalyzed by cytochrome P-450. The mechanism by which the reaction occurs remains to be elucidated, but it may occur either by a double hydrogen abstraction mechanism, or through the formation of a carbonium ion followed by the release of a hydrogen ion.

Naphthalene metabolism. Buckpit et al. have discovered that naphthalene causes a lesion in the pulmonary bronchiolar epithelium of mice, but not in that of rats or hamsters, and offered evidence suggesting that the lesion was caused by a chemically reactive metabolite. Kinetic analysis of their data suggested the possibility that different isozymes of cytochrome P-450 may convert naphthalene to different stereoisomers of naphthalene-1,2-oxide which may have different toxicological properties. As a first step in evaluating this possibility, we have isolated two isozymes of cytochrome P-450 from liver microsomes from untreated mice that catalyze the conversion of naphthalene to α-naphthol, and raised polyclonal antibodies against them. Unfortunately, in their impure state, the antibodies cross react and thus they require further purification.

Kinetic Studies of Formation of Chemically Reactive Metabolites

Differential equations for pharmacokinetic models representing the formation and elimination of chemically reactive metabolites are frequently so complex that they cannot be integrated to provide an explicit solution. Under certain circumstances, however, the equations may be simplified to forms that may be integrated. During the past year we have derived an equation that under specified conditions may be useful in relating the formation of chemically reactive metabolites in organs to the depletion and repletion of endogenous nucleophiles. The specified conditions are that virtually all of the reactive metabolite formed in the organ combines with the endogenous nucleophile by a second order reaction to form the conjugate and that all other processes follow first-order-kinetics. We have applied the equation to the depletion and repletion of glutathione in liver after the subcutaneous administration of subtoxic doses of acetaminophen to hamsters. With the data, we can calculate a clearance for the formation of the chemically reactive metabolite in liver, which may be used to estimate the in vivo activity of the enzymes that catalyzes the formation of the metabolite within the organ. The kinetic parameters thus obtained may be used to simulate events that may occur following the administration of toxic doss of the drug.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00617-10 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

The mechanism of carrageenan induced inflammation in rat

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

Laboratory of Chemical Pharmacology

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Upon incubation with pharmacologically relevant concentrations of carrageenan (100 µg/ml), isolated pleural macrophages previously labeled with [^{14}C]arachidonic acid released 2.5 times the radioactivity of control cells. The main components of the released radioactive materials were arachidonic acid and, to a much lesser extent, PGE2 and leukotriene C4, both of which are known to be vasoactive. In the absence of carrageenan, indomethacin (20 µM) augmented the release of arachidonic acid but suppressed the formation of PGE2, implying that the cyclooxygenase in resting pleural macrophages is capable of converting the released arachidonic acid to PGE2. That carrageenan can stimulate release of arachidonic acid from pleural macrophages indicates that activation of phospholipase A2 is involved in the carcageenan action and that PGE2 and LTC4 may mediate the inflammatory response to carrageenan in vivo. Furthermore, our findings suggest that the macrophages may play a role in the initiation of the early event in the inflammatory response.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00937-04 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Biochemical mechanisms of mast cell degranulation: PI breakdown and Ca signal

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Dr. Nicholas Dean, NCI
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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.)*

Receptor-mediated hydrolysis of inositol phospholipids in 2H3 cells, unlike that in other secretory cells, is dependent on extracellular Ca2+. Several lines of evidence, however, suggest that this hydrolysis may mediate the influx of Ca^{2+} across the plasma membrane. Studies with covalently cross-linked oligomers of IgE, for example, have shown that such hydrolysis and the increase in cytosol $[Ca^{2+}]_i$ are closely correlated with the number of IgE receptors aggregated and that with saturating concentrations of oligomer the generation of these early signals exceeds that required for maximal secretion of histamine. Excess signalling capacity was evident also in studies with monoclonal anti-DNP IgE and DNP24 -BSA (1 mole bovine serum albumin conjugated with 24 moles of dinitrophenol). Furthermore, the hydrolysis of phospholipids appeared to be a consequence of receptor aggregation and not of Ca2+ mobilization. For example, disaggregation of receptors by displacement of DNP24BSA with DNP-lysine resulted in abrupt cessation of hydrolysis and secretion. Antigen stimulated hydrolysis of phospholipids, but not secretion, became increasingly less dependent on external Ca2+ with time. Finally, analysis of antigen stimulated cells by HPLC revealed multiple isomers of the insoitol phosphates but correlations in the pattern of hydrolysis and the increase in [Ca2+]i were established.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00962-04 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)*

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Dr. Tamiko Takemura and Dr. V.J. Ferrans, Ultrastructure Section, Pathology Branch,NHLBI; Sandra Jelenich, Anesthesiology Section, Clinical Center, NIH; James Neuberger and John Kenna, King's College Hospital, London, England.

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Laboratory of Chemical Pharmacology

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Enzyme Drug Interaction

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.9	0.4

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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.)*

We have previously reported that halothane's reactive oxidative metabolite, trifluoroacetyl halide (CF3COX), forms trifluoroacetylated (TFA) covalent adducts within and on the outer surface of hepatocytes, when halothane is administered to rats or humans. It was additionally found that certain individuals, who have had halothane-induced hepatotoxicity, possesses anti-TFA antibodies in their sera. This finding suggested that the toxicity may have been initiated by a sensitization aganist TFA cellular proteins. In order to investigate this idea, we began elucidating the identify of the TFA adducts. Last year we reported that the major TFA adduct within the cells of phenobarbital treated rats that were administered halothane was identified as a 54 kD form of microsomal cytochrome P-450. We have now developed a general immunoaffinity purification procedure for isolating TFA proteins and have applied it to purify the TFA proteins found in the liver microsomal fraction of normal rats treated with halothane. One major (Mr 59,000) and two minor (Mr 76,000 and 92,000) TFA protein fractions were isolated by this method. Preliminary studies suggest that the 59 kD protein maybe a form(s) of cytochrome P-450 that has not been previously identified. The physiological function of this enzyme as well as the potential role of all three TFA proteins as immunogens in halothane-induced hepatotoxicity is currently being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00967-04 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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. Helen W. Davies	Staff Fellow	LCP	NHLBI
Others:			
Lance R. Pohl	Section Chief	LCP	NHLBI
Kaori Maeda	Vist. Fellow	LCP	NHLBI
John W. George	Chemist	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Pharmacological Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.8	0.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have previously reported that the suicide inactivation of cytochrome P-450 by CCl4 is caused by a novel pathway which involves the irreversible binding of products of the heme prosthetic group to the protein moiety of the enzyme. It was initially believed that this mechanism of enzyme inactivation was solely mediated by lipid hydroperoxides produced by carbon tetrachloride metabolites. During the last year, however, we have discovered that CCl4 as well as several structurally diverse drugs and environmental chemicals can destroy cytochrome P-450 and produce heme-derived protein adducts independent of lipid hydroperoxides. This pathway appears to involve the initial reductive or oxidative metabolism of the chemical by cytochrome P-450 into a radical intermediate, which subsequently either activates the heme or protein moiety of the enzyme leading ultimately to bound heme-derived products. Not only is the enzyme irreversibly inactivated by this process, but it also appears to be "tagged" for catabolism by cellular proteases. Therefore, a general pathway of chemically-induced irreversible inactivation and degradation of cytochrome P-450 has been discovered, which appears to have importance in the regulation of the activity of this ubiquitous family of enzymes and ultimately in the design of safer and more specifically acting drugs and environmental agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00973-02 LCP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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.: Jose R. Cunha-Melo	Guest Researcher	LCP	NHLBI
Others:			
Michael A. Beaven	Deputy Chief	LCP	NHLBI
Katazutaka Maeyama	Visiting Fellow	LCP	NHLBI

COOPERATING UNITS *(if any)*

T.R. Hesketh and J.C. Metcalfe, Dept. Biochemistry, Univ. of Cambridge, Cambridge, England

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The aim of this study was to determine whether activation of protein kinase C reinforced or modulated the Ca2+ signal produced in response to antigen on IgE-primed 2H3 cells. When the concentrations of antigen or the Ca2+ ionophore A23187 were such that both elicited the same increase in cytosol Ca2+ concentration ([Ca2+]i), antigen but not A23187 induced secretion. A23187 and the phorbol ester 12-o-tetradecanoyl phorbol 13- acetate (TPA) together stimulated histamine release, whereas TPA alone had no effect. Both the Ca2+ signal and activation of protein kinase C appear, therefore, to be obligatory for secretion. In antigen stimulated cells, however, TPA blocked the antigen-induced [Ca2+]i responses and the release of inositol phosphates, but had little effect on histamine release. Thus the possibility exists that a cryptic signal is generated by antigen independently of protein kinase C activation, the [Ca2+]i response, or the release of inositol phosphates. Suppression of the [Ca2+]i signal and the release of inositol phosphates occur with low concentration of TPA (1-20 nM). Further studies suggest that this suppression results from modification of membrane G-protein that allow coupling of IgE receptors to the catalytic unit, phospholipase C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00974-02 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

ATP dependency of signal generation and secretion in rat basophil leukemic cells

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

PI:	Theresa N. Lo	Research Chemist	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
1.4	0.8	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.)*

Rat leukemic basophil (2H3) cell line was stimulated to secrete histamine either with calcium-specific ionophores or by aggregation of plasma membrane receptors for IgE. The ionophore, A23187, at concentrations (< 100 nM) well below those stimulating secretion elicited large increases in [Ca2+]i and at higher concentrations (200-1000nM) stimulated hydrolysis of membrane radiolabeled inositol phospholipids as well. The hydrolysis was dependent on the concentration of ionophore and presence of external Ca2+ and was correlated with the secretory response. The results pointed to generation of diacylglycerol rather than of inositol phosphates as a critical factor in the action of A23187. When secretory responses were plotted as a function of percent hydrolysis of inositol phospholipid, the curve was shifted leftwards in the presence of the phorbol ester, TPA, which, like diacylglycerol, is an activator of protein kinase C. Paradoxically, the increases in $[Ca^{2+}]_i$ in response to both antigen and A23187 were highly dependent on intracellular ATP levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00975-02 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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 Investigators:			
Michael A. Beaven	Deputy Chief	LCP	NHLBI
Elizabeth WoldeMussie	Staff Fellow	LCP	NHLBI
Jose R. Cunha-Melo	Guest Researcher	LCP	NHLBI

COOPERATING UNITS *(if any)*

Dr. Reuben Sirganian, National Institute of Dental Research

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

NHLBI, 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.)*

Antigen mediated histamine release from cultures of RBL-2H3 cells is associated with increase in cytosol Ca++ levels (Ca signal) and substantial hydrolysis of membrane inositol phospholipids. Several clones of the RBL-2H3 cell line showed varied responses to antigen that ranged in extent from undetectable(BUDR 1A3, 2B1 and 1B3) to about 80% of those in 2H3 cells (TG 2B6). The initial rate of response in the partially responsive clones was similar to that of 2H3 cells but the maximal respones were blunted. In most of these clones, as in the 2H3 cells, the Ca signal (as measured by quin 2 fluorescence) and hydrolysis of the phospholipids were correlated. However, TG 1B3, which showed very little Ca signal, still showed modest phospholipid hydrolysis and histamine release. Phospholipase C activity towards all inositol phospholipids was present in extracts and membranes of all the clones tested. Moreover, activity in the nonresponsive clones was 3 to 5 times higher than that in 2H3 cells. Studies with phorbol ester and Ca2+ ionophore also indicated the presence of protein kinase C activity in the 1A3 and 1B3 clones. These data point to no obvious defect in the genetic expression of enzymes involved in the inositol phospholipid cascade system in the nonresponsive clones. Our preliminary indications are that these clones have defective coupling of IgE receptors to phospholipase C through G-protein(s).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00976-02 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Drug-induced peroxisomal proliferation

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

P.I.: Y. Singh	Vist. Fellow	LCP	NHLBI
Others:			
G. Krishna	Chief, Section	LCP	NHLBI
C.T. Liu	Chemist		

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Tissue Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md. 20892

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

Valproic acid increased markedly both carnitine acetyltransferase (CAT) and carnitine palmyitoyltransferase (CPT) in a dose dependent fashion in rat hepatocytes. A maximal increase of 800% of CAT and 200% of CPT was induced by 3 mM valproic acid in 72 h. Even though valproic acid increased the peroxisomal marker enzyme there was no increase in the number of peroxisomes in cells as examined by electron microscopy. However, there was a marked increase in the number of mitochondria which could account for the increase in CAT and CPT. Another peroxisomal marker, namely a 80 kD protein, was not increased by valproic acid indicating that valproic acid is not an inducer of peroxisomes. Valproic acid did not change cytochrome P-450 but increased markedly liver cell GSH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HL 00981-02 LCP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation and interaction of cytochromes P-450 in liver microsomes

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

Kiyoshi Nagata	Visiting Fellow	LCP	NHLBI
Other Investigators:			
James R. Gillette	Chief	LCP	NHLBI
Henry A. Sasame	Chemist	LCP	NHLBI

COOPERATING UNITS (if any)

Frank Gonzalez - Staff Fellow, National Cancer Institute

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Enzyme Drug Interaction

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

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

We have purified seven cytochrome P-450 isozymes (UT-2,UT-3,Ut-4,UT-5, UT-7, UT-11 and UT-12) from male untreated rats and five cytochrome P-450 isozymes (Dex I, Dex II, Dex III, Dex IV, and female Dex II) from male and female rats, treated with dexamethazone. UT-5, however, appears to be identical to Dex IV.

These eleven isozymes are different isozymes judging from many characteristics. We have previously reported another five different isozymes purified from 3-methylcholanthrene and phenobarbital-treated rat liver. In all we have purified sixteen different isozymes from rat liver. Four of the eleven isozymes represent a new group of isozymes (UT-12,Dex I, Dex II and Dex III). One isozyme (Dex III) possesses catalytic activity for testosterone 6 beta-hydroxylation. Purified Dex III, however, is easily denatured, but anti-rabbit antibody raised against it inhibited testosterone 6 beta-hydroxylation.

2) We determined the coding nucleotide sequence of the mRNA for cytochrome P-450 UT-2 of rat liver by sequence analysis of cloned cDNAs. The amino acid composition of the deduced sequence also agrees well with that determined from the purified protein. Computer-aided analysis was carried out to compare the complete primary structure of another species of cytochrome P-450. The influence of age, sex and inducers on the expression of the isozymes was evaluated by hybrization of mRNA, catalytic activity and immunochemical reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00983-01 LCP

PERIOD COVERED: October 1, 1985 through September 30, 1986

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.: Y. Singh	Vist. Fellow	LCP	NHLBI
Other:			
G. Krishna	Chief, Section	LCP	NHLBI

COOPERATING UNITS (if any)

Dr. B.K. Sinha, Clinical Oncology Branch, NCI, NIH.

LAB/BRANCH: Laboratory of Chemical Pharmacology

SECTION: Drug Tissue Interaction

INSTITUTE AND LOCATION: NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced dose and time dependent cell death in hepatocytes in culture. MPTP was converted to MPP+ (1-methyl-4-phenylpyridinium ion) before cell death occurred. MPP+ when added to the medium also caused cell death. High doses (1 mM) of both compounds caused cell death within 4-6 h while low doses (100-200 μM) required 24-48 h to induce death. MPTP was taken by the cells and converted to MPP+ by a specific monoamine oxidase (MAO-B). The conversion to MPP+ was markedly reduced by treatment of cells with 10 μM deprenyl which is a specific MAO-B inhibitor. With the marked reduction in MPP+ production, the early cell death induced by MPTP was completely prevented.

MPTP and MPP+ induced a number of cytochemical changes, such as shape change, cell aggregation and cell blebbing, which was observed even before massive LDH leakage. MPTP induced glutathione leakage but only after LDH leakage had occurred. The reason for the unusual delay in glutathione leakage is obscure.

MPP+ when incubated with NADPH and NADPH cytochrome P-450 reductase under anaerobic conditions did not produce any radical. However, when incubated in air, MPP+ produced both superoxide and hydroxyl radicals. Whether these reactive oxygen radicals are the cause of cell death remains to be elucidated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 00984-01 LCP

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A unique testosterone metabolite: 17β-hydroxy 4,6-androstadiene-3-one

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

Henry A. Sasame	Chemist	LCP	NHLBI
Other Investigators:			
Kiyoshi Nagata	Vist. Fellow	LCP	NHLBI
James R. Gillette	Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*
Dr.William Trager, Univ. of Washington
Dr. Frank Gonzalez, Staff Fellow, NCI

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Enzyme Drug Interaction

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

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

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

Immunochemical and biochemical evidence indicates that an isozyme of cytochrome P-450 in rat liver microsomes catalyzes the formation of a previously unidentified metabolite, 17β-hydroxy 4,6-androstadiene-3-one. The identify of the metaoblite was confirmed by HPLC, uv spectroscopy and thermospray, mass spectrometry. The metabolite appears to be formed by isozymes of cytochrome P-450 that catalyze the 6α-hydroxylation of testosterone. When animals were treated with various inducers the ratios of 6-hydroxytestosterone over 17β-hydroxy 4,6-androstadiene-3-one remained constant. Furthermore, a known 6β-hydroxylase inhibitor, 16α-methylprogesterone, inhibited the formation of the metabolite as well as 6β-hydroxytestosterone. An antibody raised against a P-450 isozyme isolated from liver microsomes of rats pretreated with dexamethasone also inhibited the formation of both the metabolite and 6β-hydroxytestosterone to the same extent.

PHS 6040 (Rev. 1/84) GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00985-01 LCP

PERIOD COVERED

October 1, 1985 through September 30, 1986

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 Investigator:			
James R. Gillette	Chief	LCP	NHLBI
Kiyoshi Nagata	Vist.Fellow	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

0.6 0.6

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NIH, NHLBI-IR-LCP, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Three isozymes of cytochrome P-450 have been isolated from liver microsomes of untreated mice. Two of the isozymes catalyze the conversion of naphthalene to alpha-naphthol. Antisera have been produced in rabbits against both isozymes, but in their impure state each antiserum reacts with both isozymes. Both antisera, however, inhibit the metabolism of naphthalene by mouse lung microsomes. They thus may be useful in helping to determine whether the two isozymes form different stereoisomers of naphthalene-1,2-oxide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00986-01 LCP

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Pharmacokinetic models for the study of reactive metabolites

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

P.I. Ruth Chen	Staff Fellow	LCP	NHLBI
Other Investigator: James R. Gillette	Chief	LCP	NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Enzyme Drug Interaction

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Pharmacokinetic models of the metabolism of foreign compounds may be used to identify relevant parameters that govern the time course of chemically reactive metabolites at putative action sites and to aid in the development of experiments by which these parameters may be estimated. During the past year pharmacokinetic equations have been derived to describe the formation and elimination of the chemically reactive metabolite of acetaminophen in liver. The equations include not only the elimination of acetaminophen by various pathways, but also the effect of the reactive metabolite on the concentration of glutathione in liver. These equations revealed ways of estimating the hepatic clearance for the formation of the reactive metabolite in living animals. They thus illustrate a way of comparing the in vitro and in vivo activities of the enzymes that catalyze the formation of certain kinds of chemically reactive metabolites, which would be difficult, if not impossible, to obtain by direct experimentation

ANNUAL REPORT OF THE
LABORATORY OF CHEMISTRY
SECTIONS ON CHEMICAL STRUCTURE AND STRUCTURAL
NUCLEAR MAGNETIC RESONANCE
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

October 1, 1985, through September 30, 1986

The Laboratory now consists of the above two sections concerned with isolation, elucidating the structure and studying the properties of biologically important compounds. All of Dr. John Pisano's group have either relocated or left government service except for Dr. Hiroshi Nonoguchi who is currently working on peptide mediators in the regulation of renal tubular cyclic nucleotide metabolism and water and electrolyte transport under Dr. V. Manganiello (LCN,NHLBI).

Synthesis is a continued interest in the Laboratory under Dr. S. Miller who has first elucidated the structure and then synthesized N(5)-(1-carboxyethyl)ornithine, a novel amino acid from S. lactes (J. Thompson, LMI, NIDR). He has also successfully attached to a resin of the alcohol dehydrogenase inhibitor of 4-(3-aminopropyl) pyrazole (synthesized last year) (P. Rathnagini, LG, NIAAA).

The two new NMR spectrometers (Varian XL-200 and XL-300) installed last year are working well and provide high quality data. The older Nicolet 360 mHz system has suffered a breakdown in the magnet requiring its return to the factory. The GE 4.7 tesla magnet system located in Bldg. 1 is in full operation under Dr. R. Balaban (LCEM, NHLBI) and at this time involves little attention from our laboratory.

NMR studies by Ferretti and his coworkers have resulted in a useful technique to suppress strong interfering solvent signals (e.g. H_2O), often a problem in NMR examination of biological systems. Spectra of peptides have been obtained at 5-10 mM concentrations in water. Two new 2D proton methods have been developed, one providing connectivity information on spins (protons) coupled via scalar interactions, the other providing information on spatial proximity via cross relaxation. The latter technique useful especially for large molecules. NMR combined with laser methods (S. Strauss (FDA), I. Levin (LCP:NIADDK)) have been used to study phase transition properties of phospolipid bilayers. Preliminary results indicate addition of small amounts of exogenous lipids do not strongly affect ordering.

An extensive NMR study of peptide lactones related to actinomycin D J. Ferretti (A.B. Mauger (Washington Hospital Center)) has revealed the presence of two conformations, depending on solvent. Energy calculations by J. Silverton (using new programs operating on the IBM PC) on the peptide conformations agree well with the NMR results, lending credence to the latter.

Other NMR studies by Ferretti, using new pulse methods and nuclear Overhauser effects, have allowed the complete assignment of protons and many internuclear distances in several linear peptides (Substance P, 1-10 1-14 fragments of ACTH, oxytocin, vasopressin and bovine neurophysin II). The results show that linear peptides are highly flexible in water. Since knowledge of accuracy and precision in measurement of peaks areas are critical in correctly interpreting such data, Ferretti, G. Weiss (PSL,DCRT) and A. Byrd (FDA) continue to explore the optimum procedures for this estimation. One immediate result is that assumption

of uncorrelated spectrometer noise is not always valid.

Highet and his coworkers, using 2D NMR Overhauser effects have confirmed their earlier proposed structure for a metabolite of Alterneria, positively identifying for the first time a naturally occurring dihydroanthracene structure. The structures of all four stereoisomers of a synthetic 3,5-dialkylpyrrolizidine one of which is found in the ant C. antarcticus, have been elucidated by NMR and the tribisulfite addition compound of phyloroglucinol continues to be studied. Although it still resists isolation it is clear from NMR it is the all cis isomer in solution.

E. Sokoloski with G. Krishna (LP, NHLBI) has developed a P-31 NMR method to monitor B. pertussis adenylate cyclase mediated conversion of ATP to CAMP and detected variations in rate with calmodulin and mellitin. Several secondary reactions, not ordinarily seen in regular chemical rate analysis, were observed. With E. Obarzanek (LCS,NIMH) he has also developed an infrared method for analyzing HOD used in diagnosis of patients suffering from anorexia nervosa and bulemia.

In X-ray diffraction, J. Silverton has collaborated with IBM and DCRT in evaluation of a new vector processor, expected to greatly increase computation speed on such programs as XTAL used in single crystal analysis. The results have cast new light on the programs themselves and after revising certain program areas, speed up by a factor of two was achieved. Silverton has also examined and applied molecular mechanics programs on the IBM PC-AT and further plans to combine other laboratory PCs into a local area network for greater efficiency with less duplication of peripherals such as printers. In single crystal work, he has solved the structures of racemic colchicine, the optically active 2-acetyl derivative, a hexahydropyridine, a bridged nicotine, triglycine, disinomenine, camphor chlorosulfenone and the most potent known carcinogen (a diol epoxide). Work is in progress on a large nucleotide, small to medium ring compounds, a synthetic intermediate and several drugs used in AIDS therapy.

H. Lloyd and H. Fales have examined compounds related to brunfelsamidine (pyrrole-3-carboxamidine) and found that its N-methyl derivative, but not other closely related compounds, is fully active. They are also examining an anti-leukemia factor from A. belladonna and discovered two new alkaloids whose structures are being studied. They have also thoroughly characterized commercial digitonin, separating it into its components by HPLC and CCCC, and identifying the sapogenins by Cf-252 PDMS, chemical degradation and sugar analysis. One new glycoside was discovered whose structure is proposed to be desglucotigonin. Lloyd has examined the allergen-producing S. terebenthifolius and found a new terpene, probably a bis-narengenin.

In mass spectrometry, the Cf-252 plasma desorption system continues to produce spectra on a wide assortment of compounds including inorganic, metalloorganic, peptide, carbohydrates, etc., brought to it from all over NIH and other institutions. It is operated by E. Sokoloski who runs about 7-21 samples daily. L. Pannell, LB-NIADDK) maintains a vital interest in the system and makes necessary program and hardware changes as well as collaborating with Fales in fundamental studies of the phenomena. A new digitizer and ion mirror has been ordered recently, which should both increase its resolution and allow study of ion fragmentation processes.

Fales, with J. Showell (NSF) has found that the spectra of mixtures analyzed by the system do not necessarily reflect their correct bulk stoichiometry since the technique detects only those molecules in the surface layer. Using this fact, a technique has been developed, allowing increased detection of volatiles by chemically reacting them with nonvolatile substrates directly on the sample holder. With J. DeBlas (SUNY Stoneybrook) a compound has been isolated from mammalian brain of untreated rats that is unequivocally desmethyl-diazepam, a known metabolite of diazepam having the same physiological activity. Its source is uncertain but the most obvious possibility, sample contamination, appears to be ruled out.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01002-12 CH

PERIOD COVERED

October 1, 1985, to September 30, 1986

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

Application of Nuclear Magnetic Resonance to Biochemical Systems

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

Edward A. Sokoloski Chemist CH NHLBI

COOPERATING UNITS *(if any)*

Dr. Gopal Krishna, Section on Drug Tissue Interaction, Laboratory of Pharmacology, NHLBI

Dr. Eva Obarzanek, Section on Biomedical Psychiatry, LCS:NIMH

LAB/BRANCH

Laboratory of Chemistry

SECTION

Nuclear Magnetic Resonance

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.)*

A P-31 Nuclear magnetic resonance technique was developed to monitor the reaction kinetics of the Bordetella pertussis adenylate cyclase conversion of adenosine triphosphate to 3'5' cyclic adenosine monophosphate and pyrophosphate. Simultaneous monitoring of the reaction by earlier methods and this method provide excellent correlation of reaction times. The NMR method allows hands-off monitoring of both reactants and products for extended periods. Calmodulin activator and mellitin inhibition were measured by the separation and NMR techniques. The use of infrared spectroscopy as a possible analytical tool to monitor changes in body composition of patients with anorexic and bulemic disorders is being explored. Early studies gave reproducible calibration curves, but patient samples have given some questionable results. Sources of error are being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-HL-01003-14 CH

PERIOD COVERED

October 1, 1985 to September 30, 1986

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)*

P.I.	H.M. Fales, Ph.D	Chief, Laboratory of Chemistry, NHLBI,CH
OTHER	Y.M. Yang	Visiting Fellow Shanghai, PRC)
	E. Sokoloski	Laboratory of Chemistry, NHLBI
	J. Showell, Ph.D	National Science Foundation

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Chemical Structure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	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 Cf-252 plasma desorption system has been redesigned for higher resolution and detection of neutrals and metastables. Surface phenomena leading to abnormal surface concentrations in mixtures have been detected with the method. Propylene oxide and ethylene oxide adducts of digitonin and cyclodextran have been examined successfully along with trehalosemycolates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01004-15 CH

PERIOD COVERED

October 1, 1985 to September 30, 1986

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: H. A. Lloyd Research Chemist CH NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Chemical Structure

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

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

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01005-15 CH

PERIOD COVERED

October 1, 1985, to September 30, 1986

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)*

J. V. Silverton Research Chemist CH NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Chemical Structure

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20205

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

Solid state and computational work of the Laboratory of Chemistry, NHLBI concerns structural and configurational studies of biologically-interesting compounds. Drug action, chemical synthesis and biological function of natural and synthetic drugs, peptides and nucleotides have been investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 01006-15 CH

PERIOD COVERED: October 1, 1985, through September 30, 1986

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

The Characterization of Natural Materials and Metabolic Products

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

P.I. Robert J. Highet	Research Chemist	CH NHLBI
OTHER: G. W. Perold, Ph.D.	Visiting Fellow, University of Witswatersrand Johannesburg, South Africa.	
I. V. Ekhato, Ph.D.	Visiting Fellow	

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Structural Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

NMR Studies have elucidated the structures of metabolites of the mold Alternaris alternans, alkaloids of the ant Chelaner antarcticus, synthetic fulgides, and the sodium bisulfite addition complex of phloroglucinol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 01027-04 CH

PERIOD COVERED

October 1, 1985, to September 30, 1986

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

Nuclear Magnetic Resonance Spectroscopy on Biologically Important Molecules

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

James A. Ferretti, PhD	Research Chemist	NHLBI CH
Donald G. Davis, PhD	Senior Staff Fellow	NHLBI CH
Kathleen S. Gallagher, MA	IPA Fellow	NHLBI CH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Structural Nuclear Magnetic Resonance Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Research involves the development and application of multiple pulse Fourier transform methods in Nuclear Magnetic Resonance Spectroscopy, including solvent suppression and two dimensional techniques. Applications include conformational properties of peptides and small proteins in solution, studies in the precision of the methodology, and physical properties of lipid bilayers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 01028-02 CH

PERIOD COVERED

October 1985 - September 30, 1986

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

Synthesis and Characterization of Bioactive Materials

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

Stephen P. Miller Research Chemist CH NHBLI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Chemical Structure

INSTITUTE AND LOCATION

NHLBI:NIH, Bethesda, MD. 20892

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

Bioactive compounds were studied by a combination of chemical synthesis and instrumental analysis.

A previously unknown amino acid, isolated from Streptococcus lactis, was studied. Its structure was determined to be N(5)-(1-carboxyethyl)-ornithine by NMR and GC/MS analysis. This was confirmed by chemical synthesis.

New synthetic approaches to alcohol dehydrogenase (ADH) inhibitors based upon 3-alkyl-tetrahydrothiophene sulfoxides and 4-alkyl-pyrazoles have been explored. These enzyme inhibitors are desired for use in purification of mammalian ADHs by affinity chromatography.

Studies continue on the mass spectral analysis of growth inhibitory saponins (MW 1000-1800) by Californium PDMS, and insect pheromone samples by microchemistry/EIMS.

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Annual Report of the Clinical Hematology Branch
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

The research of this Branch is directed toward understanding the underlying causes and developing effective treatment for major hematological disorders, primarily those affecting the red cell. Red cell disorders that produce significant morbidity and mortality include thalassemia, severe hemoglobinopathies of which sickle cell anemia is the most common, and the various syndromes of bone marrow failure.

Patients with either severe beta-thalassemia or sickle anemia could benefit from increased production of fetal hemoglobin. Fetal hemoglobin (HbF=α_2 γ_2) produced in utero, is replaced during the perinatal period with the adult type of hemoglobin (HbF=α_2 β_2). At the gene level, this switch reflects turn off of the gamma globin and turn on of the beta globin gene. If both beta genes are defective, the switch leads to the onset of disease. Earlier studies in the laboratory had shown that 5-azacytidine activates gamma globin genes (see individual project: "Pharmacological Manipulation of Fetal Hemoglobin Synthesis"). Although this drug, that affects DNA structure by inhibiting methylation, and others that act by perturbing erythroid progenitor and precursor differentiation, share the ability to stimulate HbF synthesis, major clinical benefit has not yet been demonstrated. Needed is a greater understanding of the mechanisms that regulate globin gene expression.

Several individual projects are pertinent to this objective. These include "Tissue and Developmental Specificity of Globin Promoters", "Identification of Cis and Trans-Acting Elements that Regulate Human Gamma Gene Expression", and Regulation of Hemoglobin Switching During Development: Characterization of the Human Gamma Globin Gene Promoter". We have learned that the globin sequences confer tissue and developmental specificity on reporter genes to which they are linked. A dissection of the promoter region has begun. One distal or "upstream" segment is clearly involved in developmentally specific gene expression. This segment includes sequences that have both positive and negative effects on promoter function. Point mutations within a small segment of this promoter region that increase fetal hemoglobin synthesis in vivo, have been shown to affect DNA conformation in vitro. A major objective in the future is to identify and characterize proteins that interact with these regulatory sequences.

The mechanisms of regulation of two other types of genes are also being defined. Trans-activation of immunoglobulin gene regulatory sequences has been achieved using cytoplasmic constituents present in differentiated immunoglobulin producing cells (see individual project: "Enhancer and Promoter Specificity of Immunoglobulin Genes"). The human dihydrofolate reductase gene is an example of a constitutively expressed gene that undergoes cell-cycle specific modulation to meet the needs for DNA synthesis. An analysis of its promoter structure in DNA and chromatin has revealed both distal and proximal regulatory elements and suggest the presence of multiple proteins (see individual

project: "Characterization of the Gene for Human Dihydrofolate Reductase").

Achievement of the differentiated state characteristic of hematopoietic precursors involves selective and coordinated expression of many genes. The cellular homologues of viral onocogenes apparently have important roles in cellular differentiation. One such gene, designated c-fms, encodes for a hematopoietic growth factor receptor. We have cloned a portion of the c-fms gene and compared its structure to the transforming retroviral homologue. Modifications at the C-terminal end appear to be involved in acquistion of transforming potential. This observation provides a model to investigate a potential role for this gene in human leukemias (see individual project: "Function of Proto-Oncogenes in Human Hematopoietic Cells").

Another strategy to define the role of certain genes in hematopoietic differentiation involves the introduction of sequences that generate "anti-sense" transcripts complementary to normal mRNA. Transcriptional units in which sequences complementary to the c-myc and c-fos proto-oncogene mRNA have been introduced into mouse fibroblasts. Induction of the anti-sense transcript inhibits cell growth. In addition, inhibition of cellular differentiation in an embryonal carcinoma cell line has also been observed. This approach offers the opportunity to dissect the role of these critical genes in hematopoietic differentiation.

Efficient and reproducible transfer of genes into hematopoietic cells may ultimately prove useful for genetic therapy and for modifying the genetic makeup of normal cells in vivo (see individual project: "Use of Viral Regulatory Sequences to Facilitate Gene Transfer and Analysis of Gene Function"). A retroviral vector has been constructed that contains an intact human globin gene. Transfer, expression, and normal regulation of this gene have been documented in mouse erythroleukemia cells. Production of human protein has also been observed. Analogous retroviral vectors can be used to modify the genetic makeup of primary hematopoietic cells. For example, we have shown that introduction of the v-abl gene into mice stem cells results in the generation of mast cell lines (see individual project: "The effect of v-abl and IL-3 Genes on Hematopoietic Stem Cell Differentiation"). A vector containing the normal regulator, IL-3, results in vector independent hematopoietic colony formation and the generation of analogous cell lines. Currently we are investigating the effects of IL-3 expression in stem and progenitor cells on normal hematopoietic differentiation and in collaborative studies, will attempt to correct certain genetic anemias in mice. One limitation to the retroviral approach to gene transfer is the possibility that all cells of the marrow are infected. Targeting to specific cells might be a great advantage. We have designed experiments to determine whether the polypetide sequences of normal hematopoietic regulators can be incorporated into retroviral envelopes leading to targeting of the vectors specifically to cells bearing receptors to these hemopoietins (see individual project: "Modification of Retroviral Targeting via Hybrid Envelope Proteins).

A major focus of clinical interest of the laboratory has been plastic anemia. Previous studies have indicated an immunological mechanism for bone marrow suppression in a large proportion of patients with this disease. Our own studies have shown that many patients with aplastic anemia have an abnormal number of suppressor T-cells, indentifiable by flow microfluorometry, and that these cells produce gamma interferon, a potent suppressor of hematopoiesis in vitro and in vivo. The production of this lymphokine by a specific lymphokine population almost certainly provides the explanation for the large number of previously published experiments showing a suppressive effect by cells or sera of patients with aplastic anemia in tissue culture. Our current studies have tested the hypothesis that these immunologic abnormalities are pathogenetic rather than epiphenomenal (see indivdidual project: "Lymphocytes and Lymphokines in Aplastic Anemia"). Cell phenotype and gamma interferon levels have been determined in a large number of cases prior to and following therapy with anti-thymocyte globulin, a horse serum preparation which produces hematologic remissions in approximately 50% of cases. Activated suppressor lymphocyte number and gamma interferon levels consistently fall in all patients treated with ATG. However, in patients who respond hematologically, suppressor lymphocyte levels are always in the normal range, whereas in patients who are hematologic failures, a large proportion continue to show circulating abnormal T-cells. These results therefore are consistent with a primary role for this T-lymphocyte population in aplastic anemia. Conversely, the ability of interferon to suppress hematopoiesis in patients with hyperproliferative bone marrow syndromes has also been tested, in a trial of gamma interferon in stable phase chronic myelogenous leukemia (see individual project: "Treatment of Chronic Myelogenous Leukemia with Recombinant Interferon-Gamma"). Patients with chronic myelogenous leukemia in chronic phase show a regular depression of platelet number with gamma interferon therapy, but gamma interferon at high doses has been sucessful in modulating disease in only a minority of cases. One possible explanation for these results is that gamma interferon acts in concert with other modulatory lymphokines to suppress hematopoiesis. In vitro, we have documented remarkable degrees of synergy between small amounts of gamma interferon and the leukocyte factor, alpha interferon, as well as the monocyte factor, tumor necrosis factor. The synergy between gamma interferon and tumor necrosis factor is particularly marked, as small quantities of either molecule, inactive alone in suppressing hematopoietic colony formation, together can abolish hematopoiesis in vitro.

Because activated lymphocytes bear the interleukin-2 receptor antigen (Tac), we have experimentally treated three patients using a monoclonal antibody to this antigen, anti-Tac. In these three cases, anti-Tac treatment failed to improve hematopoiesis and flow microfluorometry studies showed that the abnormal lymphocyte level was either only transiently decreased or the cells were coated in vivo with the non-complement fixing antibody. Future therapy may require more cytotoxic monoclonal antibodies. In parallel studies anti-thymocyte globulin and the European preparations of anti-lymphocyte globulin have been extensively analyzed for their

active properties. Significant differences have been delineated between ATG and ALG, and a restricted number of antigens that they recognize have been identified. A second possible mode of therapy in aplastic anemia may be the development of more selective polyclonal sera.

Studies of aplastic anemia have been expanded by collaborations with colleagues in Japan, Thailand, and China. An initial field trip established that the frequency of this disease was almost certainly increased in the Far East at least by a factor of three in comparison to the West. Serum samples from China and Thailand analyzed in our laboratory show similar lymphokine abnormalities to those we have described in American patients. Future studies will be directed at defining whether a chemical or viral basis is responsible for the epidemiologic differences between the Orient and the West.

The immunological abnormalities present in aplastic anemia are similar to those described with chronic viral diseases in humans caused by retroviruses and herpes viruses (see individual project: Viruses and Aplastic Anemia). Projects designed to elucidate a viral etiology of aplastic anemia have fallen into two major categories. First, we have sought a retrovirus in patients with aplastic anemia by analysis of a virus specific enzyme, reverse transcriptase, and looked for retroviral type particles in cultured blood and bone marrow cells from patients. The paucity of cells from aplastic patients and the possibly transient nature of the initial viral infection may be responsible for the negative results obtained to date. The situation in patients with severe aplastic anemia may be similar to attempting to isolate a retrovirus from patients with end- stage acquired immunodeficiency syndrome. Current efforts are directed at the culture of cells from patients with more moderate disease, better subjects for both practical and theoretical reasons. Studies of the cat retrovirus, feline leukemia virus, have shown only minimal effects of the virus on cat hematopoiesis in vitro.

In the second major category of studies attention has been focused on the family of Parvoviridae. The B19 parvovirus, discovered only 10 years ago, has been shown to be the cause of transient aplastic crisis of chronic hemolytic disease and fifth disease, a common childhood exanthem. We have previously shown that the B19 parvovirus interacts specifically with an erythroid progenitor cell in human marrow by colony culture study. Further studies of the B19 virus have been impeded by the lack of an adequate culture system. We have developed a productive culture system, using bone marrow cells from patients with sickle cell disease, which is rich in erythroid progenitor cells. In these suspension cultures, B19 virus, a single stranded DNA virus, replicates through chracteristic double stranded intermediates that are linked by terminal hairpins. B19 propogation is highly dependent on the erythroid cell content of the cultures and the hormone erythropoietin, and the virus produces in vitro a characteristic abnormal morphology similar to that described in the bone marrow of patients with aplastic crisis. At optimal virus input, greater than 200 times the output virus can be obtained from infected

cultures. B19 virus produced in suspension cultures is at least as potent as the virus obtained from the limited serum stocks drawn from acutely infected patients. At a molecular level, B19 RNA and protein have been examined in detail. The transcription map of the B19 virus is distinctive in its complexity and novel in comparison to other parvoviruses. In particular there are multiple short transcripts of unknown function which are polyadenylated and probably regulatory. Also in contrast to other Parvoviridae, all the mRNA transcripts do not co-terminate at the 5' viral end. Finally, the right-handed transcripts appear to utilize an unusual sequence as a promoter. The viral proteins produced in vitro include two capsid proteins, similar to those observed in Western gel analysis of infected serum. In addition there may be as many as three non-capsid proteins, again almost certainly serving regulatory functions. In clinical studies, a persistent B19 viral state has been sought in patients with chronic arthritidies and chronic bone marrow depression syndromes; so far, there is evidence of viral persistence in the peripheral blood mononuclear cells or bone marrow of these patients. However, a child with an underlying chronic immunodefiency syndrome has been studied in whom parvovirus has been isolated from the serum on three occasions at six month intervals. This patient also suffers from chronic, transfusion-dependent anemia. In studies of the related cat parvovirus, called feline panleukopenia virus, a different pattern than that of the B19 virus has emerged. The cat virus inhibits both myeloid and erythroid colony formation equally and is much less specific for hematopoietic compared to other tissues of the cat. These in vitro studies are in excellent agreement with the earliest studies of the behavior of the cat virus in infected populations. Some parvoviruses clearly are capable of causing generalized bone marrow failure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02208 12 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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, Chief, Clinical Hematology, CHB, NHLBI

Others: Patricia Griffith, Clinical Nurse Specialist, CHB, NHLBI
Timothy Ley, M.D., Senior Investigator, CHB, NHLBI
W.F. Anderson, M.D., Branch Chief, LMH, NHLBI
Gary Brittenham, M.D., Division of Hematology, Cleve. Gen Hosp.
H. Strawczynski, M.D., Dir., Chronic Care Clinic, Montreal Children's Hospital, Montreal, Quebec, Canada
Evan Tucker, M.D., Senior Investigator, CB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, 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.)*

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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02307 07 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Use of Viral Regulatory Sequence to Facilitate Gene Transfer and Analysis of Gene Function

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

PI: Stefan Karlsson, M.D., Ph.D., Visiting Associate, CHB, NHLBI
Others: Stefan Schweiger, B.S., Research Assistant, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI
Yakov Gluzman, Ph.D., Cold Spring Harbor Laboratory, New York
Kevin Van Doren, Ph.D., Cold Spring Harbor Laboratory, New York
Thalia Papayannopoulou, University of Washington, Seattle
George Stamatoyannopoulos, University of Washington, Seattle
Dusty Miller, Fred Hutchinson Cancer Research Center, Seattle

COOPERATING UNITS *(if any)*

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
University of Washington, Seattle
Fred Hutchinson Cancer Research Center Seattle

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

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.)*

The immediate goal of these studies is to develop methods for efficiently introducing human globin genes into hemopoietic cells both in order to study their tissue specific regulation. A hybrid SV40 virus construct, which contains the prokaryotic gene coding for chloramphenicol acetyl transferase (CAT), transiently expressed the CAT gene in hemopoietic cell lines and fresh bone marrow cells of humans and other species into which it had been transferred. We have made use of helper free double recombinant adenoviruses containing the neo gene and human globin genes. These viruses can successfully transfer both genes into K562 and other cell lines. Adenoviral mediated transfer of a γ-β hybrid globin gene and a β globin gene resulted in expression of the γ-β but not the β gene. Neither globin gene is efficiently expressed in fibroblasts demonstrating tissue-specific expression in human cell lines. As adenoviral vectors have not proven useful for gene transfer into stem cells of bone marrow, retroviral vectors containing a human globin gene and a selectable marker gene have been constructed. A γ-β hybrid globin gene has been efficiently expressed in MEL cells following retroviral mediated gene transfer, showing high levels of normally initiated, spliced, and terminated γ-β mRNA. Protein expression is also seen. This high titer globin containing retroviral vector is now being used to achieve transfer of globin genes into hemopoietic progenitor-and stem cells of mouse, monkey, and man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02310 06 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Characterization of the Gene for Human Dihydrofolate Reducatase

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

PI: Takashi Shimada, M.D., Visiting Associate, CHB, NHLBI
Others: Koiti Inokuchi, M.D., Visiting Fellow, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The structure and function of the promoter of the human dihydrofolate reductase (DHFR) gene have been studied. An extensive mapping study of RNA from methotrexate resistant HeLa cells, using a single strand RNA probe, identified a cluster of minor initiation sites about 400 bp upstream from the major initiation site for DHFR mRNA. In addition, we identified about 300 nucleotides of RNA which initates at position -90 and is transcribed from the opposite strand to that coding for DHFR mRNA. Another opposite strand transcript initiated at position -600 was detected using an in vitro transcription system. A series of deletion mutants of the DHFR gene promoter were fused to the DHFR coding sequence (DHFR minigene) or to the bacterial chloramphenicol acetyltransferase gene (DHFR-CAT). RNA analysis of monkey kidney Cos cells transfected with the DHFR minigene showed the 72 bp upstream sequence is sufficient for correct initation. Deletional analysis using the DHFR CAT vectors identified two activation sequences from -610 to -360 and from -109 to -72. All these cis-acting regulatory elements were found to be located in the previously defined nucleosome free region. The promoter binding proteins, which have important roles in establishing and maintaining the nucleosome free structure, were partially purified and characterized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02313 04 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Regulation of Hemoglobin Switching During Development: Characterization of the Human γ Globin Gene Promoter

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

PI: Henry J. Lin, M.D.

Others: Nicholas P. Anagnou, M.D., Guest Worker, CHB, NHLBI
Tim Rutherford, M.D., Visiting Associate, CHB, NHLBI
Stefan Karlsson, M.D., Visiting Associate, CHB, NHLBI
Austine Moulton, Research Assistant, CHB, NHLBI
Amanda Cline, Research Assistant, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our aim is to identify DNA sequences in the fetal and adult globin gene promoters that participate in the developmental regulation of these genes. We constructed composite promoters containing portions of the fetal γ and adult β 5′ flanking regions and assessed their function in stably transformed human K562 cells, a cell line that expresses the γ globin gene but not the β. A 270 base pair fragment from the γ flanking region activated the β globin promoter. The reversed sequence of this γ fragment did not activate the β promoter. A corresponding region of the human ζ globin gene, which is also expressed by the K562 line, appeared to function in a similar way. Further dissection of the γ flanking region may delineate specific sequences involved in globin gene regulation in these cells.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02314 04 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Function of Proto-oncogenes in Human Hematopoietic Cells

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

PI: Philip J. Browning, M.D. Guest Worker Others: A.W. Nienhuis, M.D.,Branch Chief, CHB NHLBI
Others: H.F. Bunn, M.D., Fogarty Scholar, CHB NHLBI
T.V. Gopal, Ph.D., Senior Staff Fellow, CHB, NHLBI
A.Cline, Chemist, CHB, NHLBI
M.Shuman, Guest Worker, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

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.)*

Proto-oncogenes when transduced by retroviruses may undergo structural modifications that render their gene products oncogenic. The c-fms gene encodes a transmenbrane protein with tyrosine kinase activity that is very similar or identical to the receptor for the monocytic-colony stimulating factor (M-CSF). The transforming homologue of this oncogene (v-fms) in the McDonough strain of the feline sarcoma virus causes fibrosarcomas in cats. Molecular cloning and sequence analysis of the cDNA that encodes the cytoplasmic domain of the human c-fms gene has shown that the product of the transduced viral homologue, v-fms , is truncated at the C-terminal end. The forty amino acids at the C-terminal of the c-fms gene product are replaced in the v-fms gene product by 11 amino acids encoded by the retroviral genome. Hybrid v-fms/c-fms genes, in which either the entire cytoplasmic domain or the C-terminal coding sequences of the v-fms gene were replaced by the corresponding segments of the c-fms gene, had a reduced ability to transform fibroblasts despite a high level of the encoded protein of the cell surface. These data indicate that the C-terminal modifications contribute to the transforming potential of the v-fms viral oncogene product.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02315 04 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Lymphocytes and Lymphokines in Aplastic Anemia

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

PI: Neal S. Young, M.D., CHB, NHLBI
Others: Leonidas Platanias, M.D., Visting Fellow, CHB, NHLBI
Eric Raefsky, M.D., Medical Staff Fellow, CHB, NHLBI
Eileen Leonard, M.D., Guest Worker, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

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.)*

Previous studies from our laboratory have implicated a population of activated suppressor lymphocytes which produce an inhibitory lymphokine as pathogenic in patients with bone marrow failure. These cells are detectable in abnormally high numbers in the circulation of patients with aplastic anemia. Production of gamma interferon by activated suppressor cells probably explains other laboratories' previous results showing inhibition in co-culture by patients' cells of normal hematopoiesis. Current studies have been directed at the changes in lymphocyte phenotypes and lymphokine production in patients that follow treatment with antithymocyte globulin (ATG); the mechanism of action of ATG in aplastic anemia; and the interaction in vitro of gamma interferon with with other soluble mediators of immune function. The scope of studies of aplastic anemia has been broadened by collaborations with investigators in the Far East, where aplastic anemia is a more common disorder than in the United States or Western Europe.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02318 03 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Enhancer and Promoter Specificity of Immunoglobulin Genes

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

PI: T. Venkat Gopal, Staff Fellow, CHB, NHLBI
Others: Arthur W. Nienhuis, Branch Chief, CHB, NHLBI
Ann Baur, Research Assistant, CHB, NHLBI
Takashi Shimada, Visiting Associate, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	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.)*

Tissue-specific promoters and enhancers play a major role in the control of developmentally regulated gene expression during development. Expression of rearranged immunoglobulin (Ig) genes introduced into both lymphoid and non-lymphoid cells has led to the identification of tissue-specific transcriptional enhancer sequences in the major intron between the J and C region of the Ig gene. We have shown that the Ig promoter also contributes to tissue-specific expression of mouse Ig kappa gene. Tissue-specificity of Ig gene enhancers and promoters is thought to be due to their interaction with trans-acting regulatory factors. We have designed a genetic approach to study and clone such B cell specific trans-acting factors. Plasmid vectors we've constructed that contain the bacterial neomycin resistance gene linked to the mouse immunoglobulin kappa gene promoter and a neutral enhancer or Ig heavy chain gene enhancer. By introducing these hybrid genes into non-lymphoid mouse 3T3 and L cells, we have created recipient cell clones in which the hybrid gene is stably integrated and non-functional. The hybrid gene could then be activated by cell fusion and by DNA transfer methods. Our results suggest that this approach can be used to directly isolate genes that code for putaitve trans-acting regulatory factors. Isolation of these regulatory genes will greatly enhance our abiltiy to understand the regulation of tissue-specific genes at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02319 03 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Viruses and Bone Marrow Failure

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

PI: Neal Young, M.D.
Others: K. Ozawa, M.D., Visiting Fellow, CHB, NHLBI
G. Kurtzman, M.D., Medical Staff Fellow, CHB, NHLBI
L. Platanias, M.D., Visiting Fellow, CHB, NHLBI
E. Raefsky, M.D., Medical Staff Fellow, CHB, NHLBI
M. Harrison Biologist, Research Assistant, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
03	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.)*

Two types of viruses have been examined for their role in the etiology of bone marrow failure in animals and man. The first, the Parvoviridae, are the smallest DNA containing animal viruses. The B19 human parvovirus causes selective erythroid aplasia in individuals with underlying hemolysis. Feline panleukopenia virus is a common agent of cat disease and capable of causing a true pancytopenia and bone marrow aplasia. The human parvovirus was discovered only 10 years ago and a major stumbling block to its investigation has been an adequate in vitro cell culture system. Using suspension cultures of erythroid bone marrow cells from patients with sickle cell disease, we have acheived the first propagation of the B19 agent. This virus is highly selective for erythroid in comparison to myeloid progenitor cells. Events associated with the replication, transcription, and protein production of this virus have been elucidated in detail. The feline virus, in contrast, is less selective in vitro, with a strong inhibitory action on the proliferation of both erythroid and myeloid cells. This virus can also be propagated in suspension cultures of cat bone marrow. Studies of the cat retrovirus, feline leukemia virus, have shown that its effect on in vitro colony formation by cat cells is minimal. In animal studies, feline leukemia virus had marginal effects on hematopoiesis. Extensive studies of human patients of a possible role for a retrovirus in human aplastic anemia had failed to reveal consistent reverse transcriptase activity or retroviral particles by electron microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02320 03 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Pharmacological Manipulation of HbF Synthesis

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

PI: Timothy J. Ley, M.D., Senior Investigator, CHB, NHLBI
Others: Lyn Mickley, Medical Technologist, CHB, NHLBI
Brian Agricola, Animal Technician, Section on Animal Surgery CSB, NHLBI
Joseph E. Pierce, D.V.M., Chief Section on Animal Surgery, CSB, NHLBI
Arthur W. Nienhuis, Chief, Clinical Hematology, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

These studies were designed to determine the potential for various compounds to induce HbF synthesis in experimental animals. Three drugs, 5-azacytidine, cytosine arabinoside, and hydroxyurea, have been studied extensively in rhesus monkey and baboons. 5-Azacytidine is consistently the most active of the three compounds, although significant differences between species and among individuals of a given species have become evident.

An individual with severe beta thalassemia, untransfused because of the presence of multiple allo-antibodies, has been treated with 5-azacytidine. An increase in hemoglobin from 5.7 gm/dl to 9.5 gm/dl has been documented after two five day courses of treatment. Therapy is to be continued by the oral route. This represents the first thalassemic patient to achieve significant clinical benefit from the use of 5-azacytidine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02321 02 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Inhibition of Oncogene Expression with Antisense RNA

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

PI: Jeffrey Holt, M.D., Staff Fellow, CHB, NHLBI
Others: Robert Redner, M.D., Staff Fellow, CHB, NHLBI
T. Venkat Gopal, Ph.D., Senior Staff Fellow, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

These studies are designed to analyze the effects of oncogene expression on cell growth and differentiation. The functions of the proto-oncogenes c-fos and c-myc will be studied by observing the phenotype of both benign (immortalized) and malignant cells when oncogene expression is inhibited. Recombinant DNA vectors designed to produce "antisense RNA" have been introduced into mouse fibroblast and embryonal teratocarcinoma cell lines. These vectors contain a steroid inducible (MMTV) or metal inducible (Metallothionein) promoter which should allow regulated production of the antisense RNA. Inhibition of c-fos or c-myc expression with these antisense constructs blocks proliferation of fibroblasts. Preliminary results suggest that antisense c-fos can also inhibit teratocarinoma differentiation. Such selective inhibition of c-fos or c-myc expression may further elucidate the role of these proto-oncogenes in normal cellular growth and differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02322 02 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Molecular Defects in Beta Thalassemia

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

PI: Jeffrey Holt, M.D., Staff Fellow, CHB, NHLBI

Others: Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI
Nicholas P. Anagnou, M.D., Ph.D., Visiting Fellow, CHB, NHLBI
George Stamatoyannopoulos, M.D., and Thalia
Papayannopoulou, M.D., Division of Medical
Genetics, University of Washington, Seattle, Wa.
Janet Ash Tobian, Ph.D., Staff Fellow, HFB, NICHHD
Michael Zasloff, M.D., Branch Chief, HGB, NICHHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, 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.)*

These studies are designed to define and precisely characterize various molecular lesions occurring in the beta-globin gene cluster in patients with beta thalassemia or with syndromes associated with increased HbF production in adult life, such as delta-beta thalassemia or hereditary persistence of fetal hemoglobin (HPFH). To investigate the mechanism by which premature termination codons cause a quantitative deficiency of beta globin mRNA, precursor and spliced mRNA molecules are generated in vitro and in vivo. The processing and nuclear to cytoplasmic transport of these RNA molecules are studied by microinjection into nuclei of Xenopus oocytes.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02323 02 CHB

PERIOD COVERED

Ocotber 1, 1985- September 30, 1986

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

Characterization of the Bone Marrow Defect in PNH

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

PI: Jeffrey G. Moore, Chemist, CHB, NHLBI
Others: Neal S. Young, M.D. Chief, Cell Biology Section, CHB, NHLBI
Michael M. Frank, M.D., Clinical Director, LCI, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.25	0.75

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.)*

Two populations of red blood cells coexist in the circulation of patients with paroxysmal nocturnal hemoglobinuria (PNH), one normal and one that is abnormally sensitive to complement-mediated lysis. We have previously shown that these two populations are not maintained by two distinct stem cells populations in the bone marrow. Rather, it appears that any PNH progenitor cell may have the potential to generate both normal and abnormal red cells during clonal expansion. In our previous experiments, we studied the generation of abnormal cells during differentiation by using in vitro colony culture assays and an antibody to a complement regulatory protein, decay accelerating factor (DAF), which is missing on the complement-sensitive red cells and may account for the susceptibility of PNH cells to complement action. Using the antibody to DAF and flow cytometry, we also found that all cells in the circulation express DAF at varying amounts. Compared to normal individuals, lymphocytes, monocytes, granulocytes, and platelets from PNH patients had lower DAF expression, but it was not possible to detect two distinct populations as was seen with red cells. A small population of DAF- cells are seen in the blood, bone marrow, and cells in the in vitro colony assays in normal individuals. The presence of DAF- cells in normal individuals may provide a clue to the development of PNH: these infrequent cells may exist normally and their proliferation may be favored followed marrow insult, resulting in the development of PNH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02324 02 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Identification of Cis and Trans-acting Elements that Regulate Human Gamma Gene Expression

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

PI: David Bodine, Ph.D., Guest Worker, CHB, NHLBI

Other: Timothy J. Ley, M.D., Senior Investigator, CHB, NHLBI

Peter C. Hoppe, Ph.D., Jackson Laboratory

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In addition to providing insight into the developmental regulation of eukaryotic gene expression, studies of the human gamma globin gene may also suggest means by which these genes, which are normally inactive in adult life, could be reactivated in individuals with disorders of the adult beta globin gene. This laboratory has undertaken a number of projects to analyze the molecular behavior of the human gamma globin genes in response to both cis and trans-acting factors. Studies of a tissue specific cis acting element located just 3' to the A gamma gene have been shown that this fragment has all the properties of an enhancer element and that it may have a role in the regulation of the gamma genes. To study both cis and trans-acting factors during hemoglobin switching, three lines of transgenic mice have been generated carrying 4-8 copies of a construct, consisting of an A gamma gene linked to a human beta globin gene. Several laboratories have demonstrated that point mutations in the gamma promoter (usually in the region approximately 200 bp upstream from the CAP site) are associated with hereditary persistence of fetal hemoglobin (HPFH), implying that this region is a target for trans acting factors in erythroid cells. Deletion analysis of the gamma promoter performed in this laboratory have shown that normal expression of the gamma gene requires the presence of this region. Mutation within this region can increase transcription dramatically, but the only mutations that are effective are those that are associated with HPFH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02325 02 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Treatment of Chronic Myelogenous Leukemia with Recombinant Interferon-gamma

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

PI: Gary J. Kurtzman, M.D., Medical Staff Fellow, CHB, NHLBI

Others: Neal S. Young, M.D., Section Chief, CHB, NHLBI
Arthur W. Neinhuis, M.D., Branch Chief, CHB, NHLBI
Keiya Ozawa, M.D., Ph.D., Visiting Scientist, CHB, NHLBI
Eric Raefsky, Medical Staff Fellow, CHB, NHLBI
Stephen A. Sherwin, M.D. Genentech, Inc.
Sam Saks, M.D., Genentech, Inc.

COOPERATING UNITS *(if any)*

Genentech, Inc., South San Francisco, California

LAB/BRANCH

Clinical Hematology

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

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

Chronic myelogenous leukemia (CML) is characterized by increased formation of granulocytes and other blood forming elements. Despite the name, CML is almost invariably fatal, with a median survival time of approximately three years. Conventional chemotherapy for the initial chronic phase has failed to prevent transformation to acute leukemia or to significantly affect survival. Although bone marrow transplantation is successufl in a minority of patients, clearly more effective regimens, to be administered during the chronic phase, need to be developed. We previously demonstrated that gamma interferon (IFN-gamma) has a potent suppressive effect on hematopoiesis in vitro and have provided evidence implicating IFN-gamma in the patogenesis of the hematopoietic supression observed in aplastic anemia. Another of the interferons, alpha interferon (IFN-alpha), has shown promising clinical results in the treatment of chronic phase CML. Based on theoretic advantages and in vivo suppression of myelopeiesis observed in patients treated with recombinant IFN-gamma (rIFN-gamma) for other disorders, we embarked on a clinical study of patients with both chronic and accelerated phases of CML with rIFN-gamma appriximately one year ago.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02326 02 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Cloning Characterization of a Sequence of Human DNA with Homology to Adenovirus 5 and 12

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

PI: Neal D. Epstein, M.D., Medical Staff Fellow, CHB, NHLBI
Others: Stefan Karlsson, M.D., Ph.D., Visiting Associate, CHB, NHLBI
Arthur W. Nienhuis, M.D., Branch Chief, CHB, NHLBI
Steve O'Brien, Ph.D., Branch Chief, LVC, Frederick, Md.

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Hematology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	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.)*

During the course of gene transfer experiments into K562 cells using a modified adenovirus vector, we noted that control K562 cell DNA showed a positive signal when probed with the entire adenovirus genome. This signal was also present in all normal human genomic DNA tested. The adenovirus genome was resolved into three fragments which, when used as probes in Southern blot analysis showed evidence for homology between the 5'9 kb of adenovirus and a 2.5 kb Sst I fragment of human DNA. Because the 5' adenovirus fragment contains coding sequences for proteins involved in DNA replication, transcriptional control, and cell transformation, we chose to clone the human sequence homologous to the fragment. In cloning this sequence out of a human cosmid library, we identified a moderately repetitive DNA sequence family consisting of tandem arrays of 2.5 kb members. A member was sequenced and several non-adjacent, 15-20 bp G-C rich segments with homology to the left side of adenovirus were discovered. The copy number of 400 members is highly conserved among humans. Southern blots of partial digest of human DNA have verified the tandem array of the sequence family. The chromosomal location was defined by somatic cell genetics and in situ hybridization. Tandem arrays are found only on chromosomes 4 (q31) and 19 (q13.1-q13.3). Homologous repetitive sequences are found in DNA of other primates but not in cat or mouse. Thus we have identified a new family of moderately repetitive DNA sequences, unique because of its organization in clustered tandem arrays, its length, its chromosomal location, and its lack of homology to other moderately repetitive sequence families.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02327 01 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

The effect of v-abl and IL3 genes on hemopoietic stem cell differentiation

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

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Others: Siu-Wah Chung, PhD, Fogarty Fellow, Lab of Genetics, NCI
Timothy M Browder, MD, Guest Worker, CHB, NHLBI
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COOPERATING UNITS *(if any)*

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Clinical Hematology Branch

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	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.)*

Retroviral mediated gene-transfer of important and relevent genes such as v-abl (an oncogene) and IL3 (the growth factor for hematopoietic stem cells) into hemopoietic stem cells will provide information about regulation of hemopoietic stem cell behaviour. Recently, we have established an in vitro system which allows growth of hemopoietic colonies consisting of up to 90% stem cells with self-renewal capacity. Infection of colonies with v-abl virus resulted in differentiation of these early stem cells to tumorigenic, immortalized mast cells which are growth factor independent. A number of recombinant IL3 and GM-CSF retrovirus have also been constructed. On infection of a factor-dependent cell line with these viruses, factor-independent growth was observed. Using cells for primary tissues such as fetal liver and adult mouse bone marrow, the results indicate that fetal liver contains a higher frequency of IL3 virus target stem cells. Our preliminary studies indicate that these infected stem cells can also reconstitute lethally irradiated receipients and express the viral IL3 gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02328 01 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Modification of Retroviral Targeting via Hybrid Envelope Proteins

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

PI: Timothy M. Browder,M.D.,Guest Worker,CHB,NHLBI
Others: Arthur W. Nienhuis,M.D.,Branch Chief,CHB,NHLBI
Peter Wong,Ph.D.,Guest Worker,CHB,NHLBI
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

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☐ (a1) Minors
☐ (a2) Interviews

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

These experiments are designed to test whether structural alterations of retroviral envelope glycoproteins can direct virus targeting for tissue-specific gene transfer. Ecotropic and xenotropic viruses are modified for expression of a hybrid envelope gene which encodes for a murine pluripotent colony stimulating factor, interleukin 3 (IL3). These viruses are further constructed so that the only mechanism to gain entrance into murine cells is through the IL3 receptor, present only on bone marrow cells. If such specificity of infection targeting can be demonstrated, the psi sequence could be deleted in order to create packaging cell lines which encapsidate (pseudotype) other retroviral vectors within its own mutant envelope proteins but cannot package its own RNA. Such packaging cell lines would then allow in vivo recombinant gene transfer via IL3-pseudotype retroviral infection.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02329 01 CHB

PERIOD COVERED

October 1, 1985- September 30, 1986

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

Tissue and Developmental Specificity of Globin Promoters.

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

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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 purpose of this project is to examine the interaction of trans regulators of globin gene transcription with the globin gene promoters, and potentially to clone the genes responsible for the developmental regulation of globin genes. To this end we have made recombinant DNA constructs in which an antibiotic resistance gene, neo, is transcribed from different globin gene promoters. By transfection of these constructs into different cell types we have shown that they are transcribed in a tissue specific and developmental stage specific manner. Since expression of the neo gene can be selected for using antibiotic G418, these recombinant genes can be used to study globin gene regulation by genetic selection experiments. In particular by linking these genes to other selectable markers (TK, prt) we have been able to stably introduce them into non-expressing cell lines. We intend to study whether these non-expressed genes can be reactivated in trans (i) by promoter competition, (ii) by transfection of activated oncogenes, (iii) by cell fusion with erythroid cells, or (iv) by transfection with normal human DNA sequences including the putative trans regulatory genes.

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ANNUAL REPORT OF THE
LABORATORY OF EXPERIMENTAL ATHEROSCLEROSIS
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1985 through September 30, 1986

Atherosclerosis is the underlying basis of most coronary artery disease, the leading cause of death in the United States. Because pathological cholesterol accumulation is central to the development of the atherosclerotic lesion, we have chosen to study this aspect of atherosclerosis.

Work in the Laboratory of Experimental Atherosclerosis is continuing in the examination of mechanisms of cellular cholesteryl ester accumulation that may be relevant to accumulation of cholesterol within cells of atherosclerotic lesions. In this regard, we have extended our studies concerning platelet-mediated cellular cholesterol accumulation. We previously reported that when platelets are activated with thrombin, they induce cholesterol accumulation in co-cultured cells. This finding suggests that activated platelets release cholesterol that can be taken up and accumulated by cells.

Over the past year we have demonstrated that platelets do release substantial amounts of cholesterol (50 nmoles per 30×10^8 platelets) when activated by a variety of platelet agonists (thrombin, collagen, A23187). Release of cholesterol occurs over a relatively slow time course (2.5 hours) as compared with other platelet functions that occur within minutes after activation. Release of cholesterol can be dissociated from release of the cytoplasmic marker lactate dehydrogenase suggesting that cholesterol release does not result from simple platelet lysis.

Characterization of the cholesterol moiety released from human and rat platelets has revealed that released cholesterol is contained within large (> 600 angstrom) cholesterol-phospholipid vesicles. The vesicles have a unique appearance in that they are studded with 190 angstrom rod-shaped projections. The vesicles also have a significant protein content that is currently under investigation. The relative cholesterol content of the vesicles increases when rats are fed a high-cholesterol diet or when vesicles are incubated for 18 hours before being removed from platelets.

Platelet factor 3 is a phospholipid factor that is released from activated platelets. This factor accelerates the formation of fibrin by the extrinsic coagulation pathway. It is possible that the cholesterol-phospholipid vesicles that we have described as being released from activated platelets are related to platelet factor 3. This possibility is supported by the fact that the time course of platelet cholesterol release we observed and the time course of platelet factor 3 release previously reported are similar.

Preliminary experiments have indicated that the platelet cholesterol-phospholipid vesicles can induce cellular cholesterol accumulation when incubated with cultured human monocyte-derived macrophages. Thus, it does appear that activated platelets release cholesterol in a form that can be accumulated by cells. This mechanism may explain the origin of some portion of the cholesterol that accumulates within cells of thrombi, which are known to contribute to the growth of atherosclerotic lesions.

Work has also continued in our investigation of unique lipid vesicles which accumulate within human and experimentally induced atherosclerotic lesions. These vesicles are comprised of phospholipid and cholesterol which is in a predominantly unesterified form. We reported last year that accumulation of these vesicles in the subendothelial space is the first detectable structural change in developing atherosclerotic lesions and thus may be important in inducing subsequent pathological events such as migration of monocytes and smooth muscle cells into the subendothelial space. These unesterified cholesterol-containing vesicles may also provide a source of cholesterol that these cells accumulate.

We have used cultured fibroblasts from patients with Type C Niemann-Pick disease as a model system to help determine the origin of the unesterified cholesterol-containing lipid vesicles. In collaborative studies with Dr. Pentchev and Ms. Comly of NINCDS and Dr. Butler of NICHHD, we have shown that fibroblasts from these patients accumulate large amounts of unesterified cholesterol (rather than esterified cholesterol as normal fibroblasts accumulate) when incubated with human low density lipoprotein. Our studies have revealed that this occurs because of a metabolic block in the conversion of unesterified cholesterol to cholesteryl ester in these cells. The unesterified cholesterol accumulates within vesicles that have similar physical and chemical characteristics to the lipid vesicles that we have isolated from atherosclerotic lesions. This finding suggests that plasma LDL can be a precursor of unesterified cholesterol-phospholipid vesicles and that these vesicles are generated when accumulated cellular cholesterol fails to be esterified.

Thus, the pathogenesis of cholesterol deposition in atherosclerosis may be postulated as an elevated level of cellular uptake of plasma lipoproteins associated with their conversion to large, unesterified cholesterol-rich lipid particles that accumulate in the vessel wall. The fact that cholesterol-phospholipid vesicles accumulate directly under the endothelium suggests the possibility that vesicles are produced by and released from endothelial cells. It is intriguing to consider the possibility that these aortic lipid vesicles may function analogously to the platelet-derived vesicles (described above) in promoting coagulation in the vessel wall as a response to injury. In both cases cholesterol-phospholipid vesicles may be taken up by cells leading to "foam cell" formation, the hallmark of atherosclerotic lesions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02827-04 EA

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Release of cholesterol from activated platelets

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

PI: H.S. Kruth Senior Investigator LEA, NHLBI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Experimental Atherosclerosis

SECTION

Vascular Physiology Section

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.)*

Cholesterol accumulation by cells in the blood vessel wall or in thrombi associated with blood vessels is the hallmark of the atherosclerotic lesion. Previous work in our laboratory has shown that platelets can mediate cholesterol accumulation in vascular smooth muscle cells. This study shows that when platelets are activated, they release cholesterol.

Washed human or rat platelets, activated with the potent platelet agonist thrombin, released 50 nmoles of cholesterol per 3 billion platelets. This amount represents approximately 20 percent of the platelet cholesterol content. The calcium ionophore, A23187 (5 μm) was as effective as thrombin in stimulating cholesterol release, whereas, collagen (200 μg/ml, type I) was somewhat less effective. ADP (200 μm), a less potent platelet agonist, was ineffective in stimulating cholesterol release.

The release of cholesterol from activated platelets was relatively slow (>90% released within 2 hours) when compared with the rapid release of serotonin (>90% released within 5 min) a constituent of dense granules. Release of cholesterol was inhibited by the platelet antagonist forskolin, an agent that elevates platelet cAMP. Release of cholesterol could be dissociated from release of a cytoplasmic marker lactate dehydrogenase suggesting that cholesterol release did not result simply from platelet lysis.

These findings suggest that platelets, in addition to their known function of stimulating proliferation of cells, are also a source of cholesterol that may accumulate in cells. Both of these platelet functions are relevant to the pathogenesis of atherosclerosis, a disease in which cell proliferation and cellular accumulation of cholesterol are characteristic.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02828-01 EA

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Characterization of cholesterol-containing vesicles released from platelets

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

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SECTION

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2	1	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.)*

The purpose of this project is to isolate and characterize the cholesterol moiety that we have shown to be released by activated human and rat platelets (see report Z01 HL 02827-04 EA). Platelet-free supernatants were prepared from incubated thrombin-activated human and rat platelets. Unique appearing cholesterol-phospholipid vesicles (greater than 600 angstroms in diameter) with numerous rod-shaped projections were isolated from these supernatants.

Chemical analysis showed that vesicles released from activated human or rat platelets contained 9% cholesterol, 34% phospholipids, 1% triglycerides, and 56% protein. The molar ratio of cholesterol to phospholipid in these vesicles was 0.5, whereas, the molar ratio of cholesterol to phospholipid was 1.0 in vesicles released from activated platelets obtained from rats fed a high-cholesterol diet. In addition, prolonged incubation of activated platelets (18 hrs rather than the usual 2 hrs) also resulted in vesicles with a substantially higher cholesterol to phospholipid molar ratio (0.9). All vesicles demonstrated a density of 1.14 when centrifuged isopycnically in a continuous sucrose density gradient.

This research has been directed towards identifying how platelets can contribute directly or indirectly to the formation of atherosclerotic lesions which characteristically contain cells with large amounts of accumulated cholesterol. When macrophages were incubated with the platelet-derived cholesterol-containing vesicles, a substantial increase in their cholesteryl ester content was found in comparison with control cultures. Thus, these studies demonstrate that activated platelets release cholesterol that can be accumulated by cells. This process may represent a new mechanism to explain the deposition of cholesterol within cells of thrombi and possibly atherosclerotic lesions.

ANNUAL REPORT OF THE
HYPERTENSION-ENDOCRINE BRANCH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1985 through September 30, 1986

This year the Hypertension-Endocrine Branch has continued its basic and clinical research into many aspects of the causes and therapy of hypertension. These studies have focused on some of the neurohumoral and vasoactive systems that control the circulation.

I. Catecholamines and the sympathetic nervous system. The role of the sympathetic nervous system in the onset and maintenance of essential hypertension is still a subject for extensive research by many scientists. We have developed and applied methodology for measuring regional and total body release and neuronal uptake of norepinephrine (NE), the sympathetic neural transmitter, and have assessed the effects of various stimuli, i.e., isoproterenol (Iso.), clonidine, manipulations of dietary salt, on sympathetic activity. Sympathetic and cardiovascular responses to psychological stress are more likely to uncover abnormalities in neural circulatory control in hypertension than measuring sympathetic and cardiovascular responses at rest. We have developed and begun to use an electronic game as a stimulus which increases blood pressure, pulse rate and plasma levels of NE and epinephrine (E). The response pattern is similar to that which would be expected during "defense" reactions, where neurogenic hypertension occurs with patterned increases in sympathetic nerve activity to the kidney, heart, and viscera, but decreases in activity to skeletal muscle. This new technology should be very useful in allowing us to study responses in both hypertensive and normal-subjects, and in normotensive offspring of either hypertensive or normotensive parents. We have shown that the clonidine suppression test is useful in the delineation of the neurogenic component of hypertension. Thus in patients with essential hypertension, there was a good correlation between the resting level of plasma NE and the magnitude of the depressor response and the decrease in mean arterial pressure three hours after a single oral dose of clonidine. However, the question was raised about whether or not this response was affected by the patient's dietary sodium intake. When this was evaluated in a series of patients on both a high- and low-sodium diet, there was no significant change in the responses obtained during the clonidine suppression test. Thus this procedure can be used without regard to acute alterations in dietary sodium. We infused Iso. into healthy subjects and patients with essential hypertension in order to determine if presynaptic beta-adrenoreceptors modulate NE release from sympathetic nerve endings, and thereby whether Iso. would act in vivo indirectly as an alpha-adrenoreceptor agonist. Linear concentration-response relationships were observed between plasma Iso. and cardiac index and heart rate. Plasma NE increased as a function of plasma Iso. Thus Iso. stimulates presynaptic beta-adrenoreceptors to enhance NE release. There was no effect on plasma E levels even though Iso. can cause anxiety. There was also no increase in plasma ACTH levels.

We have continued in the development of methodologies for measuring plasma, urine, tissue, and cerebrospinal fluid levels of the catecholamine precursor, dihydroxyphenylalanine (DOPA). Now DOPA, the catecholamines NE, E, and dopamine (DA), and the deaminated catecholamine metabolites, dihydroxyphenylglycol (DHPG) and dihydroxyphenyl acetic acid (DOPAC), can be

measured simultaneously using liquid chromatography with electrochemical detection. DOPA is the product of tyrosine hydroxylase and the first step in the conversion of tyrosine to NE. Venous plasma DOPA in humans averaged 3200 pg/ml. Virtually all healthy subjects had an arterial venous increment in DOPA, but sympathectomized patients did not. Administration of clonidine decreased the level of plasma DOPA, while tilt, Iso., yohimbine, and trimethaphan had no effect on it. General anesthesia in animals decreased DOPA by 34%, while inhibition of tyrosine hydroxylase decreased DOPA by 62%. Thus plasma DOPA derives extensively from sympathetic nerves and may indicate activity of the rate-limiting step in catecholamine biosynthesis. DHPG is produced by the action of monoamine oxidase on NE. Venous plasma DHPG of humans averaged 878 pg/ml. Yohimbine and tilt increased DHPG, while desipramine decreased baseline DHPG and abolished the DHPG responses to tilt or yohimbine without attenuating NE responses. These results support the hypothesis that DHPG is formed intraneuronally in humans after reuptake of endogenously released NE into the axonal cytoplasm. Comparison of responses of DHPG and NE may enable separation of altered neuronal uptake of NE from altered NE release as determinants of changes in plasma NE. We have found that plasma DOPA levels were markedly increased in 9 patients with neuroblastoma and in 8 of 14 patients with malignant pheochromocytoma, whereas all patients with either benign pheochromocytoma (n=15) or essential hypertension had normal plasma levels of DOPA. Plasma levels of NE and E are normal in patients with neuroblastoma and elevated in patients with benign or malignant pheochromocytoma. Thus measurements of circulating levels of DOPA and catecholamines appear to indicate differentiation of tumors of neural crest origin and explain the clinical finding that neuroblastoma is an aggressive pediatric tumor unassociated with hypertension, whereas pheochromocytoma is a slow-growing tumor of adults and children which is associated with hypertension. We made the first simultaneous measurements of DOPA and catecholamines in samples of human cerebral cortical tissue obtained from patients undergoing excisions of epileptogenic foci. DOPA, NE and DA concentrations from affected cortical regions were all about double those in control areas. These results indicate increased catecholamine synthesis in epileptogenic foci and thus confirm earlier observations which suggested increased levels of tyrosine hydroxylase activity. Another question of importance has arisen over the source of DA in the urine. Thus when tritiated DOPA was infused into the renal artery of anesthetized dogs, we found that the delivery rate of endogenous DOPA was about 100 times that of endogenous DA. About 60% of arterial DOPA appeared unchanged in the renal vein. We calculated that about half of urinary DA derived from circulating DOPA. Thus while only a small portion of circulating DOPA is converted to DA in the kidney, since there is a large delivery rate of endogenous DOPA, this conversion can account for a substantial portion of urinary DA. When we studied the effects of surgical stress in monkeys, we were able to demonstrate that enhanced sympathetic neural activity was able to maintain circulatory homeostasis in animals that were adrenalectomized previously.

Biochemical evidence has been adduced to support the hypothesis that adrenergic neurosecretion can be mediated by the outward transport of NE from synaptic vesicles that are fused or attached to the plasma membrane of the nerve ending. These data suggest that a driving force for the uptake and retention of NE by vesicles is the electrochemical gradient of hydrogen established by the activity of magnesium-ATPase, and that these effects occur in the presence of high concentrations of Cl-. Apparently nonexocytotic

secretion can occur in the presence of physiologic concentrations of Cl-. In studies of catecholamine release from isolated and cultured bovine chromaffin cells, blockade of the voltage-dependent potassium current by triethylammonium (TEA) causes a dose-dependent increase in catecholamine secretion. This increase is blocked by both organic and inorganic calcium antagonists (nifedipine and high magnesium). These data support our earlier findings of the importance of voltage-dependent calcium channels in exocytotic release of catecholamines from chromaffin cells.

Previous studies in our laboratory as well as in those of others have demonstrated two subsets of patients with normal renin essential hypertension. We have now applied a similar study to a pilot group of normal subjects and found that some, like the hypertensives, show the phenomenon of salt sensitivity of blood pressure. Compared to the salt-resistant normal subjects, the salt-sensitive normal subjects had a lower stimulated plasma renin activity, lower urinary DA, greater cumulative sodium retention and a higher plasma NE that showed a smaller change in response to a high sodium intake. Mean urinary DA content correlated inversely with both cumulative sodium retention and the percent change in plasma NE. Thus the differences between these two subsets appeared to be attributable, in part, to differences in dopaminergic activity in the kidney. These findings further suggest that salt-sensitive normal subjects are qualitatively similar to patients with salt-sensitive hypertension in many respects and may represent the population at risk for development of salt-sensitive essential hypertension. Results also suggest that features that characterize the salt-sensitive hypertensive subject represent modifications of normal physiology and do not appear to be acquired as a consequence of the hypertensive process.

II. Atrial natriuretic peptides. Mammalian atrial myocytes contain biologically active peptides within specific secretory granules. These atrial natriuretic peptides (ANP) have potent natriuretic, diuretic and vascular smooth muscle relaxant activities. They have potential importance in controlling blood pressure, yet little is known about the regulation of their secretion into the blood stream. We have developed sensitive radio-immunoassays for the different ANPs found in rat and man. We have shown that atrial distention (e.g., increased perfusion pressure in the rat heart-lung preparation), as well as acute volume expansion in anesthetized rats, causes a marked increase in circulating ANP. Administration of pharmacologic doses of arginine vasopressin, oxytocin, and angiotensin II also induces profound increases of circulating ANP. Basal and stimulated release of ANP were significantly blunted in hypophysectomized animals. Administration of alpha- or beta-adrenergic antagonists had no effect on the response to atrial dilatation. Supplementation of hypophysectomized rats with vasopressin, gluco- or mineralocorticoids, or prolactin did not restore the response of ANP to atrial distention. When various lobes of the pituitary gland were transplanted to the kidney, only the anterior lobe restored the ANP response to atrial distention. These data are surprising since they strongly suggest that the anterior pituitary may produce an "atriotrophic" factor which has a permissive role on the release of ANP by mechanical stretch from the atria. Studies are underway to see if this factor can be extracted from the anterior lobe of the pituitary and to determine its structure. One area where ANP might have important effects is in renal failure. We therefore studied plasma ANP levels in rats with renal failure of differing severity and duration produced by surgical reductions in renal mass. At 48 hours bilaterally

nephrectomized rats had a two-fold increase in ANP. Rats subjected to 5/6 nephrectomy had no change in ANP at 1,2,3 and 4 weeks postoperation, but had a six-fold increase in ANF at five months. These changes in ANP were not directly related to the degree of fluid retention or the severity of renal failure or hypertension. Clearly more work is necessary in this area. Measurements of ANP in man have indicated that just as in the rat, it takes large infusions of volume to produce significant increases in circulating levels of ANP. While spontaneous atrial tachycardia has been reported to increase levels of ANP in man, we were unable to show any increase in ANP during infusions of isoproterenol that produced a doubling of heart rate. In studies of ANF binding, we have been able to demonstrate specific binding in both the stellate and celiac ganglia, subfornical organ, choroid plexus, area postrema and in the pituitary. We have also shown ANF binding in the medulla and outer cortex of the kidney. This is of great interest, as all of these areas have been demonstrated to be involved in circulatory control.

III. Studies in spontaneously hypertensive rats. We have shown in the past that dietary protein is able to retard the rate of rise of blood pressure and the increase in calcium uptake activity of the sarcoplasmic reticulum from hypertensive hearts. We therefore examined the mechanism for such dietary effects with emphasis on the influence of diet on the activities of protein kinase. In the presence of cAMP, the activity of the Type I protein kinase in the low protein diet group was only 25-33% of that of the SHR on either high protein or a methionine-supplemented diet. Type II protein kinase exhibited similar activities in the presence and absence of cyclic AMP regardless of the diet. cAMP-binding activities in the cardiac fractions from animals on the standard control diet and the high-protein and methionine-supplemented diets correlated with protein kinase activities; however, cAMP-binding activities in the cardiac fractions from the low-protein diet group were higher than enzyme activities. Such changes in protein kinase isozymes may affect the degree of phosphorylation of cardiac regulatory proteins and might thus explain the impaired cardiac physiology noted in the increased stiffness, impairment of diastolic function and left ventricular hypertrophy of hypertension.

IV. Studies of receptor regulation and function. The glutamate and adenosine-type receptors are apparently supramolecular entities, consisting of a transmitter recognition site coupled to a calcium channel. Thus we have been able to show that 3H-nitrendipine binds with high affinity to brain membranes and its binding sites seem to be part of the voltage-dependent calcium channel complex. In rat brain, these CA2+ channel complexes are mainly located in intrinsic neurons. After the injection of kainic acid, an endogenous ligand that binds to a receptor of its own, into the caudate nucleus of rat brain, the number of nitrendipine binding sites and the veratridine-elicited increase of calcium uptake were ablated. After long-term treatment of mice with a calcium channel blocker, the density of nitrendipine binding sites was significantly reduced in caudate nucleus, hippocampus, and cerebral cortex. Further evidence indicates that there is an endogenous ligand in rat brain that modulates nitrendipine binding sites. Attempts to isolate and characterize this endogenous ligand are underway. Studies of γ-aminobutyric acid (GABA) receptors in canine adrenal glands have indicated that catecholamine release may be triggered by direct stimulation of GABA-receptors on chromaffin cells, presumably causing membrane depolarization by a burst of chloride channel opening. This depolarization of chromaffin cells may be responsible for obtunding the subsequent depolarizing effect of

nicotinic receptor stimulation. We have studied the effect of the drug BHT 920, which modulates dopaminergic transmission by selectively regulating tyrosine hydroxylase activity. When BHT 920 was injected subcutaneously into the rat, it produced a dose dependent reduction in striatal levels of tyrosine hydroxylase activity. The drug had no effect on enzyme activity in other brain areas. The effect in the striatum was attenuated by pretreatment with the dopaminergic blocker, haloperidol. This suggests that BHT 920 acts specifically on D2-dopamine receptors. These findings are important since they show that BHT 920, which is highly effective in the treatment of schizophrenia, can selectively slow down the rate-limiting step of dopamine biosynthesis. This gives credence to the view that schizophrenic symptoms may be due to impaired dopaminergic transmission. Studies have shown the development of neuropeptide Y (NPY)-containing neurons in the developing rat fetus. Studies of the mRNA for NPY indicate that the arcuate nucleus of the hypothalamus is the key region for the central actions of this peptide. NPY and NE are contained within and co-released from sympathetic nerves innervating vascular and cardiac tissues. Our work in the rat shows that NPY decreases cardiac output, stroke volume and heart rate and increases mean arterial pressure and total peripheral resistance. Thus NPY possesses both negative inotropic and chronotropic activity and may modulate the cardiac response to NE which has both positive inotropic and chronotropic activity.

V. The kallikrein-kinin system. The precise role for the kallikrein-kinin system in blood pressure regulation, circulatory homeostasis and renal function remains unknown. The catholic strain of Brown-Norway rat lacks the substrates for kinin generation, i.e., both high and low molecular weight kininogen and could therefore serve as an excellent model for studies of the physiology and pathophysiology of the kallikrein-kinin system. However, when we characterized the KKS system in the catholic strain of the Brown-Norway rat, we found that the urinary and plasma levels of glandular kallikrein are not different from the normal Brown-Norway rat. Also the urinary kinin levels are normal during salt restriction. This may be related to the activation of a third kininogen (T-kininogen) which is present in these rats. We are currently involved in determining the enzyme responsible for the physiologic activation of T-kininogen, as well as the metabolic fate of the generated T-kinin. We will be comparing the response of various inflammatory stimuli in the catholic strain of Brown-Norway rats vs. the normal intact BNR. We have made one other interesting finding: T-kinin has biologic activities similar to bradykinin in both the rat uterus and guinea pig ileum. However, the effects of T-kinin are not simply additive to bradykinin, but rather T-kinin appears to modify the effects of bradykinin. This causes us to generate a new hypothesis in which simultaneously generated similar peptides (kinins) can modulate the effects of each other with a physiologic stimulus/effect coupling that would partially depend on the different metabolism of the individual peptides involved. Further work is underway to explore this possibility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01965-03 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Biochemical Methods for Vasoactive Substances

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

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	J. Tate	Bio. Lab. Tech.	HE NHLBI
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	H.R. Keiser	Chief	HE NHLBI
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COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD

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.)*

We have established methodology for measuring plasma, urine, tissue, and cerebrospinal fluid levels of the catecholamine precursor dihydroxyphenylalanine (DOPA), the catecholamines norepinephrine (NE), epinephrine (E), and dopamine (DA), and the deaminated catecholamine metabolites dihydroxyphenylglycol (DHPG) and dihydroxyphenylacetic acid (DOPAC) simultaneously using liquid chromatography with electrochemical detection (LCED). We also have developed radioimmunoassay methodology for measuring specifically the 1-28 form of human ANP and measuring angiotensin II (AII) using liquid chromatography with radioimmunoassay (LC/RIA). Plasma DOPA derives extensively from sympathetic nerve endings and may indicate activity of the rate-limiting step in catecholamine biosynthesis. Simultaneous measurement of DHPG and NE provides more information about noradrenergic function than measuring either substance alone. Immunoreactive ANP increases with salt loading and decreases with standing upright, and there is an arteriovenous decrement in immunoreactive ANP in the human arm.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01968-03 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Collaborative Studies of Neuroendocrine Pharmacology and Physiology

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

PI:	David S. Goldstien	Senior Investigator	HE NHLBI
Others:	R. Zimlichman	Visiting Associate	HE NHLBI
	R. Stull	Chemist	HE NHLBI
	S.D. Averbuch	Staff Investigator	CPB COP DCT NCI
	D.T. George	Staff Fellow	LCS NIMH
	W.H. Kaye	Staff Associates	LCS NIMH
	H.R. Keiser	Chief	HE NHLBI
	I.J. Kopin	Scientific Director	OD NINCDS

COOPERATING UNITS *(if any)*

Surgery Branch, NCI, Neurobiology and Anesthesiology Branch, NIDR; Children's Hospital, Washington, D.C.; Univ. of Michigan; Dept. of Neurol., USUHS.

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Using methodology we developed we found increasd plasma DOPA in all patients studied with neuroblastoma and in most patients with malignant pheochromocytoma. DOPA, norepinephrine (NE), and dopamine (DA) are detectable in vivo in human cerebral cortex, and levels of these substances are increased in epileptogenic foci. Neuroendocrine responses to a real-life stress (removal of third molars) in humans include increases in plasma beta-endorphin NE, but only the beta-endorphin response is attenuated by pre-treatment with naloxone. Bulimics hve decreased baseline levels NE, with normal responses of NE and augmented responses of heart rate to isoproterenol, suggesting decreased sympathetic neural activity and up-regulated cardiac beta-1 adrenoceptors. During surgical stress in adrenalectomized monkeys, ewnhanced sympathetic neural activity maintains circulatory homeostasis. Angiotensin II increases intracellular calcium and stimulates catecholamine release from cultured bovine adrenomedullary cells, providing the first demonstration that adrenomedullary cells contain functioning AII receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01989-02 HE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hormonal responses to salt in normal and essential hypertension

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

PI: J.R. Gill, Jr. Senior Investigator HE NHLBI

COOPERATING UNITS *(if any)*
Dept. of Psychiatry, USUHS, Bethesda, MD (C.R. Lake)
Dept. of Pathology, St. Paul-Ramsey Med. Ctr., St. Paul, MN (D.J. Lakatua)

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION
Experimental Therapeutics

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In previous studies of normal renin essential hypertension two subsets were identified. One subset showed salt-sensitivity of blood pressure, subnormal stimulation of plasma renin activity, normal urinary dopamine, supranormal cumulative sodium retention and no change in plasma norepinephrine during a high sodium intake. The other subset showed salt-insensitivity of blood pressure, normal stimulation of plasma renin activity, supranormal urinary dopamine, normal cumulative sodium retention and a significant decrease in plasma norepinephrine during a high sodium intake. Since mean urinary dopamine correlated inversely with both cumulative sodium retention and the percent change in plasma norepinephrine, the differences between these two subsets appeared to be attributable, in part, to differences in dopaminergic activity. Results of similar studies of normal subjects indicate that some, like the hypertensives, show the phenomenon of salt-sensitivity of blood pressure. compared to the salt-resistant normal subjects the salt-sensitive normal subjects had a lower stimulated plasma renin activity, lower urinary dopamine, greater cumulative sodium retention and a higher plasma norepinephrine that showed a smaller change in response to a high sodium intake. These initial findings suggest that salt-sensitive normal subjects are qualitatively similar to patients with salt-sensitive hypertension in many respects and many represent the population at risk for development of salt-sensitive essential hypertension. The results also suggest that many features that characterize the salt-sensitive hypertensive subjects represent modifications of normal physiology and do not appear to be acquired as a consequence of the hypertensive process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01990-02 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Clinical Physiology of Hypertension.

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:	C.J. Folio	Clin. Nurse Tech.	OD, NHLBI
	R. Chadwick	Biomedical Engineer	BEIB, DRS
	B. Chidakel	Electronics Technician	BEIB, DRS
	M. Maxwell	Biomedical Engineer	BEIB, DRS
	H. R. Keiser	Chief	HE, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

Experimental Therapeutics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have found that young patients with essential hypertension have defective modulation of the brachial arterial dicrotic wave. A circulatory model which we developed explains the abnormality in terms of increased arterial rigidity and decreased vasodilator responsiveness. The magnitude of the defect was related to sympathetic neural activity as reflected by arterial plasma concentrations of norepinephrine (NE), the sympathetic neurotransmitter. Using arterial pulse wave velocity and forearm plethysmography to indicate brachial arterial stiffness and mean arteriolar caliber, we plan to determine the separate contributions of arterial rigidity and vasodilatory failure to the vascular abnormalities which occur early in the development of essential hypertension. Pilot results suggest the possibility that arterial stiffness -- true arteriosclerosis -- may cause overestimation of intra-arterial pressure based on measurements by cuff, and this stiffness may have a neurogenic component.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01991-02 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Plasma Catecholamines and Sympathetic Activity in Clinical Hypertension

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:	R. Zimlichman	Visiting Associate	HE NHLBI
	R. Stull	Chemist	HE NHLBI
	G. Eisenhofer	Visiting Fellow	NINCDS
	B. Chidakel	Electronics Technician	BEIB DRS
	C. J. Folio	Nurse	OD NHLBI
	I. J. Kopin	Scientific Director	OD NINCDS
	H. 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:

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 developed and applied methodology for measuring regional and total body release and neuronal uptake of norepinephrine (NE), the sympathetic neurotransmitter, and have assessed the effects of various stimuli (isoproterenol, clonidine, manipulations of dietary salt) on sympathetic activity. Total body spillover of NE into the circulation is increased by playing an electronic game, which also increases blood pressure, pulse rate, cardiac output, and forearm blood flow. Applying these methods to patients with essential hypertension should allow testing of the long-standing hypothesis that excessive sympathetic responses to mental challenge characterizes young patients with hypertension or subjects at risk for developing established hypertension. The clonidine suppression test can be conducted without controlling dietary salt intake and can be used to define the neurogenic contribution to hypertension. Isoproterenol stimulates NE release without affecting plasma epinephrine (E) and may therefore be helpful in studying pre-synaptic beta-adrenoceptor function in hypertensive patients. Circulating beta-adrenoceptor agonists do not increase adrenocorticotropin (ACTH).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01992-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Renal Vasoconstriction by Acetylcholine in Indomethacin-treated dogs

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

PI:	J. Yun	Guest Worker	HE NHLBI
Others:	J.R. Gill, Jr.	Senior Investigator	HE NHLBI
	H.R. Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

Experimental Therapeutics

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 mechanism for the renal vasoconstriction induced by acetylocholine (ACh) in idomethacin (Indo)-treated dogs was examined in dogs receiving an infusion of either verapamil (V) or dibutyryl cyclic AMP (db-AMP). Renal arterial infusion of V (60 ug/min) attenuated, but did not eliminate, the fall in renal plasma flow induced by ACh (40 ug/min) in Indo-treated dogs (5 mg/kg). Renin secretory rate, however, did not show a rise. Renal arterial infusion of db-cAMP (6 mg/min) restored the natriuretic and vasodilatory effects of ACh in Indo-treated dogs. The data suggest that ACh causes an increase in intracellular CA++ concentration by stimulating Ca++ influx and the release of Ca++ from intracellular storage sites in Indo-treated dogs. The increase in cytosolic Ca++ concentration then leads to contraction of vascular smooth muscle resulting in renal vasoconstriction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 HL 01993-01 HE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptor alterations induced by acute methylene dioxymethamphetamine administration.

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

PI: D.R. Gehlert Staff Fellow NIGMS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION
Experimental Therapeutics

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

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

I have found that animals treated acutely with psychotic agent methylene dioxymeth-amphetamine (MDMA, "Ecstasy") undergo a number of neurochemical alterations in the brain. Acute administration of MDMA results in a rapid decrease in the levels of serotonin and its metabolites in the brain. In addition, dopamine concentrations rapidly increase. These alteractions persist for up to 1 week after administration. In response to these changes in transmitter levels I have seen a selective and persistent increase in serotonin type-1 receptor binding, while dopamine type-1 receptors show a marked decrease in binding. No change is seen in seratonin type-2 receptor binding. These results indicate that MDMA administration may result in neurotoxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01994-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Localization of neuropeptide Y mRNA by in-situ hybridization

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

PI: D.G. Gehlert Staff Fellow HE, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

Experimental Therapeutics

INSTITUTE AND LOCATION

NHLBI, 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.)*

I have demonstrated the distribution of neuropeptide Y (NPY) mRNA in the brain. NPY mRNA containing cell bodies are found primarily in the arcuate nucleus of the hypothalamus but can also be found scattered in the cortex, caudate-Putamen and nucleus accumbens. The distribution of NPY mRNA containing neurons correlates well with the distribution of immunohistochemically detected NPY. These results indicate that the arcuate nucleus of the hypothalamus is a key region for the central actions of NPY.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01995-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanisms of uptake of Norepinephrine in adrenergic storage vesicles in situ

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

PI: Dr. Donald F. Bogdanski Pharmacologist HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Biochemical evidence has been adduced to support the hypothesis that adrenergic neurosecretion can be mediated by the outward transport of norepinephrine (NE) from synaptic vesicles that are fused or attached to the plasma membrane of the nerve ending. Moreover, theories of uptake and retention of NE by isolated vesicles incubated in sucrose-based media seem to be verified by our experiments in which the vesicles are located within the nerve endings in heart ventricle slices in vitro. The results suggest that a driving force for the uptake and retention of NE by vesicles is the electro-chemical gradient of H+ established by the activity of Mg-ATPase. However, these effects of Mg-ATPase activity in situ occur in the presence of high concentrations of Cl-. In isolated vasicles incubated in sucrose with ATP, Cl-inhibits uptake and induces lysis of vesicles. The uptake of NE by vesicles in situ is blocked by Li+, N-ethylmaleimide (NEM), and Dicyclohexylcarbodiimide (DCCD) which are all inhibitors of Mg-ATPase. Uptake is also inhibited by 2,4-dinitrophenol (2,4-DNP) a dissipator of H+ electrochemical gradients, (NH4)2SO4 which neutralizes an established pH gradient across the vesicle membrane, and nigericin, which facilitates an electrically neutral exchange of H+ and K+ across the membrane. Apparently, non-exocytotic secretion can occur in the presence of physiological concentrations of Cl-.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01996-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Modification of ANF and AII receptors in hypertension

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

PI:	C. Gonzalez	Visiting Fellow	HE, NHLBI
Others:	J.M. Saavedra	Chief, Unit on Preclinical Neuropharmacology Section on Clinical Pharmacology	LCS, NIMH
	H.R. Keiser	Chief	HE, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

Experimental Therapeutics

INSTITUTE AND LOCATION

NHLBI, 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.)*

We have been studying the presence of receptors for atrial natriuretic factor (ANF) and Angiotensin II (AII) in the central nervous system (CNS), and in several peripheral tissues in a high renin hypertensive rat model (2 kidney, 1 clip) as well as in the human adrenal gland and pheochromocytoma.

We found specific ANF and AII binding on several areas of the brain that are associated with circulatory control and we are working to determine if there is any difference in this binding between control and hypertensive animals.

In the human adrenal gland preliminary results suggest that there are ANF receptors in the glomerulosa of the adrenal gland but they are absent on the medulla. We plan to characterize this receptor further.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01997-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The role of K+ channels on catecholamine secretion

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

PI:	C. Gonzalez-Garcia	Visiting Fellow	HE NHLBI
Others:	H. R. Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	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.)*

Preliminary results suggest that isolated and cultured bovine chromaffin cells possess a potassium conductance that plays an important role in the maintenance of resting membrane potential. Blockade of this current would lead to depolarization of the chromaffin cell and opening of voltage-dependent calcium channels, calcium entry and catecholamine(ca) secretion. The fact that CA secretion evoked by tetra ethylammonium (TEA) from chromaffin cells is calcium-dependent, blocked by high magnessium and organic calcium antagonists supports the previous sequence of events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01998-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Biochemical changes in hearts of spontaneously hypertensive rats following various dietary proteins

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

PI: Martina Diolulu Research Fellow HE NHLBI

Other: Donald F. Bogdanski Pharmacologist HE NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NIH, NHLBI, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	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.)*

cAMP-dependent protein kinase isozymes (Type I and Type II) were isolated from the soluble fractions of cardiac tissue from 10-month-old spontaneously hypertensive rats (SHR) which had been maintained for nine months on one of four experimental diets: low protein (LP) (19% protein), standard (STD) (24% protein), high protein (HP) (32% protein) or high methionine (1.9% methionine) (MET) by DEAE-cellulose chromatography. The activity and/or levels of these isozymes were determined to examine the influence of diet on the enzyme's effect on the phosphorylation of cardiac regulatory proteins. In the presence of cAMP the activity of the Type I protein kinase was reduced by 3 and 4-fold in the LP diet group compared to the SHR on 32% and methionine diet groups respectively. Type II protein kinase from all four diet groups exhibited similar activities in the presence and absence of cAMP. While cAMP-binding acivities in the cardiac fractions from STD, HP and MET groups of rats correlate to protein kinase activities, cAMP-binding activities in the cardiac fractions from the LP group of rats by contrast were higher than enzyme activities. In the phospholamban (sarcoplasmic reticulum (SR) protein whose phosphorylation regulates CA2+ transport mediated by [Ca2+-Mg2+]-ATPase) phosphorylation study, addition of cAMP significantly stimulated phospholamban phosphorylation in all diet groups but the extent of stimulation was highest in the MET group of animals and lowest in the LP groups was not significant. The decrease in the activity of Type I isozyme found in the cardiac fractions from SHR fed low protein diet may be due to a defect in response of the enyme to cAMP or a reduction in the number of enzyme molecules. Such a condition may affect the degree of phosphorylation of cardiac regulatory proteins, thus impairing cardiac physiology in hypertension.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01999-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The Kallikrein-Kinin System in Circulatory Homeostasis and Inflammation

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

PI:	Peter Ohman	Visiting Associate	HE NHLBI
Others:	E. Marks	Guest Worker	HE NHLBI
	H.R. Keiser	Chief	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The precise role of kinins in blood pressure regulation, circulatory homeostasis and renal function is unknown. The Brown Norway Rat (BNR) / Catholic strain lacks the substrates for kinin generation, i.e., high and low molecular weight kininogen (HMWK and LMWK) and could serve as an excellent model for studies of the physiology and pathophysiology of kinins. The Catholic strain has not been completely characterized regarding the different components of the kallikrein-kinin system.

We have found that the urinary and plasma glandular kallikrein levels are not different in the Catholic strain from normal BNR. Also the urinary kinin levels are normal during salt restricted conditions. This may be related to the activation of a third kininogen (T-kininogen) which is present in rats, also in both strains of the BNR. The enzyme responsible for the physiological activation of T-kininogen is unknown as is the metabolic fate of the generated T-kinin, which we are now investigating.

The acute response of the kinin system to inflammatory stimuli in the BNR/Catholic strain is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02000-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Tissue and Cellular Interactions Between Bradykinin and T-Kinin

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

PI:	Peter Ohman	Visiting Associate	HE NHLBI
Others:	Daniel Goldstein	Guest Worker	HE NHLBI
	H. R. Keiser	Chief	HE NHLBI
	T. Ropchack	Biologist	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The substrates for the generation of kinins by kallikrein are high (HMWK) and low molecular weight kininogen (LMWK) in humans and all animals examined except for the rat. The rat has a third trypsin activatible kininogen which generates T-kinin (Ile-Ser-Bradykinin). The pharmacological effects of T-kinin in relation to and combined with Bradykinin (BK) have been investigated in whole animal and bioassay preparation. Preliminary results indicate that T-kinin is biologically active but that the effects are not simply additive to BK but rather that T-Kinin modifies the effects of BK. This gives rise to a new hypothesis in which simultaneously generated similar peptides (kinins) can modulate the effects of each other, with a physiological stimulus/effect coupling that would partially depend on the different metabolism of the peptides involved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03520-06 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Dopamine Receptor Regulation in Schizophrenic Illness

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

PI:	Ingeborg Hanbauer	Parmacologist	HE NHLBI
Others:	H. Michael Jennewein	Guest Scientist	HE NHLBI
	Enrico Sanna	Guest Scientist	HE NHLBI
	Eleanor Bruckwick	Chemist	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NIH, NHLBI, 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.)*

Radiological binding studies with BHT 920 indicated a specific interraction of this compound with D2 dopamine receptor in caudate nucleus of nets. Furthermore, BHT 920, when injected subcutaneously, reduced tyrosine hydroxlase activity specifically in stratal tissue. This decrease in enzyme activity was attenuated by haloperidol. BHT 920 failed to interact with postsynaptic dopamine receptor and did not change basal or dopamine-sensitive adenylate cyclase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03552-02

PERIOD COVERED

October 1, 1986 to September 30, 1986

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

Regulatory Mechanisms for Voltage-dependent CA2+ Channels in Rat Brain.

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

PI:	Enrico Sanna	Guest Scientists	HE NHLBI
Others:	Jack Grebb	Clinical Associate	PP NIMH
	Ingeborg Hanbauer	Pharmacologist	HE NHLBI
	Arthur G. Wright	Chemist	HE NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NIH, NHLBI, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	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.)*

3H-Nitrendipine binds with high affinity to brain membranes, and its binding sites seem to be part of the voltage-dependent calcium channel complex.

In rat caudate nucleus, these (Ca2+ channel complex) are mainly located in intrinsioc neurones; after injection of Kainic acid in caudate nucleus, the number of 3H-nitrendipine binding sites and the veratridine-elicited increase of 45Ca2+ uptake were ablated.

After long-term treatment of mice with Nifedipine or Verapamil, the density of 3H-Nitrendipine binding sites was reduced by 40% in caudate nucleus, hippocampus and cerebral cortex.

Previous evidence indicates the presence in rat brain of an endogenous ligand that modulates 3H-Nitrendipine binding sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03553-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Anterior pituitary-atrial regulation: A novel endocrine axis

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

PI:	N. Zamir		HE NHLBI
Others:	M. Haass	Visiting Fellow	NIB NINCDS
	Z. Zukoowska-Grojec	Guest Researcher	

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Hypertension-Endocrine

SECTION

INSTITUTE AND LOCATION

NHLBI, 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.)*

Mammalian atrial myocytes contain biologically active peptides within specific secretory granules. These peptides collectively termed atrial natriuretic peptides (ANP), have potent natriuretic, diuretic and vascular smooth muscle relaxant activities and thus are of potential importance in controlling blood pressure. Little is known about the regulation of ANP secretion into the blood stream. Atrial distension by increased perfusion pressure causes release of ANP in rat heart-lung preparation, and acute volume expansion in rats also causes a marked increase in circulating ANP. Administration of pharmacological doses of arginine-Vasopressin and oxytocin induced a profound release of ANP into the circulation. The stimulated release of ANP apparently was related to increased arterial blood pressure and could be mimicked by bolus injection of the pressor agents angiotensin II and phenylephrine. In a series of experiments we examined the role of the pituitary gland in basal and stimulated (acute volume expansion) release of ANP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03554-01 HE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Effects of Neuropeptide Y on cardiac function

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

PI:	E.S. Marks	Guest Worker	HE, NHLBI
Others:	Z. Zukowska-Grojec	Guest Researcher	NIB, NINCDS
	H.R. Keiser		

COOPERATING UNITS *(if any)*

LAB/BRANCH

Hypertension-Endocrine Branch

SECTION

Experimental Therapeutics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Summary:

Neuropeptide Y (NPY) is a 36 amino acid peptide which is contained in and co-released with norepinephrine (NE) from sympathetic nerves innervating vascular and cardiac tissues. Experiments performed with conscious Sprague Dawley rats demonstrated that an intravenous infusion of NPY decreased cardiac output (CO), stroke volume (SV), heart rate (HR) and increased mean arterial pressure (MAP) and total peripheral resistance (TPR). An infusion of NE increased SV, MAP, TPR, with a decrease in HR and no change in CO. Intraventricular pressure measurements in anesthetized rats showed that with an equivalent increase in MAP, the concomitant increase in left ventricular end diastolic pressure was twice as great with NPY as compared to NE. NPY decreased dP/dt while NE induced a significant increase.

In contrast to NE, NPY possesses both negative inotropic and chronotropic activity and may modulate the cardiac response to NE.

SYY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03555-01 HE

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cardiac effects of Atriopeptin III and its plasma level in renal failure

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

PI:	E.S. Marks	Guest Worker	HE, NHLBI
Others:	Z. Zukowska-Grojec	Guest Researcher	NIB, NINCDS
	K. Peter Ohman		
	D. Goldstein		
	N. Zamir		
	H. Keiser		

COOPERATING UNITS *(if any)*

LAB/BRANCH
Hypertension-Endocrine Branch

SECTION
Experimental Therapeutics

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

CHECK APPROPRIATE BOX(ES)

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

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

Summary:

Extracts from mammalian atrial tissue contain peptides referred to as atrial natriuretic factors(s) (ANF) that possess natriuretic, diuretic, and vasorelaxant properties. ANF circulates in the blood and the measured level appears to be related to volume homeostasis. Our studies have shown that the decrease in mean arterial pressure produced by atriopeptin III (AP III) is due to a decrease in cardiac output secondary to a fall in stroke volume caused by lowered ventricular filling pressure. Preliminary data indicate that exogenous atriopeptin III enhances baroreceptor sensitivity as tested by phenylephrine infusion.

Experiments designed to define the ANF and catecholamine responses to renal failure of differing severity and duration caused by reduction in renal mass demonstrated that renal failure when chronic (5 months) is associated with increased ANF and norepinephrine. Acute renal failure (4 weeks) induced by partial nephrectomy did not increase levels of ANF while an increase did occur at 48 hours following bilateral nephrectomy. Adrenergic activity was increased in both clinical situations.

ANNUAL REPORT OF THE
LABORATORY OF KIDNEY AND ELECTROLYTE METABOLISM
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
October 1, 1985 to September 30, 1986

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 control transport and metabolism related to transport.

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 during the past year using this method are as follows:

Nonoguchi, Manganiello and Knepper measured the effect of atrial natriuretic factor (ANF) on accumulation of cyclic guanosine monophosphate (cGMP) in isolated nephron segments and glomeruli from rat kidneys. ANF is a peptide hormone that plays a role in the regulation of sodium chloride excretion by the kidney. cGMP accumulation in inner medullary collecting ducts increased greatly in response to ANF. The threshold concentration of ANF for the effect was similar to the normal plasma concentration of ANF. These studies identify the inner medullary collecting duct as a site of action of ANF, and point to cGMP as the second messenger.

Garvin, Burg, and Knepper investigated transepithelial ammonia secretion in isolated perfused rabbit proximal straight tubules. Ammonia excretion during antidiuresis requires that it be concentrated in the urine. This is achieved by countercurrent multiplication of ammonia in the renal medulla. Countercurrent multiplication requires both reabsorption of ammonium by the ascending limb of Henle's loop (previously demonstrated in this laboratory) and secretion of ammonium into the descending limb. The proximal straight tubule is part of the descending limb. The investigators found that proximal straight tubules spontaneously acidified their luminal fluid and secreted ammonia into the lumen. Because of the acidification, NH_3 was lower in the lumen than in the peritubular bath, providing a gradient for passive ammonia secretion. The measured NH_3 permeability was high enough to account for all of the ammonia secretion by passive diffusion. NH_4^+ permeability was much lower, but still substantial. Thus, in proximal straight tubules spontaneous ammonia secretion occurs by passive NH_3 diffusion, and the NH_4^+ permeability is high enough that significant passive backflux of NH_4^+ also occurs.

Kurtz, Star, Balaban, Garvin and Knepper compared acid-base transport in the middle part (S-2) of rabbit proximal tubules to that in the last part (S-3). Proton secretion in S-2 lowered luminal bicarbonate well below the level in the peritubular bath. There was no disequilibrium pH, implying that S-2 like S-1 (the first part) has endogenous luminal carbonic anhydrase. In S-3, on the other hand, bicarbonate concentration did not fall below that of the bath, but luminal pH did fall approximately 0.5 units, owing to a pH disequilibrium. In contrast to S-2, therefore, S-3 lacks endogenous luminal carbonic anhydrase. S-3 secreted ammonia spontaneously as a result of its disequilibrium pH. Thus, the acidic luminal disequilibrium pH in S-3 should enhance countercurrent multiplication of ammonia in the intact kidney by increasing ammonia secretion into the descending limb of Henle's loop.

Star, Burg, and Knepper compared acid-base transport of rabbit medullary outer stripe collecting duct segments to that of inner stripe collecting duct segments. Ammonia secretion is important in collecting ducts because it is the final step in renal ammonia excretion. Both segments reabsorbed bicarbonate at similar high rates. Both also secreted ammonia, but the rate in the outer stripe was three times faster than in the inner stripe. Outer stripe segments generated a large acidic disequilibrium pH in the lumen, but there was no disequilibrium in the inner stripe. Thus, inner stripe, but not outer stripe medullary collecting ducts have endogenous luminal carbonic anhydrase. The lower luminal pH in the outer stripe segment (due to pH disequilibrium) accounts for its higher rate of ammonium secretion.

Strange and Spring developed and utilized a computer controlled, video, light microscope technique to measure the size and shape of the cells in isolated perfused rabbit cortical collecting ducts. By following the rate of change of cell volume in the first seconds after a step change in the concentration of the perfusate or bath, they measured the osmotic water permeability of the apical and basolateral membranes of the two cell types (principal and intercalated) in this epithelium. The basolateral water permeability was very high in both cell types, regardless of the conditions studied. The apical water permeability, on the other hand, was low until stimulated by vasopressin. These are the first precise direct measurements of this important parameter in cortical collecting ducts.

Leader and Spring studied ion transport mechanisms in the malpighian tubule of the mosquito, Anopheles. Ion transport is of interest in this tissue because these insects carry malaria, and the development of the parasites depends on the ionic composition of the insect's body fluids. The malpighian tubules control the ionic composition of the body.

Regulation of cell volume and solute 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 some 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 studied solute and water transport by Necturus gall bladders and toad skins. In epithelia that transport large quantities of salt and water the cells are subject to significant osmotic stress. Therefore, the mechanisms which minimize changes in cell size and shape while solutes and fluid are moving in and out of them are important. The investigators have developed and used a combination of light microscopic, video, computer, and electrophysiologic methods to study cell volume and intracellular ions. They found that the epithelial cells in these tissues had high osmotic water permeability, and that the cell volume was directly related to cell solute content. They have therefore been analyzing the factors which control solute movements across the cell membranes.

Hermannson and Spring analyzed the mechanisms for K entry and exit from Necturus gallbladder epithelial cells. They found K channels in both apical and basolateral cell membranes but no evidence of carriers that cotransport K. They showed that cell swelling in high K solutions was due to the depolarizing effect of K and not to K entry into the cells, as previously believed.

Larsen, Ussing and Spring investigated the route of NaCl transport across toad skin, using optical techniques. They found that sodium passed through one kind of cell and chloride through another kind of cell. Sodium was transported through the principal cells, always present in the frog skin. Chloride, on the other hand, passed through specialized cells rich in mitochondria. The number of mitochondrial rich cells varied with the demand for NaCl transport.

Sands, Spring, and Knepper adapted the techniques developed for other planar epithelia to study ion transport in the papillary surface epithelium of the rabbit kidney. They found that Na and K were transported actively out of the cells by Na-K-ATPase present in the basolateral cell membranes. Na and K entered the cells via a coupled Na-K-Cl pathway in the apical membrane. The latter transporter was inhibited by as little as 1 nanomolar bumetanide.

Harris and Handler are isolating the water permeability channels that are inserted into the apical plasma membrane of toad urinary bladder epithelial cells in response to vasopressin. The channels are contained in intracellular vesicles (aggrephores) that undergo vasopressin-induced cycles of fusion with the apical plasma membrane. The investigators first characterized the cycling of aggrephores in vasopressin treated cells, identifying the aggrephores by otherwise impermeant marker molecules that the aggrephores took up when they left the apical surface following vasopressin withdrawal. Of interest, the aggrephores (and their water channels) were endocytosed when the transepithelial water flow caused by vasopressin was large. This is a feedback mechanism that serves to limit water flow through the cells when there are large osmotic gradients. The investigators isolated the marked aggrephores from broken cell preparations, using a new technique of density-shift gradient centrifugation. The purified vesicles contained only few protein bands (including, presumably, the water channels) on SDS-polyacrilamide gel electrophoresis. Antibodies are being prepared against the proteins to identify and further purify the channels.

Cell culture of epithelia. Although the technique of perfusing kidney tubules in vitro has provided an overall description of their transport properties, it has been difficult to extend the studies to subcellular and molecular levels. Chemical and physical methods for studying transport require much larger amounts of homogeneous tissue than are present in single tubules. Handler, Burg and their colleagues have been using cultures of renal epithelial cells to overcome this difficulty. In addition, epithelia in culture can be readily maintained for prolonged periods of time under conditions not obtainable in intact tissues, and the cultures are more amenable to study by number of the powerful techniques of cell and molecular biology.

Handler and his colleagues studied epithelia formed by continuous cell lines derived from kidneys. A6 cells (from kidney of Xenopus laevis) expressed transporters similar to those of cortical collecting ducts. Preston and Handler prepared apical plasma membrane vesicles from A6 cells to study renal sodium channels. Treatment of A6 epithelia with vasopressin or aldosterone increased sodium flux through channels in vesicles isolated from the cells. The vesicles are now being used to study the mechanisms by which the sodium flux is controlled. Spiegel, Handler, and Fishman found that application of certain gangliosides to the apical surface of A6 epithelia amplified the increase in sodium transport caused by cyclic AMP. The added gangliosides remained in the apical plasma membrane, suggesting that they directly affected the sodium channels.

Guggino and Green studied potassium channels in GRB-MAL1 epithelia in culture. GRB-MAL1 is a continuous line of cells derived from rabbit thick ascending limbs in this laboratory. Patch clamps and cellular impalements with microelectrodes were used to measure cellular voltages and ion currents. Barium and furosemide changed the cellular voltage in a manner consistent with their known actions on the K channels and Na,K,Cl carriers responsible for transepithelial Na, K, and Cl transport in thick ascending limbs. The K channels were Ca-activated, maxi-K channels.

Burg, Bagnasco, Uchida, Balaban, Bedford and Kador studied the response of GRB-PAP1 cells to hypertonicity. GRB-PAP1 is a continuous line derived in this laboratory from rabbit renal inner medullary epithelium. A strain of these cells (PAP-HT25) grew continuously in hypertonic medium. Non-mammalian cells are known to accumulate osmotically active organic intracellular solutes when their environment is hypertonic. These "osmolytes" protect the cells from dehydration. Most mammalian tissues are not normally hypertonic and do not normally express osmolytes. The exception is the renal inner medulla which is hypertonic as part of the renal concentrating mechanism. Sorbitol was one of osmolytes found in rat and rabbit inner medullas in vivo and was also present in the PAP-HT25 cells growing in hypertonic medium. When the cells were switched from isotonic to hypertonic medium, cell sodium, potassium, and water did not change, but sorbitol accumulated in them. The sorbitol was synthesized from glucose, catalyzed by the enzyme aldose reductase. Cellular aldose reductase activity greatly increased in hypertonic medium, as did the amount of aldose reductase protein. The investigators purified the aldose reductase and prepared antiserum against it. When the hypertonic medium was changed back to isotonic, aldose

reductase decreased slowly over 1 week, but intracellular sorbitol fell within one day because of release of sorbitol to the medium. mRNA from the induced cells is being used to clone the gene for aldose reductase.

Uchida, Coon and Burg studied modifications in phenotype and karyotype that occurred when GRB-PAP1 cells changed to the PAP-HT25 strain in hypertonic medium. The modifications were of interest since they did not revert when the cells were returned to isotonic medium and therefore might represent a model of commitment to differentiation. The PAP-HT25 strain had much larger cells which were often multinuclear and were more resistant to hypertonicity than were the wild type cells. Also, the PAP-HT25 strain also was polyploid, whereas the wild type cells were pseudodiploid. Studies of cloned lines proved that the change was adaptation, not selection. Somewhat similar changes were previously reported following exposure of chick embryo fibroblasts to high NaCl. The chicken cells exhibited persistently altered DNAaseI hypersensitivity, suggestive of structurally altered gene regulation. The investigators propose to elucidate the genetic basis of these changes caused by hypertonicity and to examine primary cultures for similar changes in order to determine whether native inner medullary cells are somehow protected from this phenomenon.

Nakanishi, Balaban, Bagnasco and Burg searched for additional lines of renal cells that might grow in hypertonic medium. In general, only cells that expressed osmolytes and maintained near normal levels of Na, K, and water were able to grow in hypertonic medium. None of these other cell lines accumulated sorbitol as did GRB-PAP1 cells. The osmolytes in the other cells have not yet been completely identified, but include at least betaine, choline, inositol, and various amino acids. The investigators propose to determine the source of these other osmolytes and study how they are controlled.

Metabolism associated with solute transport. A large fraction of the metabolism of renal epithelial cells is utilized to produce energy for transepithelial transport. Balaban and his co-workers have been using the noninvasive techniques of nuclear magnetic resonance and optical spectroscopy to investigate the general mechanisms that coordinate cellular energy metabolism with work in the kidney and heart.

Balaban, Katz, Kantor, Koretsky, Briggs and Metz studied the role of high energy phosphate compounds in hearts. Previously, cardiac energy metabolism was generally believed to change with cardiac work because of alterations in ATP, ADP and creatinine phosphate (CrP). The investigators measured these compounds in intact dog hearts by ^{31}P NMR. Contrary to previous theory, the concentrations of ATP, ADP and CrP did not change during the cardiac cycle or when cardiac work was increased by increasing heart rate. Thus, factors in addition to these phosphorus compounds must regulate cardiac energy metabolism. The investigators then studied NADH by fluorescence spectroscopy to see whether it was involved. They found that both in perfused hearts and isolated cardiac mitochondria the concentration of NADH controlled respiration independent of the levels of ATP, ADP, and CrP under some conditions. The investigators are continuing along these lines to establish the relative importance of the various factors under different conditions.

Balaban and Lynch investigated the importance of respiration versus glycolysis in providing energy for ion transport in cultured epithelial cells. When sodium and potassium transport were inhibited by ouabain, glycolysis decreased more than respiration. Conversely, when transport was stimulated by adding potassium, glycolysis increased more than respiration. Also, when metabolism was shifted from respiration to glycolysis by altering the metabolic substrates, K transport increased. Thus, glycolysis was more closely coupled to sodium and potassium transport than was respiration. The investigators are now studying plasma membranes isolated from these cells to see whether sodium and potassium pumps (Na-K-ATPase) and glycolytic enzymes coexist in the membranes, explaining their functional coupling.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01224-09 KE

PERIOD COVERED

October 1, 1986 to September 30, 1986

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

Control of sodium and potassium transport by the nephron

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

P.I.:	M.A. Knepper	Senior Investigator	LKEM, NHLBI
Others:	Hiroshi Nononguchi	Visiting Fellow	LKEM, NHLBI
	V. Manganiello	Laboratory of Cellular Metabolism, NHLBI	

COOPERATING UNITS *(if any)*

Laboratory of Cellular Metabolism, NHLBI

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:
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.)*

The effects of atrial natriuretic factors (ANF) on cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) production by isolated renal tubules and glomeruli from rats has been investigated. ANF (1 micromolar) increased cGMP accumulation in isolated glomeruli 35-fold and in isolated inner medullary collecting ducts 20-fold. Small increases in cGMP accumulation were seen in response to 1 micromolar ANF in other nephron segments: proximal convoluted tubules, proximal straight tubules, thin descending limbs, medullary thick ascending limbs and cortical collecting ducts. Dose response studies revealed that the threshold for an increase in cGMP accumulation was 0.1-1 nanomolar ANF in inner medullary collecting ducts, and was 10-100 nanomolar in glomeruli. The threshold concentration for a response in inner medullary collecting ducts was approximately the same as reported circulating levels of ANF in control rats. ANF (1 micromolar) did not alter cAMP accumulation in the absence or presence of vasopressin in medullary thick ascending limbs or in inner medullary collecting ducts. Based on these results, we have initiated transport studies in isolated perfused inner medullary collecting ducts to determine whether ANF affects urea, water, or sodium chloride transport.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01237-08 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Hormonal control of transport in kidney epithelia in culture

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

P.I.:	Masahiro Yanase	Visiting Fellow	LKEM, NHLBI
	Agnes S. Preston	Research Chemist	LKEM, NHLBI
Others:	Chester Williams	Biology Lab. Tech.	LKEM, NHLBI
	Sarah Spiegel	Staff Fellow	DMN, NINCDS
	Peter Fishman	Section Chief	DMN, NINCDS
	Joseph S. Handler	Section Chief	LKEM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

Membrane Metabolism Section

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	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 mechanism of action of hormones is studied in epithelia formed in culture. To understand the responses better, the cell biology of the epithelia is studied as well as transport specific events. The amiloride sensitive sodium channel in the apical plasma membrane of A6 epithelia is studied in vesicles enriched in apical membranes. Amiloride sensitive sodium flux is increased in vesicles prepared from epithelia stimulated with vasopressin. The sodium transport response of intact epithelia to vasopressin, cholera toxin, and cAMP is enhanced following the incorporation of exogenous gangliosides into the apical plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01246-06 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Primary and continuous culture of epithelial kidney cells

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

P.I.	Maurice Burg	Chief	LKEM, NHLBI
Other:	Nordica Green	Chemist	LKEM, NHLBI
	Shunya Uchida	Visiting Fellow	LKEM, NHLBI
	Sandra Guggino		NIA, GRC
	Robert Balaban	Staff Fellow	LKEM, NHLBI
	Serena Bagnasco	Visiting Associate	LKEM, NHLBI
	Jenifer Bedford	Guest Worker	LKEM, NHLBI
	Takeshi Nakanishi	Visiting Fellow	LKEM, NHLBI
COOPERATING UNITS *(if any)*	Michael Horster	Visiting Scientist	LKEM, NHLBI
	Peter Kador		LMOD, NEI
	Hayden Coon		LG, NCI

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:
6.5	6.5	

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.)*

Continuous lines of cells have been established in tissue culture from rabbit renal medullary thick ascending limbs and papillary pelvic epithelium and are being used for studies of the their transport function and resistance to hypertonicity. The same methods are being applied to establish continuous lines from other renal epithelia.

561

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01247-06 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Urea transport and the urinary concentrating mechanism

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

P.I.:	Mark A. Knepper	Senior Investigator	LKEM, NHLBI
Others:	Jeff Sands	Medical Staff Fellow	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:
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.)*

The papillary surface epithelium has been proposed to play an important role in the urinary concentrating mechanism. We undertook studies to determine what transporters are present in this epithelium. The papillary surface epithelium was dissected from the surface of the rabbit renal papilla and mounted in a perfusion chamber which allowed both sides to be perfused independently. Cell volume was measured at 25 degrees C using computerized quantitative microscopy which allows continuous measurement of cell volume of the living tissue. Addition of ouabain (0.1 mM) to the basolateral side of the epithelium induced a 20% volume increase. This volume increase was completely inhibited by removal of apical bath NaCl, sodium, chloride, or potassium, but not by removal of urea. As little as 1 nanomolar bumetanide in the apical bath completely inhibited the ouabain-induced cell swelling. Changes in apical osmolality resulting from the addition or removal of NaCl caused cell volume changes that were greater than could be accounted for by osmotic water flow alone. This hyper-response was blocked by bumetanide and was stimulated by vasopressin. These observations are consistent with the presence of Na-K-ATPase on the basolateral membrane and a bumetanide-sensitive, vasopressin-responsive Na-K-Cl cotransporter in the apical membrane. These results are consistent with transpithelial transport of sodium chloride by this epithelium which could modify the composition of the pelvic urine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01250-06 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Acidification and bicarbonate transport by renal tubules

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

P.I.:	M.A. Knepper	Senior Investigator	LKEM, NHLBI
Others:	Jeff Garvin	Guest Worker	LKEM, NHLBI
	Robert Star	Medical Staff Fellow	LKEM, NHLBI
	Ira Kurtz	Visiting Fellow	LKEM, NHLBI
	M.B. Burg	Chief	LKEM, NHLBI
	Raymond Mejia	Mathematician	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:
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.)*

Studies of ammonia and bicarbonate transport are being conducted in isolated, perfused tubules from rats and rabbits. Studies in rabbit proximal straight tubules showed that 1) ammonia secretion occurs spontaneously by diffusion of NH_3 down a concentration gradient generated by luminal acidification; 2) the S-3 portion of the proximal staight tubule generates a spontaneous disequilibrium pH which enhances ammonia secretion; 3) the permeability of the S-2 proximal straight tubule is 2 x 10E-2 cm/s which is adequate to account for ammonia secretion down the measured NH_3 concentration gradient; 4) the NH_4^+ permeability is 5 x 10E-5 cm/s which is consistent with a significant lumen-to-bath backflux of NH_4^+ in vivo. Experiments in outer medullary collecting ducts from rabbits show 1) the outer stripe portion generates a luminal disequilibrium pH which enhances ammonia secretion by increasing the transepithelial concentration difference driving NH_3 diffusion into the lumen; 2) the inner stripe portion does not generate a spontaneous disequilbrium pH despite a rapid rate of proton secretion implying the presence of endogeneous carbonic anhydrase in the apical membrane; 3) both the outer stripe and the inner stripe segments secrete protons at rates that are rapid compared with the cortical collecting duct. Experiments in rabbit cortical collecting ducts show: 1) that this segment lacks endogenous luminal carbonic anhydrase and can generate a spontaneous luminal disequilibrium pH; and 2) that the NH_3 permeability is 7 x 10-3 cm/s, a value high enough to account for ammonia secretion by passive diffusion of NH_3 down a transepithelial NH_3 concentration gradient generated by luminal acidification.

SL7

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01266-04 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters 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:	Kevin Strange	Guest Worker	LKEM, NHLBI
	Hans Ussing	Guest Worker	LkEM, NHLBI
	Erik H. Larsen	Visiting Scientist	LKEM, NHLBI
	John P. Leader	Guest Worker	LKEM, NHLBI

COOPERATING UNITS *(if any)*

Depatment of Biological Chemistry, University of Copenhage; Dept. of Physiology University of Otago.

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:
4.2	4.2	

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 cortical collecting tubule of the rabbit, the toad skin, malpighian tubules, on the renal papillary epithelium, and on mosquito.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01276-02 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The water permeability channel regulated by anti-diuretic hormone.

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

P.I.:	H. William Harris, Jr.	Guest Worker	LKEM, NHLBI
Others:	Helen Murphy	Chemist	LKEM, NHLBI
	Chester Williams	Biologist	LKEM, NHLBI
	James Wade	Associate Prof.	Univ. Maryland
	Joseph S. Handler	Section Chief	LKEM, NHLBI

COOPERATING UNITS *(if any)*

Department of Physiology, University of Maryland School of Medicine

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

Membrane Metabolism Section

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	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.)*

Aggrephores, the vesicles that are thought to contain water permeability channels that are inserted in the apical plasma membrane in response to vasopressin, are studied in toad urinary bladder with the use of macromolecules with fluorescent tags. In addition, membrane proteins are labeled with radioactive iodine to identify proteins in the aggrephore membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01279-01 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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)*

P.I.:	Robert S. Balaban	Research Physiologist	LKEM, NHLBI
Others:	Alan Koretsky	Staff Fellow	LKEM, NHLBI
	Lawrence Katz	Medical Staff Fellow	LKEM, NHLBI
	Ronald Lynch	Guest Worker	LKEM, NHLBI
	Richard Briggs	Associate Professor,	Univ. of PA

COOPERATING UNITS *(if any)*

University of Pennsylvania, Hershey, PA

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
3	3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The control of energy metabolism within intact tissues is being investigated using a variety of techniques and tissues. The relation between the rates of energy conversion, via mitochondrial oxidative phosphorylation and glycolysis, and work output is being measured in the heart in vivo, isolated perfused heart, isolated mitochondria and cultured cells lines. In all of these preparations a tight coupling between the rate of work and the rate of energy conversion has been observed. In order to gain insight into the mechanism of this coupling, several of the key metabolic intermediates are also being determined as a function of work output using non-invasive techniques. Adenosine di- and tri-phosphates, inorganic phosphate, creatine phosphate and pH are being monitored using 31P NMR. Mitochondrial NAD redox state is monitored using fluorescence spectroscopy. Classical models concerning the control of energy conversion within cells involve the intracellular concentrations of adenosine di- and tri-phosphates as well as inorganic phosphate. However, in our in vivo and perfused heart studies we have demonstrated that no change in intracellular adenosine phosphates or inorganic phosphate occurs with large changes in cardiac work output. Further, in both the isolated perfused heart and mitochondria studies we have demonstrated that the redox state of NADH can control the rate of mitochondrial respiration and that the NAD redox state does change appropriately (i.e. becomes more reduced) when the isolated perfused heart is stimulated to do more work. These data suggest that redox state of NAD may be a key intermediate in the coupling of work output with mitochondrial energy converison in the heart.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01280-01 KE

ERIOD COVERED

October 1, 1985 to September 30, 1986

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

Non-invasive techniques for monitoring cellular function and structure

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

P.I.:	Robert S. Balaban	Research Physiologist	LKEM, NHLBI
Others:	Alan Koretsky	Staff Fellow	LKEM, NHLBI
	Larry Katz	Medical Staff Fellow	LKEM, NHLBI
	Robert Bowman	Chief	LTD, NHLBI
	David Lu	Medical Staff Fellow	CB, NHLBI
	Marty Leon		CB, NHLBI

OOPERATING UNITS *(if any)*

AB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

ECTION

ISTITUTE AND LOCATION

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

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

UMMARY 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 cellular structure and function. Two general techniques are being used: Nuclear magnetic resonance(NMR), and optical spectroscopy. Over the last year improvements in the NMR transmitter-receiver coil have been made by charaterizing the effects of surface area contact. In addition, a flexible catheter NMR probe was developed. Using optical spectroscopy, a procedure was developed for the determination of fatty plaques on human artery walls using surface fluorescence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01281-01 KE

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cloning the gene for aldose reductase

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

P.I.:	Arlyn Garcia-Perez	Guest Worker	LKEM, NHLBI
	Shunya Uchida	Visiting Fellow	LKEM, NHLBI
Others:	Toshimichi Shinohara	Section Chief	LMDB, NEI
	Joseph S. Handler	Section Chief	LKEM, NHLBI
	Maurice B. Burg	Laboratory Chief	LKEM, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Kidney and Electrolyte Metabolism

SECTION

Membrane Metabolism Section and Renal Mechanisms Section

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, 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.)*

The project is designed to isolate and study the gene for aldose reductase in the kidney. The starting material is a continuous cell line derived from rabbit renal medulla. The cells produce increased levels of aldose reductase when grown in a hypertonic medium.

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Annual Report
Laboratory of Molecular Cardiology
National Heart, Lung, and Blood Institute
October 1, 1985 through September 30, 1986

Actin and myosin are the two major contractile proteins present in all vertebrate cells. The Laboratory of Molecular Cardiology studies the regulation of these contractile proteins in vertebrate smooth muscle, nonmuscle (e.g. intestinal brush border and platelets) and cardiac muscle cells. We, along with other laboratories, have shown that regulation of contractile activity in smooth muscle and nonmuscle cells is quite different than it is in striated muscle. In the former case contractile activity is initiated by calcium binding to calmodulin and the calcium-calmodulin complex activating the enzyme myosin light chain kinase. This activation results in the phosphorylation of the 20,000-dalton light chain of myosin and is followed by the initiation of contractile activity. In vitro, the phosphorylation of myosin in smooth muscle and nonmuscle cells results in a marked increase in the actin activated MgATPase activity of myosin.

Our laboratory continues to explore the mechanism by which phosphorylation by two different kinases, myosin light chain kinase and protein kinase C regulates the activity of smooth muscle and nonmuscle myosin. In addition we have started research programs in two new areas, closely related to the regulation of contractile proteins. One involves cloning the gene for the enzyme myosin light chain kinase from vertebrate smooth muscle and nonmuscle cells as well as the gene for myosin from vertebrate nonmuscle cells. The second area involves studying the contractile proteins of smooth muscle cells grown in culture in order to understand the various factors regulating myosin and myosin light chain kinase expression. Our purpose is to understand the role of myosin and myosin light chain kinase in regulating contractile activity in smooth muscle cells, where their primary function appears to involve muscle contraction, as well as in nonmuscle cells, where they play an important role in cell motility, cytokinesis and other basic cellular functions. We are also interested in the mechanism by which smooth muscle cells may cease to express smooth muscle myosin (and myosin kinase) and instead express the gene for nonmuscle myosin and myosin kinase. This may relate to the ability of these cells to proliferate under certain pathological condtions.

The regulation of contractile activity of the heart requires an understanding of the mechanism by which calcium and the regulatory proteins troponin-tropomyosin influence the actin-activated MgATPase activity of cardiac myosin. As outlined below, this has also been a major area for research during the past year.

Growth and Differentiation of Smooth Muscle Cells (S. Kawamoto). Smooth muscle cells from a number of different sources, such as rat aorta, vas deferens and uterus, were found to contain two different myosin heavy chains as judged by their migration in 5% polyacrylamide using SDS-polyacrylamide gel electrophoresis. Two-dimensional peptide analysis of

these myosin heavy chains showed no major differences. In contrast, extracts of postconfluent primary cultures of rat aorta cells were found to contain three different myosin heavy chains, including the same two that were isolated from intact rat aorta. The new myosin heavy chain migrated more rapidly than the other two smooth muscle myosin heavy chains and resembled nonmuscle myosin heavy chains when analyzed by two-dimensional peptide mapping. We are studying the various factors influencing the expression of these two different classes of myosin.

Placing rat aorta smooth muscle cells in culture appears to influence the expression of the enzyme myosin light chain kinase, since primary culture cells show evidence for two different molecular weight enzymes (130,000 and 85,000), whereas intact aorta only contains the 130,000-dalton form and late passage (i.e. 30 or more passages) cells, as well as nonmuscle cells, only contain the 85,000-dalton form.

The Role of Protein Kinase C in the Regulation of Contractile Proteins (A. R. Bengur, J. Sellers). The amino acid sequence around, and including, the phosphorylated threonine residue is being determined following phosphorylation of turkey gizzard heavy meromyosin with protein kinase C. The possibility that a serine residue, in addition to the threonine, is also phosphorylated by protein kinase C, is being investigated. The ability of protein kinase C to phosphorylate human platelet myosin was confirmed and studies are underway on the kinetics and effect of this phosphorylation.

Finally dephosphorylation of turkey gizzard heavy meromyosin which has been previously phosphorylated by protein kinase C is being studied. The enzyme being used is a purified phosphatase which does not have activity toward the site phosphorylated by myosin light chain kinase. These studies are being conducted in collaboration with M. Pato (Univ. of Saskatoon).

Role of Phosphorylation as a Regulatory Mechanism in Muscle Contraction (J. Sellers). The mechanism by which phosphorylation acts to regulate the actin-activated MgATPase activity of smooth muscle and nonmuscle myosin is being investigated. Preliminary observations suggest that the regulation of myosin in avian intestinal smooth muscle cells as well as mammalian nonmuscle cells is similar to that found in avian gizzard smooth muscle cells; phosphorylation alters the maximal velocity of the actin-activated MgATPase activity rather than the apparent binding constant of actin for myosin.

The role of the calcium-calmodulin binding protein, caldesmon is being studied in collaboration with J. Lash and D. Hathaway (Univ. of Indiana School of Medicine). Caldesmon has been shown to inhibit the actin-activated MgATPase of smooth muscle myosin while causing a 40-fold increase in the binding constants of both phosphorylated and unphosphorylated myosin for actin.

The phosphorylation-dependent movement of beads coated with smooth muscle and nonmuscle myosin is being studied using actin cables from the alga, Nitella.

Phosphorylation as a Regulatory Mechanism (M. A. Conti). Myosin light chain kinase from a number of sources can be phosphorylated by cAMP-dependent protein kinase with 2 moles of phosphate being incorporated into the enzyme when calmodulin is not bound and 1 mole of phosphate being incorporated when calmodulin is bound. When 2 moles of phosphate are incorporated into myosin light chain kinase there is a decrease in the apparent affinity of this enzyme for calmodulin in vitro. Previous work from this laboratory resulted in elucidation of the amino acid sequence around the serine that is phosphorylated, whether or not calmodulin is bound. Our present studies are directed towards determining the amino acid sequence around the serine that is phosphorylated only when calmodulin is not bound to myosin light chain kinase. These studies are being carried out in collaboration with M. Elzinga (Brookhaven National Laboratory).

An enzyme with a molecular weight of 150,000 that methylates CpG sequences in DNA has been partially purified by T. Bester (MIT). The enzyme can serve as a substrate for cAMP-dependent protein kinase and protein kinase C, but only low levels of phosphate are incorporated. Present studies involve an attempt to dephosphorylate this methylase using a number of different phosphatases. The effect of phosphorylation and dephosphorylation on methylase activity is being studied in collaboration with A. Razin (Hebrew University).

Molecular Cloning of Mammalian Smooth Muscle Myosin Light Chain Kinase (M. Vahey). Two different cDNA clones have been identified using affinity-purified antibodies to myosin light chain kinase, using the expression vector lambda gt11. This library was constructed using rat uterus mRNA. One of these clones contains 630 bp, and has been sequenced (M. Cashell, NICHD). Although the cDNA sequence does not code for any known amino acid sequence of myosin kinase (which is quite limited at present), a number of techniques (epitope selection and hybrid selection) suggest that this cDNA may code for myosin light chain kinase or a smaller, related protein.

Recently a 2.4 kb clone has also been identified, which does not appear to overlap with the 630 bp cDNA described above. The latter clone is presently being characterized. Preliminary experiments using Northern blot analysis suggests that it recognizes an mRNA greater than 5 kb.

The Regulation of Cardiac Myosin (L. Tobacman). The nature of the cooperative activation of cardiac myosin subfragment-1 MgATPase activity by calcium is being studied using a complex of cardiac actin-tropomyosin-troponin. The experiments were designed to exclude myosin subfragment-1 as the cause of the cooperative response, and the source of the cooperativity is being analyzed using the various purifed proteins that compose the thin filament, with emphasis on troponin C, the calcium-binding subunit.

In related experiments, two different isoforms of troponin T were found to give subtly different responses to calcium activation of the actin-activated MgATPase activity. This is the first reported functional difference between these two forms of this regulatory protein. (Work by

others has shown that the amino terminal region of the troponin T subunit can be varied by alternative splicing patterns during mRNA processing). The different responses of the two forms of troponin T to activation by calcium, suggest that the amino terminal region of troponin T modulates the heart's response to calcium.

Regulation of Genes for Contractile Proteins in Muscle and Nonmuscle Cells (L. Weir, R. Shohet, M. A. Conti). Forty positive clones have been isolated from a rat aorta cDNA gt11 library following screening with an antibody to myosin light chain kinase (The library was supplied by B. Nadal-Ginard & M. Taubman, Harvard Medical School). These clones are presently being characterized in an effort to elucidate the structure of the messenger RNA of a nonmuscle and smooth muscle myosin light chain kinase. Previous work from this laboratory has shown that rat aorta smooth muscle cells grown in culture can express both the smooth muscle and nonmuscle forms of myosin light chain kinase. Studies will be carried out to see if a separate gene codes for these two related proteins. We are also studying the possible relationship between the genes for smooth muscle and nonmuscle myosin using human brain and rat aorta cDNA libraries.

A genomic library of mouse DNA was constructed and screened with a rat skeletal muscle myosin cDNA probe and a chicken tropomyosin cDNA probe. Positive clones have been isolated and analysed by restriction analysis and Southern hybridization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01665-11 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Growth and Differentiation of Smooth Muscle Cells

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

Sachiyo Kawamoto, M.D., Ph.D., Visiting Fellow, 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 20205

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.)*

Myosin and the enzyme, myosin light chain kinase, in primary cultures of smooth muscle cells derived from rat aorta have been studied in comparison with those in intact aorta. Postconfluent primary cultures were found to contain three different myosin heavy chains (MHCs) following SDS-polyacrylamide gel electrophores (SDS-PAGE). Based on their migration in SDS-PAGE, antigenicities, and 2-dimensional peptide maps of iodinated MHCs, the two slowest migrating MHCs have similar properties to those found in intact smooth muscle tissues, including rat aorta. The fastest migrating MHC was distinct from smooth muscle myosins but was very similar to nonmuscle myosin prepared from platelets or fibroblasts. At the subconfluent stage, the greater part of MHCs was nonmuscle myosin. These results suggest that smooth muscle cells contain predominantly nonmuscle myosin while actively growing, but at a post confluent stage, contain a mixture of both smooth muscle and nonmuscle myosins. Presently, studies are under way to determine whether a single cell in post-confluent primary culture contain both smooth muscle and nonmuscle myosins. The protein structures of two MHCs of smooth muscle is also being investigated.

Using antibodies to turkey gizzard myosin light chain kinase, two proteins having M_r of 130,000 and 85,000 were recognized on immunoblots prepared from primary culture of rat aorta smooth muscle cells. The 130,000-dalton peptide was also found in the intact aorta. On the other hand, the 85,000-dalton peptide was not detected in intact aorta but was found in nonmuscle cells as well as late passage subcultures of smooth muscle cells. The question of whether the 85,000-dalton peptide is a unique species of myosin kinase or is simply a proteolytic product of the 130,000-dalton enzyme has been raised. The enzymatic properties and protein structure of the 85,000-dalton peptide is under study.

SJB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01785 07 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Myosin phosphorylation in non-muscle cells

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

A. Resai Bengur M.D., Guest Researcher, LMC, NHLBI, Started 7/85
James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

Dr. Etore Apella, NCI,
Elizabeth Robinson, NCI

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20205

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.)*

We are investigating the role of protein kinase C phosphorylation in the regulation of contractile proteins in both smooth muscle and nonmuscle cells. The sequence of the protein kinase C phosphorylation site in the turkey gizzard myosin light chain has been nearly determined. It appears to be either threonine 9 or 10 at the N-terminal portion of the light chain. In two-dimensional tryptic peptide maps of the phosphorylated heavy meromyosin and light chain, we have found that there are two major peptides that are phosphorylated. This is in contrast to previous work in this laboratory that indicated that there was only one major phosphorylated tryptic peptide in HMM.

We have also successfully phosphorylated the nonmuscle myosin isolated from human platelets so as to incorporate 1 mole of phosphate per mole of light chain. The kinetics of phosphorylation appear to be similar to that seen with the smooth muscle myosin from turkey gizzard.

In preliminary studies with M. Pato, we have shown that smooth muscle phosphatase I will dephosphorylate HMM, the soluble two-headed subfragment of myosin, that has been phosphorylated with protein kinase C. This phosphatase does not have activity against HMM phosphorylated with myosin light chain kinase.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01786-07 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Role of Phosphorylation as a Regulatory Mechanism in Muscle Contraction

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

James R. Sellers, Ph.D., Research Biologist, LMC, NHLBI
A. Resai Bengur, M.D., Guest Researcher, LMC, NHLBI
Estelle V. Harvey, Biologist, LMC, NHLBI
William A. Anderson, Jr., Chemist, LMC, NHLBI

COOPERATING UNITS *(if any)*

Dr. Joe Lash, Indiana Univ. School of Medicine
Dr. Dave Hathaway, Indiana Univ. School of Medicine

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	1.0	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 mechanism of the phosphorylation-dependent myosin-linked regulation of smooth muscle and nonmuscle myosins is being investigated. This involves several approaches: (1) measurement of rate constants for various steps in the kinetic cycle for the hydrolysis of MgATP by heavy meromyosin (HMM), the proteolytic subfragment of myosin, in the presence and absence of actin; (2) preparation of HMM from cytoplasmic myosins and characterization of their kinetic properties; and (3) use of an *in vitro* motility system to quantitate how the velocity of movement of myosin-coated beads is affected by various factors. We are also studying the mechanism of action of caldesmon, a possible regulatory protein associated with actin filaments.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04202-05 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)*

Mary Anne Conti, Ph.D., Research Chemist, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI

COOPERATING UNITS *(if any)*

Dr. Marshall Elzinga, Brookhaven National Laboratory
Dr. Timothy Bestor, Massachusetts Institute of Technology
Dr. Aharon Razin, The Hebrew University, Jerusalem, Israel

LAB/BRANCH

Laboratory of Molecular Cardiology

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20205

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.)*

It has been determined by gel filtration under denaturing conditions (70% formic acid) that the 26,000-dalton tryptic peptide of myosin light chain kinase contains one of the two sites of phosphorylation by cAMP-dependent protein kinase. A tryptic peptide containing the second site of phosphorylation appears to be associated with the 26,000-dalton peptide and to co-elute with it on gel filtration under native conditions (0.1M NH_4HCO_3, 0.2M NaCl, pH 7.5). This second site is the one which cannot be phosphorylated when calmodulin is bound to myosin kinase and which exerts a regulatory effect on calmodulin binding and myosin light chain kinase activity. Present studies are under way to sequence the phosphorylated peptides of diphosphorylated myosin light chain kinase. One of these peptides will confirm a sequence previously determined and the second will be the site whose phosphorylation alters the ability of myosin kinase to bind calmodulin.

An enzyme (MW 150,000) that methylates DNA at CpG sequences has been partially purified by Dr. Timothy Bestor. The enzyme preparation contains endogenous kinase activity which can phosphorylate the methylase. The methylase can also be a substrate for cyclic AMP-dependent protein kinase or for protein kinase C but only low levels of phosphate can be incorporated. There is no measurable change in methylase activity versus an unmethylated DNA substrate. Neither myosin light chain kinase nor a tyrosine kinase prepared from Rous sarcoma virus-induced rat tumors can phosphorylate the methylase. Current work is directed towards measuring the effect of dephosphorylation of the methylase by nonspecific phosphatases (alkaline phosphatase and a phosphatase purified from turkey gizzard smooth muscle) on enzyme activity.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04205-04 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Molecular Cloning of Mammalian Smooth Muscle Myosin Light Chain Kinase

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

Maryanne Vahey, Ph.D., Staff Fellow, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, LMC, NHLBI
Yvette Preston, Biologist, LMC, NHLBI

COOPERATING UNITS *(if any)*

M. Cashell, NICHD

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	1.2	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.)*

We have characterized six identical clones from a rat uterus cDNA library assembled in the expression vector, lambda gt11 and screened with affinity purified antibodies to myosin light chain kinase (MLCK). That these clones contain a 630 base pair cDNA for MLCK is suggested by: (1) the clones were isolated using a highly specific antibody probe for MLCK; (2) the clones express a peptide that cross-reacts with antibodies to MLCK in an epitope selection analysis; (4) hybrid selection studies indicate synthesis of a 100,000 MW protein; (5) immunoprecipitation of in vitro translation products of uterine messenger RNA (mRNA) identify a 100,000 MW protien; and (6) the 630 bp probe hybridizes to a 2.5 kb band on a northern blot of rat uterine total RNA.

We have recently isolated a 2.4 kb cDNA from a lambda gt11 rat uterus library screened with the affinity purified antibody to MLCK. Preliminary studies indicate: (1) this cDNA does not hybridize to the 630 bp clone; (2) this clone appears positive for MLCK on epitope selection and (3) the cDNA may cross-hybridize to an oligonucleotide probe constructed on the basis of the amino acid sequence near the site phosphorylated by cAMP-dependent protein kinase in turkey gizzard MLCK.

597

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 04206-04 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

The Regulation of Cardiac Myosin

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

Larry S. Tobacman, M.D., Medical Staff Fellow, LMC, NHLBI
Robert S. Adelstein, M.D., Chief, 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 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	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 properties of a reconstituted system of bovine cardiac contractile proteins are being studied as a function of the Ca^{2+} concentration. The actin-troponin-tropomyosin complex facilitates a cooperative Ca^{2+}-induced activation of the MgATPase of cardiac myosin subfragment-1, under conditions where myosin subfragment-1 has been carefully excluded as a source of cooperativity. The details of this MgATPase activation also depend upon which one of two troponin T isoforms is present. These isoforms are known to differ near the amino terminus and are regulated in other species by alternative mRNA splicing.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 04207-01 MC

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Regulation of Genes for Contractile Protein 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., Visisting Associate, LMC, NHLBI
Ralph Shohet, M.D., Medical Staff Fellow, LMC, NHLBI
Mary Anne Conti, Ph.D., Research Chemist, LMC, NHLBI
Robert S. Adelstein, 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 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	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.)*

We have screened a number of cDNA libraries including one from human brain and one from rat aorta cells grown in culture. (This last library was supplied by M. Taubman and B. Nadal-Ginard, Harvard Medical School.) To date we have isolated 10 putative myosin clones and 40 putative myosin light chain kinase clones. We have also isolated genomic clones for tropomyosin and skeletal muscle myosin. All of these clones are in the process of being characterized by hybridization, restriction analysis and sequencing.

PHS 6040 (Rev. 1/84)

GPO 914-918

Molecular Disease Branch
National Heart, Lung, and Blood Institute
October 1, 1985 through September 30, 1986

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, the physiological role of the apolipoproteins (apo) and lipoproteins in lipid transport, the determination of the mechanisms involved in the regulation of cellular cholesterol metabolism and transport, and 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.

During the last several years the staff of the Molecular Disease Branch has developed a conceptual framework for the understanding of the dynamic processes involved in the biosynthesis, transport, and catabolism of plasma apolipoproteins and lipoproteins. Within this framework the plasma lipoproteins are conceptualized as a polydisperse collection of lipoproteins, the apolipoprotein composition of which is determined by the laws of mass action. The constituent of the lipoprotein particle which is responsible for the regulation of the lipoprotein particle transport and metabolism is the apolipoprotein moeity. The distribution of a specific apolipoprotein within plasma is governed by the relative concentration of and affinity for the individual plasma lipoproteins. This concept of plasma lipoproteins emphasizes the fundamental importance of the apolipoprotein in regulating metabolism and provides a framework for understanding apolipoprotein-lipoprotein interactions during lipoprotein biosynthesis, transport, and catabolism in normal man and in patients with dyslipoproteinemias and atherosclerosis.

The determination of specific physiological and biochemical functions of the individual apolipoproteins continues to be of major importance in our understanding of the role of apolipoproteins in lipoprotein structure, function, and metabolism. Based on our current information, apolipoproteins have been shown to be of importance in four general areas of lipoprotein metabolism: 1) cofactor for enzymes (apoC-II for lipoprotein lipase, apoA-I for lecithin cholesterol acyltransferase); 2) ligand on the lipoprotein particle for interaction with high affinity receptor sites (apoB-100 on LDL and apoE on the chylomicron remnant); 3) exchange protein for phospholipids, cholesteryl esters, and triglycerides; and 4) structural component for the lipoprotein particle (apoA-I for HDL, apoB-100 for LDL, and apoB-48 for the chylomicron remnant).

Prerequisite to our understanding of the physiological and biochemical role of apolipoproteins in lipid and lipoprotein metabolism is a detailed knowledge of the molecular structure and function of the plasma apolipoproteins. Over the last several years we have systematically evaluated the molecular structure and primary amino acid sequence of apolipoprotein A-I, A-II, apoC-I, apoC-II, apoC-III, and apoH.

Of major importance during the last year has been the completion of the entire cDNA and derived amino acid sequence of human apoB-100. ApoB-100 is the major apolipoprotein which interacts with the low density lipoprotein (LDL) receptor, and initiates receptor mediated endocytosis with catabolism of LDL. ApoB-100 is a 4536 amino acid protein with a amino acid molecular weight of 510,000. There are 25 cysteine residues in apoB-100, fifteen are located in the amino terminus providing the potential for considerable order in this region of the protein. There are 20 potential N-linked glycosylation sites, the majority of which are located in the middle of the protein. There are no linear repeating or unusual amino acid sequences in apoB-100. Computer analysis of the potential conformation of apoB-100 revealed 40% helix, 25% β structure, and 35% random structure. There are no long stretches of amphipathic helices which are characteristic of plasma apolipoproteins. There is a significant proportion of β structure, and the β structure contain segments which are amphipathic. These areas may be of importance in the tertiary structure as well as the lipid binding properties of apoB-100.

The nature of the LDL receptor binding site of apoB-100 has been of considerable interest. Detailed studies on the complete sequence of apoB-100 by computer analysis has revealed several clusters of positively charged amino acids which are complementary to the consensus sequence of the LDL receptor. A consensus sequence for the LDL binding site on apoB-100 was established which is complementary to the negatively charged consensus sequence of the LDL receptor binding domains. These results suggest that there has been genetic reduplication of the LDL receptor binding domain in apoB-100.

A single gene for apoB has been localized to chromosome 2 by hybridization of cDNA probes for apoB and a panel of human-mouse hybrids. The establishment of a single gene for apoB is of particular importance with regard to the synthesis of the two B apolipoproteins, apoB-100 and apoB-48. Previous studies in the literature have been interpreted as indicating that apoB-48 was synthesized in the intestine, and was an apolipoprotein marker for chylomicron remnants; apoB-100 was proposed to be synthesized only in the liver. Analysis of polyA$^+$ RNA of the liver and intestine with cDNA apoB probes by Northern blot analysis revealed a single mRNA for apoB in the liver of sufficient size to code for apoB-100. However, two apoB mRNA's were present in intestinal RNA. An apoB-100 mRNA and a second mRNA which would code for apoB-48. These results have been interpreted as indicating that both apoB-48 and apoB-100 are synthesized by the intestine, while only apoB-100 is synthesized by the liver. Based on these results we have proposed that the apoB is transcribed from a single gene to form a single nuclear mRNA. The mRNA then undergoes differential processing to yield both a apoB-48 and apoB-100 mRNA in the intestine, and only an apoB-100 mRNA in the liver. These results are of major importance since both apoB-100 as well as apoB-48 lipoproteins can be synthesized by the intestine, and apoB-48 can no longer be utilized as a single apolipoprotein marker for intestinal lipoproteins. The elucidation of the complete covalent structure of apoB-100 will now permit a detailed analysis of the molecular properties of apoB-100, the LDL receptor binding domain(s), and the importance of structural defects in apoB-100 in patients with dyslipoproteinemias and atherosclerosis.

Of particular interest with respect to structure and function of apoB have been recent studies on the molecular defect in abetalipoproteinemia. Abetalipoproteinemia is characterized by low plasma cholesterol, an absence of plasma apolipoprotein B-100, apoB-48, chylomicions, VLDL, IDL as well as LDL, ataxia, acanthocytosis, and atypical retinitis pigmentosa. Southern blot analysis of the apoB gene from patients with abetalipoproteinemia revealed no major insertions or deletions in the apoB gene. ApoB mRNA was evaluated by Northern blot analysis of liver mRNA from two patients with abetalipoproteinemia. The apoB mRNA was of normal size, and dot blot analysis revealed that the apoB mRNAs from the patients with abetalipoproteinemia were 5-6 fold greater than from normal subjects. The apoB-100 protein in the liver was evaluated by immunohistochemical techniques utilizing monoclonal antibodies to apoB-100. The hepatocytes contains large quantities of immunoreactive material consistent with the synthesis of the B-100 apolipoprotein. These combined results established that the apoB-100 gene is transcribed, the apoB-100 mRNA is of normal size and 5-6 fold increased, and the mRNA is translated with apoB-100 protein in the cells, but not in the plasma. These results are interpreted as indicating that the defect in abetalipoproteinemia is a defect in post-translational processing or in the secretary process of apoB-100 leading to a failure of secretion of the B apolipoproteins and the apoB containing lipoproteins.

During the last year, studies have continued on apoC-II, the cofactor for lipoprotein lipase. Extensive analysis of the apoC-II isoforms in human plasma have been performed by two-dimensional gel electrophoresis and immunoblot analysis with a monospecific apoC-II antibody. ApoC-II consists of 4 major plasma isoforms that result from post-translational processing involving glycosylation, deglycosylation, and proteolytic cleavage. Two isoforms desiginated apoC-II$_{-1}$ and apoC-II$_{-2}$ contain 1 and 2 moles of sialic acid respectively. ApoC-II$_{o}$ is the major plasma isoform and is a deglycosylated 79 amino acid protein. A fourth isoform, apoC-II$_{-1/2}$, was shown by amino acid and amino-terminal sequence analyses to be the nature 73 amino acid protein. These results have established that apoC-II is synthesized as a preproapolipoprotein. PreproapoC-II undergoes co-translational cleavage to proapoC-II which is glycosylated and secreted from the cell. ProapoC-II undergoes deglycosylation, and proteolytic cleavage to mature apoC-II. To further elucidate the rate of catabolism and conversion of apoC-II, proapoC-II and mature apoC-II were radiolabeled and the metabolism of the two isoforms studied in normal volunteers. ProapoC-II and mature apoC-II were catabolized at the same rate, and there was very slow conversion of proapoC-II to mature apoC-II. Thus there is a very slow conversion of proapoC-II to mature apoC-II in man, and apoC-II is primarily catabolized as the proapoC-II isoform.

Studies on the apoC-II gene have been continued, and the complete genomic structure of the preproapoC-II gene has been elucidated. The apoC-II gene consists of 3407 bases and contains 3 introns and 4 exons. The first intron is long and contains 2495 bases with 4 alu type repetitive sequences and a 22 dinucleotide sequence of GT repeats. The second intron interrupts the prepeptide, and the third intron which interrupts the carboxyl terminal portion of apoC-II contains a 38 bp

long sequence that is repeated 6 times within the intron. The four exons are 25 bp, 68 bp, 160 bp, and 231 bp in length. The structural organization of preproapoC-II with three introns, and four exons is similar in structure to the genes for apolipoproteins A-I, A-II, C-III, and E.

The molecular defects in patients with apoC-II deficiency have been studies in several different kindreds. Patients with apoC-II deficiency have severe hypertriglyceridemia, elevated plasma chylomicions, recurrent bouts of pancreatitis, and eruptive xanthomas. Analysis of the plasma from four separate kindreds have revealed four different defect. ApoC-II is absent in one kindred, a small (< 1% normal) quantity of apoC-II of normal molecular weight and charge is present in a second kindred, and two additional kindreds have apoC-II variants which are different in apparent molecular weight and isoelectric point than normal apoC-II. The apoC-II gene from 1 kindred has been cloned, and the complete genomic structure of this apoC-II gene nearly completed. A detailed study of the apoC-II gene in normal subjects, and in patients with apoC-II deficiency will provide major new insights into the structure, function, and physiological role of apoC-II in triglyceride and lipoprotein metabolism.

During the last year major studies have been initiated to study the expression of the apolipoprotein genes. The factors which modulate the expression of apoA-I and apoB-100 have been analyzed in Hep G2 liver cells by dot blot hybridization of apoA-I and apoB-100 mRNA and quantitation of apoB-100 and apoA-I in the culture media. Incubation in lipoprotein deficient serum was associated with a reduction in apoB-100 and an increase in apoA-I mRNA, and a corresponding reduction and increase in apoB-100 and apoA-I concentrations in the media. Incubation of Hep G2 cells with mevinolin, a new HMG-CoA reductase inhibitor, resulted in a reduction in apoB-100 mRNA and media concentration, whereas apoA-I mRNA and cellular secretion increased. The reduction in apoB-100 mRNA and cellular secretion is of major importance since it indicates that mevinolin may be a very effective drug in decreasing apoB-100 lipoprotein biosynthesis and secretion. The increase in apoA-I mRNA and apoA-I secretion suggests that this new drug may not only lower apoB-100 containing lipoproteins but also increase plasma HDL and apoA-I. It is also of particular interest in these studies that apoA-I and apoB-100 appear to be coordinately controlled and reciprocally regulated. Further studies are underway to evaluate the coordinate control of apolipoprotein gene expression.

The biosynthesis and post-translational processing of the apolipoproteins continue to be actively investigated. ApoA-I secreted by Hep G2 cells has been shown to be acylated by an ester bond to palmitate. The major apoA-I acylated is proapoA-I. Recent studies have also established that apoB-100 secreted from Hep G2 cells is acylated by both palmitate and stearate by an ester linkage. Of major importance was the finding that plasma apoB-100 in LDL was also acylated. The combined results from these studies indicate that acylation may play an important role in apolipoprotein-lipid interactions as well as the metabolism of plasma apolipoproteins as well as lipoproteins. Acylation may ultimately be shown to be a very fundamental process in apolipoprotein function and metabolism.

The intracellular transport, hydrolysis, and biosynthesis of cholesterol continues to be an active area of research within the Branch. The major rate limiting enzymes for cholesterol biosynthesis is HMG-CoA reductase. HMG-CoA reductase has been extensivley studied in our laboratory over the last several years. The major focus of this research is the short term modulation of the enzymic activity of HMG-CoA reductase by reversible phosphorylation. Both human and rat liver HMG-CoA reductase activity is modulated in vitro and in vivo by a bicyclic cascade system involving two kinases, reductase kinase and reductase kinase kinase. HMG-CoA reductase and reductase kinase undergo reversible activation-inactivation by reversible phosphorylation. The kinase responsible for the reversible phosphorylation of reductase kinase has been desiginated reductase kinase kinase. Regulation of the enzymic activity of HMG-CoA reductase by a bicyclic cascade system provides a rapid short-term mechanism for the regulation of cholesterol biosynthesis.

Recently the activity of HMG-CoA reductase was shown to be modulated by a second kinase, protein kinase C. Protein kinase, which requires calcium and phospholipids for activity, was shown to reversible phosphorylate HMG-CoA reductase. The tumor-promoting phorbol ester, phorbol 12 myristate 13 acetate (PMA) stimulated the protein kinase C catalyzed phosphorylation of HMG-CoA reductase. These latter results suggest that protein kinase C may play a role in the in vivo modulation of HMG-CoA reductase activity.

During the last year, studies have definitively established a third kinase which reversibly phorphorylates and inactivites HMG-CoA reductase. This new kinase is a calcium, calmodulin dependent protein kinase (CMK), and was purified from rat brain cytosol. The new kinase has a molecular weight is 110,000 and is different from other known calmodulin dependent kinases of molecular weight 500,000 - 600,000. This new kinase also differs in term of autophosphorylation and substrate specificity than the larger molecular calmodulin kinases. By peptide analysis the calcium calmodulin-dependent kinase is able to phosphorylate two different sites on purified HMG-CoA reductase.

Based on these data, we have now proposed that HMG-CoA reductase is modulated by reversible covalent phosphorylation involving three sperate kinase systems including reductase kinase, protein kinase C, and calcium, calmodulin-dependent protein kinase. These studies have provided new insights into the molecular mechanisms involved in the short term regulation of HMG-CoA reductase activity and cholesterol biosynthesis.

The synthesis, transport, and catabolism of plasma lipoproteins in normal subjects and patients with dyslipoproteinemias continues to a central focus of research of the Branch. As outlined above, the metabolism of proapoC-II and apoC-II provides major new insights into the processing of plasma apolipoproteins. An additional area which has continued to be of major interest and importance is the metabolism of HDL, since HDL has been identified as a negative risk factor for the development of premature cardiovascular disease. Of long standing interest is the molecular defect in Tangier disease. During the last

year a processed form of plasma apoA-I has been identified which involves the proteolytic cleavage of approximately 20 amino acids from the carboxyl-terminus of apoA-I. Processed apoA-I was identified in the plasma of normal subjects and patients with Tangier disease. Tangier patients appear to generate more processed apoA-I than normal controls during in vitro incubation of plasma. Of major importance was the kinetic study of mature and processed apoA-I in normal controls, and patients with Tangier dsease. Processed apoA-I was very rapidly cleared from the plasma ($T_{1/2}$ < 5 hrs) in both normals and Tangier patients. These studies are interpreted as indicating that processed apoA-I is rapidly cleared from plasma and may possibly represent the form of apoA-I cleared from the plasma by the apoA-I receptor system. These findings may be of critical importance to our understanding of the rapid catabolism of apoA-I in Tangier disease.

Studies on apoA-I and HDL metabolism are also being carried out in patients with hypoalphalipoproteinemia and premature cardiovascular disease. ApoA-I isolated from the subjects is currently undergoing metabolic studies to determine if the reduced levels of HDL cholesterol in these patients is due to decreased synthesis or increased catabolism. The mechanisms for the low plasma concentration of HDL cholesterol in patients with premature cardiovascular disease may ultimately lead to better methods for the diagnosis of these patients as well as innovative approaches to the therapy of this important dyslipoproteinemia.

One of the most informative areas of research in our Branch over the last several years has been the analysis of the metabolism of apoE. ApoE is coded for by three major alleles, E^2, E^3, and E^4, and several lines of in vitro and in vivo metabolic evidence suggest that the normal allele is E^3. Previous studies from our Branch have established that the product of the E^2 allele, $apoE_2$, is catabolized more slowly than $apoE_3$. These results are consistent with the delayed catabolism of remnants of triglyceride-rich lipoproteins characteristic of patients with type III hyperlipoproteinemia. The metabolism of $apoE_2$ was also extended to normolipidemic subjects homozygous for $apoE_2$. Initial studies established that normolipidemic $apoE_2$ homozygotes have a two- to three fold elevation of plasma apoE and an increase in cholesterol-rich VLDL. Analysis of apoE metabolism in these subjects revealed that the increase in plasma apoE was due to an increase in synthesis.

Recent studies on apoE metabolism have concentrated on the elucidation of the mechanisms involved in the change in catabolism of $apoE_2$ and $apoE_4$ as compared to $apoE_3$, the normal apoE allele. The modification of the cysteine residues in $apoE_2$ by methyl and aminoethyl groups resulted in the addition of a neutral and positive charge respectively. The aminoethyl adduct produced an apoE isoform with two positive charge similar to the arginine residues in $apoE_4$. As predicted based on charge the catabolism of $apoE_4$ and aminoethylated $apoE_2$ were identical. The loss of the ability of $apoE_2$ to form mixed disulfide may also have played a major role in the change in $apoE_2$ catabolism. The catabolism of the methylated $apoE_2$ was intermediate to $apoE_2$ and $apoE_4$.

The elucidation of the structure-function requirements for apoE catabolism are of primary importance, since defects in apoE metabolism result in type III hyperlipoproteinemia which is associated with premature cardiovascular disease.

One of the major aims of the staff of the Branch is the effective treatment of hyperlipidemia with the ultimate goal of reducing blood lipid levels at an early stage of atherosclerosis and preventing premature cardiovascular disease. To this end we have initiated an ongoing outpatient clinical trial for the treatment of patients with hypercholesterolemia and the type II phenotype to compare the various hypolipidemic drugs available for therapy. Drugs utilized in these clinical trails have included neomycin, niacin, and a newly developed drug mevinolin which is a competitive inhibitor of HMG-CoA reductase. Mevinolin is thus able to block cholesterol biosynthesis. Of all drugs tested, mevinolin is the most effective, and 40 mg/day was able to normalized non-familial hypercholesterolemia (FH) type II patients, and to reduced LDL cholesterol by approximately 25% in FH patients. Systematic evaluation of adrenal function established that there was no major effect on adrenal function. Detailed analysis of gonadal function is currently being completed. No significant side effects of mevinolin have been recognized, and mevinolin now appears to be the most effective new drug for the treatment of hypercholesterolemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02010-15 MDB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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
	A. Hospattankar, Ph.D.	Visiting Associate	MDB, NHLBI
	J. Hoeg, M.D.	Senior Investigator	MDB, NHLBI
	R. Ronan, B.A.	Chemist	MDB, NHLBI
	M. Meng, M.S.	Chemist	MDB, NHLBI
	C. Bishop, B.S.	Chemist	MDB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Detailed studies have been initiated to identify the LDL receptor binding site on apoB-100. The complete amino acid sequence of apoB-100 has been analyzed by computer analysis. Several positively charged domains complementary to the negatively charged consensus LDL receptor binding domain have been identified on apoB-100. The presence of several potential binding domains rather than a single receptor binding domain provides new insights into the apoB-100 LDL receptor interaction.

ApoC-II has been shown to be synthesized as a preproapolipoprotein. ProapoC-II undergoes proteolytic cleavage with loss of a hexapeptide to yield mature apoC-II. The predominate isoform in human plasma is proapoC-II, and mature apoC-II is a minor isoform in human plasma.

Human apoA-I and apoB have been shown to be acylated with palmitate, and the fatty acid is linked to the apolipoprotein by an ester linkage. The identification of covalently bound fatty acids on apolipoproteins may be of pivotal importance in our understanding of lipid-protein interactions as well as apolipoprotein-lipoprotein metabolism.

A processed form of apoA-I which has been cleaved at the carboyl-terminal region has been identified, and its structure established. The processed form of apoA-I is catabolized at a rapid rate in man and may provide major new insights into our understanding of the rapid catabolism of apoA-I in Tangier disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02012-11 MDB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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)*

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

COOPERATING UNITS *(if any)*

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, 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.)*

We have previously demonstrated that rat and human liver HMG-CoA reductase activity is modulated in vitro and in vivo in a bicyclic cascade system involving reversible phosphorylation of both HMG-CoA reductase and reductase kinase. Recently, we have also reported the modulation of the enzymic activity of both soluble purified (M_r 53,000) and native (M_r 100,000) HMG-CoA reductase involving a Ca^{2+}-activated and phospholipid-dependent protein kinase C-mediated phosphorylation.

During the past year we have purified and characterized a low molecular weight Ca^{2+}, calmodulin-dependent protein kinase (M_r 110,000) from rat brain cytosol. This purified protein kinase is different from other known calmodulin-dependent kinases (M_r 500-600,000). The new kinase also differs in terms of its degree of autophosphorylation and specificity toward other substrates including HMG-CoA reductase. Maximal phosphorylation of purified HMG-CoA reductase was approximately one mol/mol of 100,000 native HMG-CoA reductase. Dephosphorylation of ^{32}P-HMG-CoA reductase was associated with complete reactivation of HMG-CoA reductase activity and near total loss of radioactivity. Ca^{2+} calmodulin-dependent kinase is able to phosphorylate two different sites in the purified HMG-CoA reductase molecule. Phosphoaminoacid analysis of each phosphopeptide revealed that only serine residues are phosphorylated by calmodulin-dependent kinase.

Based on these results and our previous in vitro and in vivo studies, we now propose that both native and purified HMG-CoA reductase activity is modulated by reversible covalent phosphorylation involving three separate kinase systems including reductase kinase, protein kinase C, and Ca^{2+}, calmodulin-dependent protein kinase.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02019-08 MDB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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:	Loren A. Zech, M.D.	Senior Investigator	MDB, NHLBI
	Paola Roma, Ph.D.	Visiting Fellow	MDB, NHLBI
	Diane Wilson	Chemist	MDB, NHLBI
	Lila Taam	Chemist	MDB, NHLBI
	Marie Kindt	Chemist	MDB, NHLBI
	Robert Herzog	Biological Aid	MDB, NHLBI
	H. Bryan Brewer, Jr., M.D.	Chief	MDB, NHLBI

COOPERATING UNITS *(if any)*

.

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
5.5	2	3.5

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 ELISA assay has been automated and the apoA-I, A-II, and B assays are presently being run while the apoC-II and E assays are being developed. Apolipoprotein E is a polymorphic protein with 3 common isoforms, $apoE_2$, E_3, and E_4, with $apoE_2$ being catabolized the slowest and $apoE_4$ the fastest in humans. $ApoE_2$ has two reactive cysteines while $apoE_4$ has these cysteines replaced with arginines. The slow metabolism of $apoE_2$ was determined to be due to both the charge alteration of the protein resulting from the substitution of cysteine for the arginine and the slow catabolism of the $apoE_2$ disulfide dimers.

ApoA-I was isolated from a subject with hypoalphalipoproteinemia associated with a restriction fragment length polymorphism linked to the apoA-I gene. The catabolic rate of this apoA-I was normal indicating that the defect in this subject is either in the synthesis rate of apoA-I or in another gene closely linked to the apoA-I gene. Tangier disease is characterized by rapid catabolism of HDL but macrophages from Tangier disease subjects were determined to be normal for HDL binding, internalization, degradation, and resecretion. Further investigations into the etiology of the rapid HDL catabolism in Tangier disease are being pursued.

ApoC-II exists in plasma in a pro and mature form. The metabolism of both forms were studied and it was determined that both forms were catabolized relatively rapidly and at the same rate. In addition, there was a very slow conversion of the pro form to mature form of apoC-II.

Abetalipoproteinemia is characterized by a virtual absence of apoB in plasma. By utilizing sensitive methods for the detection of apoB mRNA and protein, increased amounts of apoB mRNA were assayed in liver from two study subjects and apoB protein was detected in the liver and plasma from two subjects. This indicates that in some subjects with abetalipoproteinemia, the

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02022-06 MDB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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:	H. Bryan Brewer, Jr., M.D.	Chief	MDB, NHLBI
	Juan C. Monge, M.D.	Medical Staff Fellow	MDB, NHLBI
	Wendy Farnsworth, M.D.	Medical Staff Fellow	MDB, NHLBI
	Stephen Demosky, Jr.	Chemist	MDB, NHLBI
	Santi Datta	Chemist	MDB, NHLBI
	Barbara Winterrowd	Medical Technician	MDB, NHLBI
	Briston Williamson	Lab. Technician	MDB, NHLBI

COOPERATING UNITS *(if any)*

Drs. N.N. Tandon, J.T. Harmon, G.A. Jamieson, Red Cross Research Laboratories, Bethesda, MD

Dr. T.E. Starzl, Univ. of Pittsburgh School of Med., Pittsburgh, PA

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.2	3.2	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.)*

Evaluation of cellular lipoprotein and apolipoprotein metabolism is the primary focus of this laboratory. Utilizing a variety of human cell lines in tissue culture, the ability of lipoproteins to induce delivery and egress of membrane lipids as well as the modulation of intracellular cholesterol biosynthesis and esterification has been evaluated in normal human subjects as well as in patients with a variety of inborn errors of lipoprotein, apolipoprotein, and cellular lipid metabolism. Our previous investigations of receptors for low density lipoproteins and high density lipoproteins and the intracellular enzymes acid cholesteryl ester hydrolase, neutral cholesteryl ester hydrolase, acyl:cholesteroacyltransferase, and 3-hydroxy-3-methylglutaryl coenzyme A reductase have been extended from studies conducted upon isolated cellular membranes to intact human hepatocytes and to the human hepatoma cell line Hep G2. In addition, the regulation of nascent apolipoprotein biosynthesis has been studied. Human hepatocytes regulate the secretion of lipoproteins containing apolipoprotein A-I and apolipoprotein B. The hepatic receptors for high density lipoproteins, low density lipoproteins and chylomicron remnants can alter both the level of mRNA expression for apolipoprotein A-I and apolipoprotein B as well as the secretion of newly synthesized apolipoproteins. These nascent apolipoproteins undergo a variety of post-translational modifications and we have determined that in addition to proteolytic processing, glycosylation, and deamidation, human apolipoproteins undergo covalent fatty acid acylation. The inborn errors of metabolism abetalipoproteinemia, cholesteryl ester storage disease, and familial hypercholesterolemia all have defective hepatic apolipoprotein metabolism at different points in apolipoprotein catabolism and synthesis. These insights into nascent apolipoprotein synthesis complement ongoing clinical trials in our Branch designed to modify apolipoprotein synthesis and secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02024-05 MDB

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Molecular biology of plasma apolipoproteins and lipoproteins

RINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Neme, title, leboratory, and institute effiliation)*

PI:	Simon W. Law, Ph.D.	Senior Investigator	MDB, NHLBI
Others:	H. Bryan Brewer, Jr., M.D.	Chief	MDB, NHLBI
	Silvia Fojo, M.D., Ph.D.	Medical Staff Fellow	MDB, NHLBI
	Stephen Grant, M.D.	Medical Staff Fellow	MDB, NHLBI
	Keiichi Higuchi, Ph.D.	Visiting Fellow	MDB, NHLBI
	Karl Lackner, M.D.	Visiting Scientist	MDB, NHLBI
	Ashok Hospattankar,Ph.D.	Visiting Scientist	MDB, NHLBI
	Juan Monge, M.D.	Medical Staff Fellow	MDB, NHLBI

OOPERATING UNITS *(if any)*

A. Sakaguchi & S. Naylor - Departments of Medicine and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas

AB/BRANCH

Molecular Disease Branch

ECTION

Peptide Chemistry

NSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Md 20892

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.3	5.3	1.0

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have cloned the cDNA for human apolipoprotein (apo)B-100, the ligand on low density lipoproteins which interacts with the LDL receptor and initiates receptor mediated endocytosis and LDL catabolism. The normal human liver apoB-100 mRNA is 14 kb long encoding a mature apoB-100 protein of 4536 amino acids with a molecular weight of 512,723 daltons. The delineation of the entire human apoB-100 sequence will now permit a detail analysis of the conformation of the protein, the LDL receptor binding domain(s), the structural relationship between apoB-100 and apoB-48, and may provide the basis for the study of the genetic defects of the dyslipoproteinemias.

We have also evaluated the expression of apoB mRNA in the liver and intestine by Northern blot. Human liver synthesize a single 14 kb mRNA of apoB-100, however, the intestine contained both the apoB-100 mRNA and a 7.5 kb mRNA which encode apoB-48. Result of further blot hybridization analysis with specific synthetic oligonucleotide probes showed apoB-48 mRNA contain the 5' end but not the 3' end of apoB-100 mRNA. The novel finding that human intestine synthesize both the apoB-100 and apoB-48 mRNA will now require the restructuring of the currently held concepts of human lipoprotein synthesis in normal subjects and in patients with dyslipoproteinemias. Studies on the structural organization of the apoB gene and its expression in patients with no plasma apoB (abetalipoproteinemia) have also been initiated. Southern blot hybridization showed no major rearrangement or deletion in the apoB gene of these patients. Northern blot and dot blot hybridization studies revealed apoB-100 mRNA are being produced by the liver cells of these patients at an elevated level than in normal subjects. Our data support the concept of a post-translational defect in apoB processing or secretion which leads to defective secretion of cellular lipoproteins and a virtual absence of apoB containing plasma lipoproteins in these subjects.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02028-02 MDB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Molecular Biology of the ApoC-II Gene

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

PI:	Silvia S. Fojo, Ph.D., M.D.	Medical Staff Fellow	MDB, NHLBI
Others:	Simon W. Law, Ph.D.	Senior Staff Fellow	MDB, NHLBI
	H. Bryan Brewer, Jr., M.D.	Chief	MDB, NHLBI

COOPERATING UNITS *(if any)*

Carlo Gabelli, M.D., and Giovanella Baggio, M.D. - University of Padova, Padua Italy.

LAB/BRANCH

Molecular Disease Branch

SECTION

Peptide Chemistry

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The cDNA sequence of the gene for human apolipoprotein C-II and it's localization to chromosome 19 has been previously established. In addition, the complete genomic sequence of normal human apoC-II has been elucidated from an apoC-II clone isolated from a human placental genomic DNA phage library. It consists of 3407 base pairs and like the genomic structure of other known apolipoprotein genes, it contains 3 introns and 4 exons.

The apoC-II gene from one patient with apoC-II deficiency has been cloned into an EMBL-3 lambda genomic library. Determination of the complete genomic structure of this patient is underway to determine the specific molecular defect in apoC-II in this kindred.

Total RNA from the liver of a second patient with apoC-II deficiency has been isolated. Slot blot analysis reveal decreased levels of the apoC-II message in this patient.

Analysis of the various normal apoC-II isoforms in plasma have been performed by utilizing the techniques of 2- dimensional gel electrophoresis and immunoblotting. ApoC-II consists of 4 major plasma isoforms that result from the post-translational processing of apoC-II in the form of glycosylation, deglycosylation and proteolytic cleavage. Confirmation that apoC-II is initially synthesized as a preproprotein has been obtained by amino acid composition and amino terminal sequence analysis. Similar 2-D gel studies of the plasma of 4 independent patients with apoC-II deficiency reveal 4 different abnormalities. These include: total absence of apoC-II, low levels of apoC-II with normal electrophoretic mobility, and low levels of apoC-II variants that exhibit abnormal electrophoretic mobility.

Annual Report of the
Laboratory of Molecular Hematology
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

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 genetic 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.

SECTION ON MOLECULAR GENETICS

The disease chosen as the initial candidate for human gene therapy is 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 as well as 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). A highly efficient procedure for transferring functional genes into mammaliam tissue culture cells in vitro and into bone marrow cells of mice in vivo was developed last year using these retroviral vectors as a delivery system.

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 speen 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 has been developed this year for nonhuman primates. These latter studies have been done in collaboration with the Clinical Hematology Branch, NHLBI, for studies with the rhesus monkey, and in collaboration with the Bone Marrow Transplantation Program at the Memorial Sloan-Kettering Hospital, New York City, for studies with Cynomolgus macaque. Expression of the human ADA and the prokaryotic NeoR genes at low levels has been demonstrated in several monkeys.

During the past year, this Section has achieved the following results:

(1) The human ADA gene (as well as the NeoR NPT gene) has been efficiently expressed in approximately 0.5% of the circulating mononuclear cells of one monkey and at lower levels in several other animals. The autologous BMT/gene transfer protocol that is being developed with the retroviral vector SAX is projected for use, once it is sufficiently tested, in human gene therapy clinical trials for ADA deficiency. Greater efficiency and reproducibility are still required.

(2) In utero gene transfer and expression have been demonstrated in the fetal lamb. In a collaborative study with Dr. Esmail Zanjani, Minneapolis, and Drs. Michael Harrison and Alan Flake, San Francisco, a sheep in utero

transplantation/gene transfer protocol has been successfully developed. Peripheral blood was removed from a 96 day old fetal lamb, infected in vitro with a retroviral vector, N2, carrying the NeoR NPT gene, and reinfused back into the donor fetus. After the lamb was born, bone marrow studies indicated that the NeoR gene was present and functioning.

(3) Human hematopoietic progenitor cells can be infected with the vector SAX and are resistant to G418 in a CFU-C assay at an efficiency of 1-2%. In like manner, bone marrow cells from patients with ADA deficiency can also be infected with the SAX vector. These genetically defective cells also were shown to express the NeoR gene of the SAX vector in 1-2% of the CFU-C progenitor.

SECTION ON MOLECULAR CLONING

To understand the nature and position of key transcriptional control elements which regulate differential control of gene expression, specific synthetic DNA control sequences are being constructed and their affects on transcription examined. Tissue-specific promoter elements are also being used to increase gene expression in retroviral vectors used to mediate specific gene transfer.

Proteins purified by affinity chromatography using specific synthetic DNA sequences are being used to identify transcription factor interactions with DNA control sequences and their effects on topology which regulate transcriptional initiation.

During the past year this section has:

(1) Developed rapid and efficient procedures for the synthesis and purification of specific DNA promoter elements.

(2) Constructed multicopy tandem head-to-tail arrays of specific transcriptional control sequences which have been used to a) extensively purify polypeptides required for accurate transcription, b) generate probes to identify clone and sequence translation factor genes, c) alter the structure of retroviral envelope glycoproteins to direct the tissue specific targeting of this gene vector system.

(3) Characterized the length and sequence requirements of donor DNA required for optimal T4 DNA ligase activity.

(4) Developed procedures for coupling both linear and supercoiled plasmids containing multicopy Ad2 major late promoter inserts, to a cellulose matrix for large scale purification of DNA-binding proteins.

SECTION ON RNA AND PROTEIN BIOSYNTHESIS

To understand the regulation of gene expression by RNA polymerase II, plasmids containing multiple repeats of promoter elements of the Adenovirus 2 major late transcription unit are being used to fractionate active HeLa, K562, and liver nuclear extracts into individual factors required for correct initiation.

A mouse model of β-thalassemia has also been examined to determine the molecular mechanism of the compensation achieved by the increased synthesis of β-minor globin.

The mechanisms by which adenoviruses and influenza viruses take over the translational machinery of the infected cell, and the ability of certain cell lines to prevent viral takeover, are being studied.

During the past year this section has:

(1) Identified and purified a polypeptide complex of initiation factors and RNA polymerase II capable of accurate de novo initiation of transcription.

(2) Developed DNA affinity chromatographic procedures which has allowed extensive purification of protein-DNA complexes required for transcription by RNA polmerase 2.

(3) Identified an atypical topoisomerase activity which appears to be required for transcriptional initiation.

(4) Demonstrated that the phenotypic compensation observed in murine β-thalassemia which results from an altered translational control mechanism for mRNA selection appears to involve eIF-4F.

(5) Characterized the influenza virus and adenovirus gene products which counteract interferon-mediated host antiviral activities. Prevention of activation of the dsRNA-dependent eIF-2α kinase is indicated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02213 09 MH

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Identification and Regulation of Factors Required for Transcription by RNA Polymerase II and Translation

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

PI: B. Safer, Medical Officer, LMH,NHLBI

Others: J.A. Thompson, Expert, LMH,NHLBI
T. Brendler, Staff Fellow,LMH,NHLBI
S. Sturm, Staff Fellow,LMH,NHLBI
R. Cohen, Staff Fellow,LMH,NHLBI
J. Curcio, Bio. Lab. Tech.,LMH, NHLBI
S. Garfinkel, Bio. Lab. Tech.,LMH, NHLBI
T. Boal, Bio. Lab. Tech.,LMH,NHLBI
W. Kemper, Chemist,LMH,NHLBI
L. Yang, Biologist,LMH,NHLBI
K. Anderson,Guest Worker,LMH,NHLBI
W.F. Anderson,Chief,LMH,NHLBI

COOPERATING UNITS *(if any)*

Michael Katze, Memorial Sloan-Kettering Cancer Center, NY,NY; Tom Shenk, Princeton University, Princeton, NJ; Rosemary Jagus, University of Pittsburgh, Pittsburgh, Pa.

LAB/BRANCH

Laboratory of Molecular Hematology

SECTION

Section on RNA and Protein Biosynthesis

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.6	3.6	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.)*

Regulation of gene expression occurs at the level of transcription, processing, transport, and mRNA translation. The primary goal of this section is to investigate the transcriptional and translational control mechanisms responsible for regulated gene expression.

To identify components required for transcription of genes by RNA polymerase II, stable intermediate complexes formed during assembly of specific transcription complexes are being purified and characterized using plasmids containing multiple repeats of the Adenovirus 2 major late promoter or specific promoter elements. These have been constructed to purify proteins which recognize and bind to specific DNA sequences which regulate gene activity. These constructs have been applied towards the purification of specific transcription factors, as well as the generation of cellular extracts specifically deficient in single transcription components, for functional studies.

A mouse model of B-thalassemia resulting from deletion of the entire β-major globin gene has been studied to determine the mechanism of the compensatory increase in β-minor globin gene expression. Compensation occurs almost entirely at the translational level, rather than by increased transcription and/or processing of β-minor globin mRNA. Alteration of the activity of the initiation factor eIF-4F is strongly suggested.

During infection by adenoviruses and influenza viruses, activation of host cell ds-RNA dependent eIF-2 α kinase is prevented by VA1 RNA and an unknown, but functionally similar influenza gene product. The mechanisms by which certain cell lines escape viral takeover of their translational machinery is being investigated. To understand the regulation of translation factors which participate in virus-host interactions, the genes for subunits of eIF-2 and eIF-2B are being identified and sequenced.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02216 07 MH

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Philip Kantoff, Medical Staff Fellow, LMH, NHLBI
Daniel Kuebbing, Senior Staff Fellow, LMH, NHLBI
Martin Eglitis, Senior Staff Fellow, LMH, NHLBI
Jeanne McLachlin, Visiting Fellow, LMH, NHLBI
Judith DiPietro, Biologist, LMH, NHLBI
Sheri Bernstein, Biologist, LMH, NHLBI
Robert Moen, Medical Staff Fellow, LMH, NHLBI
Jamie Zwiebel, MSF, LMH, NHLBI
Evelyn Karson, MSF, LMH, NHLBI
Robert Weider, MSF, LMH, NHLBI
Virginia Start-Vancs, Howard Hughes Fellow, LMH, NHLBI
Jane Selegue, Biologist, LMH, NHLBI

COOPERATING UNITS *(if any)*

A. Nienhuis, CHB, NHLBI; E. Gilboa, Princeton University, NJ; M. Blaese MET, NCI; R. O'Reilly, Memorial Sloan-Kettering Cancer Center, New York; E. Zanjani, VA Hospital, Minneapolis, MN; M. Harrison, U. California Medical School, San Francisco, CA.

LAB/BRANCH

Laboratory of Molecular Hematology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.2	7.4	2.8

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.)*

A highly efficient procedure for transferring functional genes into mammalian cells has been developed using retroviral vectors as a delivery system. When mouse bone marrow cells are infected in vitro with a Neo-R gene and 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 Neo-R gene. The majority of these cells produce the Neo-R gene product: phosphotransferase. Retroviral vectors containing the human gene for the enzyme adenosine deaminase (ADA) as well as the Neo-R gene have been made. Using the knowledge obtained from the murine system, a non-human primate autologous bone marrow transplantation/gene transfer protocol has been developed. Low levels of the human ADA gene have been expressed in monkey peripheral blood cells. These studies are preliminary to attempting human gene therapy in patients suffering from ADA severe combined immunodeficiency disease.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02217 01 MH

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Regulation of Gene Expression Utilizing Nucleic Acid Manipulations

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

PI: J.A. Thompson,Expert,LMH,NHLBI

Others: B. Safer,Medical Officer,LMH,NHLBI
T. Brendler,Med. Staff Fell.,LMH,NHLBI
S. Garfinkel,Bio.Lab.Tech.,LMH,NHLBI
P. Kantoff,Sen. Staff Fell.,LMH,NHLBI
J. Zwiebel,Sen. Staff Fell.,LMH,NHLBI
D. Kuebbing,Sen. Staff Fell.,LMH,NHLBI
J. DiPietro,Res. Biol.,LMH,NHLBI
R. Cohen,Med. Staff Med. Fell.
K. Anderson,Guest Worker,LMH,NHLBI
W.F.Anderson,Chief,LMH,NHLBI

COOPERATING UNITS *(if any)* R. Blakesely,Life Technologies,Inc.,Gaithersburg, Md;M. Ehrlich, Tulane Medical School,New Orleans, La;P. Browning, Guest Worker,CHB,NHLBI;T. Browder,Guest Worker,CHB,NHLBI;Robert Wells, U. of Alabama,Birmingham, Ala;L. Reid,Albert Einstein College of Medicine,Bronx,NY;and G. Zon,FDA,Bethesda, 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:
2.2	1.3	0.9

CHECK APPROPRIATE BOX(ES)

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

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

The nature and position of transcriptional control elements responsible for the differential control of gene expression in eukaryotic cells have not been precisely defined. A primary goal of this section is to develop new methods to investigate transcriptional control mechanisms, which are mediated by nucleic acid promoter elements, utilizing an active in vitro transcription system. Tissue-specific promoter elements will be used to stimulate gene expression in vivo utilizing retroviruses as mediators of specific gene transfer.

To identify components required for transcription of mRNA by RNA polymerase II, distinct transcriptional complexes have been characterized and purified using newly developed techniques of DNA affinity chromatography. Proteins associated with specific DNA sequences following affinity purification are being used to identify: 1) Transcription factors and their functionality and 2) DNA control sequences and topology necessary for regulated initiation and elongation of in vitro transcriptional intermediates.

Specific synthetic control sequences of DNA are being assembled, based on the presence of tissue-specific transcription factors and nucleic acid topology. These synthetic promoters are being used to stimulate expression of the genes for adenosine deaminase and UDP-glucuronyltransferase in bone marrow and liver cells, respectively, following cloning into retroviruses. Both in vitro and in vivo infections with these modified expression vectors are being studied as models of gene transfer into genetically deficient cells.

656

GPO 914-918

Annual Report of the
Section on Laboratory Animal Medicine and Surgery, Surgery Branch
Division of Intramural Research
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

The Section functions primarily in a support role to all laboratories of IR providing care for many species of animals, technical assistance in preparation and maintenance of animal models for various experimental regimens, and the development of animal resources not otherwise available.

Maintenance of various small animal species has been accomplished in designated areas in close proximity to IR laboratories in Buildings 3, 10, and 36. Large animal species are maintained in Buildings 3, 28, the NIHAC, and at Luray, Virginia. Postoperative intensive care and treatment of surgery patients is completed in Buildings 3, 14-E, and 28.

The animal surgery laboratory located in Building 14-E supported studies for investigative staff in the Cardiology Branch, Clinical Hematology Branch, Laboratory of Kidney and Electrolyte Metabolism, Laboratory of Technical Development, and the Surgery Branch in preparation of experimental animal models, completing cardiovascular studies and in collecting various biological specimens. The laboratory operates an x-ray catheterization suite, blood analysis laboratory, sterile operating suites, and special study suites required to meet IR requirements.

The NHLBI sheep colony continues year-round breeding of laboratory sheep. Gestation stages from 120-140 days and various age and size lambs, young adults, and aged sheep were developed for use by the LTD, LKEM and the SB and postoperative animal models have been maintained at the colony. Feed supplies have been provided to NIH to allow continued feeding of similar feed rations to sheep maintained for biomedical research studies at NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03401-10 LAMS

PERIOD COVERED
October 1, 1985 to September 30, 1986

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

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

PI:	J. E. Pierce	Chief	SLAMS, SB, DIR, NHLBI
Others:	M. Jones	Senior Surgeon	SB, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Surgery Branch

SECTION
Section on Laboratory Animal Medicine and Surgery

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	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.)*

The Newfoundland Breeding Colony has been developed as a source of laboratory dogs affected with left ventricular hypertrophy (LVH) due to infracoronary left ventricle outflow tract obstruction and other spontaneously occurring heart defects. All have some form of hereditary subaortic stenosis (SAS) and/or pulmonary outflow tract obstruction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03402-10 LAMS

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

NHLBI Laboratory Sheep Colony

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

Joseph E. Pierce, D.V.M., Chief, SLAMS, SB, DIR, NHLBI

COOPERATING UNITS *(if any)*

1. Laboratory of Developmental Neurobiology, IRP, NICHD
2. VRB, DRS

LAB/BRANCH

Surgery Branch

SECTION

Section on Laboratory Animal Medicine and Surgery

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 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.)*

The Laboratory Sheep Colony is an NIH animal resource providing varied age sheep that meet specific year-round requirements of the Laboratory of Kidney and Electrolyte Metabolism, Laboratory of Technical Development and the Surgery Branch, DIR, NHLBI; and the Laboratory of Developmental Neurobiology, IRP, NICHD. Maintenance regimens in use have resulted in successful year-round breeding and production of healthy varied age sheep.

Practices that have contributed to reduction of undesired seasonal variables include: (1) continuous prophylactic immunization of all age animal groups; (2) accurate pregnancy diagnosis during first trimester using Doppler ultrasound; (3) monitoring of animal health using various diagnostic laboratory techniques; and (4) many husbandry techniques unique to this colony. Such practices have been cost prohibitive in commercial sheep flocks that result in inconsistent availability and existence of varied states of health in animals delivered for laboratory use.

Annual Report of the Pathology Branch
Division of Intramural Research
National Heart, Lung, and Blood Institute
October 1, 1985 to September 30, 1986

As in past years, studies focused on various types of cardiovascular diseases including coronary, valvular, congenital and miscellaneous varieties.

CORONARY ARTERY DISEASE

Rupture of the heart during acute myocardial infarction appears to be increasing as death from various ventricular arrhythmias appears to be decreasing. The most frequent rupture site is left ventricular free wall, next, ventricular septum, and last, left ventricular papillary muscle. We gathered together 22 necropsy patients, aged 45-80 years (mean 64), in whom rupture of one left ventricular papillary muscle occurred during acute myocardial infarction which was fatal. The major findings in this study were 1) that rupture is nearly always the first coronary event, in that few patients had left ventricular scars; 2) rupture of the posteromedial papillary muscle is far more common than that of the anterolateral one by a 3 to 1 ratio; 3) quantitative examination of the amounts of narrowing of the 4 major coronary arteries by atherosclerotic plaque is significantly less severe in the rupture patients than in a control group of acute myocardial infarction patients without rupture; and 4) all of the hearts with papillary muscle rupture had huge amounts of subepicardial adipose tissue. The significance of this latter observation is unclear but the contrast to the control subjects was striking. Comparison of the histologic appearance of the infarct in the ruptured cases compared to the nonruptured cases disclosed no significant morphologic differences. Thus, why one patient with acute myocardial infarction has papillary muscle rupture and another does not remains unclear.

VALVULAR HEART DISEASE

A major undertaking during this past year was examination of hearts at necropsy in patients who had had simultaneous replacement of both mitral and aortic valves, or simultaneous replacement of mitral and tricuspid valves compared to patients having simultaneous mitral valve replacement and tricuspid valve anuloplasty, or combined mitral and tricuspid valve replacement for mitral valve stenosis and tricuspid valve stenosis, or simultaneous replacement of tricuspid, mitral and aortic valves. Each of these 4 groups of cases was analyzed looking specifically for anatomic causes of death less than 60 days following operation. The largest of these studies included 54 patients who died after simultaneous replacement of both mitral and aortic valves. The patients were divided into 4 groups on the basis of the presence of stenosis (with or without associated regurgitation) or pure regurgitation of each valve. Anatomic evidence of interference to movement of a poppet or disc in the aortic valve position was twice as common as anatomic

evidence of interference to poppet on disc movement in the mitral position. Interference to poppet movement is attributable to the prosthesis's being too large for the ascending aorta or left ventricular cavity in which it resided. The ascending aorta is infrequently enlarged in patients with combined mitral and aortic valve dysfunction irrespective of whether the aortic valve is stenotic or purely regurgitant. Likewise, the left ventricular cavity is usually not dilated in patients with combined mitral and aortic valve stenosis, the most common indication for replacement of both left-sided cardiac valves. Of the 54 patients, 12 had one mechanical and one bioprosthesis inserted. In our view, both substitute valves should be mechanical prostheses or both should be bioprostheses.

The second study compared 13 patients who underwent simultaneous mitral valve replacement for mitral stenosis and either tricuspid valve replacement (13 patients) or anuloplasty (17 patients) for pure tricuspid valve regurgitation. Comparison of the 13 patients having simultaneous double valve replacement to the 17 having mitral valve replacement and tricuspid valve anuloplasty disclosed similar mean age, preoperative right ventricular systolic pressure, right atrial mean pressure, left ventricular systolic pressure, average pulmonary artery wedge - left ventricular end diastolic pressure, cardiac index, heart weight, and percent with grossly visible foci of left ventricular necrosis. The causes of death early in the 2 groups, however, was different: of the 10 patients in the group having double valve replacement and dying within 60 days of operation, the cause was excessive bleeding in 5, low cardiac output of undetermined etiology in 3, dysfunction of both prostheses in 1, and cerebral insult in 1; of the 14 patients dying early after mitral valve replacement and tricuspid valve anuloplasty, none died from excessive bleeding, 4 from decreased cardiac output of uncertain cause, 5 from left ventricular inflow obstruction and 1 from left ventricular outflow obstruction.

Combined tricuspid valve stenosis and mitral valve stenosis is the least frequent of all cardiac valvular functional disturbances. Six patients who had either simultaneous replacement of the tricuspid valve and mitral valve or simultaneous mitral valve replacement and tricuspid valve commissurotomy for combined tricuspid valve stenosis and mitral stenosis were examined at necropsy. The major cause of death in all patients was inadequate cardiac output but the cause of the inadequate cardiac output was prosthetic dysfunction in only 1 patient.

Replacement of the tricuspid, mitral and aortic valves simultaneously is the least common of valvular cardiac operations. We examined at necropsy 12 patients who had underdone simultaneous triple valve replacement. Of the 10 patients dying within 60 days of triple valve replacement, 7 had the low cardiac output syndrome which in 4 and possibly in 5 was attributable to prosthetic aortic valve stenosis. In none of the 12 patients was the ascending aorta dilated and in these 4 or possibly 5 patients with low cardiac output the space between the surface of the caged poppet or margins of the tilting disc in the aortic valve position and the aortic endothelium appeared inadequate to allow unobstructed flow despite small sized prostheses in 11 of the 12 patients. Thus, aortic valve replacement in the setting of triple valve dysfunction is hazardous or potentially so. The relative small sizes of the hearts in these patients also makes valve replacement more difficult and hazardous compared to hearts with larger sized ventricles and aortas.

These 4 studies of morphologic findings after replacement of one or more cardiac valves were the first to be done focusing on the hemodynamic lesions which precipitated the need for valve replacement.

CONGENITAL HEART DISEASE

A major undertaking during this year was the examination of a large number of hearts at necropsy of patients who were found to have anomalous origin of 1 or both coronary arteries. This analysis, which is the largest to be done, focused on anomalies which allowed survival longer than 15 years of age. Of 5 patients with anomalous origin of 1 or more coronary arteries from the pulmonary trunk and origin of 1 or more coronary artery from the aorta, only 1 survived past 15 years. Origin of 1 or 2 coronary arteries from the pulmonary trunk without origin of a coronary artery from the aorta was not compatible with survival past 15 years. Anomalous origin of 1 or more coronary arteries from the aorta without origin of a coronary artery from the pulmonary trunk was the most common anomaly of coronary origin encountered. The most frequent was origin of both left main and right coronary arteries from the right aortic sinus. Five cases were encountered and each of these individuals died suddenly and unexpectedly. In contrast, origin of both left main and right coronary arteries from the left aortic sinus was encountered in 16 patients and in 2 death was attributable to this coronary anomaly. The most common of the anomalies of origin was origin of both right and left circumflex coronary arteries from the right aortic sinus (or origin of the left circumflex from right coronary artery) and of the left anterior descending coronary artery from the left aortic sinus. This anomaly was observed in 15 patients, 11 of whom were men. This anomaly in no patient appeared to cause evidence of cardiac dysfunction. A number of cases of single coronary artery were encountered but in none was this anomaly, when isolated, a cause of cardiac dysfunction.

MISCELLANEOUS CARDIOVASCULAR CONDITIONS

One study involved necropsy cases of hypertrophic cardiomyopathy to determine if the thickened cardiac walls of these patients was due to increased size or number of myocytes or to increased amounts of fibrous tissue or to both. Eight patients, aged 18 to 42 years, and 8 matched controls without heart disease were studied. Specific regions in each of the ventricular walls were evaluated for fibrous tissue by point counting; cell diameter was measured using an ocular micrometer. Cell layers were counted across the walls. The results disclosed that increased cell size, cell layers and fibrous tissue are characteristic of hypertrophic cardiomyopathy, but only in the ventricular septum were all 3 significantly increased. The fibrous tissue was most extensive in the ventricular septum but it was greater than in the controls in all 3 walls. Cell diameters were largest in the layers closest to the left ventricular cavity.

Starting about 4 years ago, we began measuring total 12-lead QRS voltage in several conditions, including aortic valve stenosis, idiopathic dilated cardiomyopathy, cardiac amyloidosis and in a few patients with hypertrophic cardiomyopathy, and found that the use of total 12-lead QRS

electrocardiographic voltage was a better criterion for left ventricular hypertrophy than any previously proposed voltage criterion. The problem with these previous studies is that a normal control group had not been examined. We examined total 12-lead QRS voltage in 30 patients who at necropsy had normal hearts. It was found that 175 mm was an appropriate upper limit of normal for total QRS voltage in all 12 leads. Having this upper limit of normal, of course, makes comparison to the abnormal hearts more meaningful.

To determine whether or not the ability of isoproterenol to induce myocardial necrosis is altered by the presence of alloxan treatment, isoproterenol-induced myocardial necrosis was examined in male mice with alloxan-induced and with genetically transmitted diabetes mellitus. Ten days after alloxan treatment, the mice had elevated blood glucose concentrations, weight loss, polyuria and decreased heart weights compared to matched-control mice. Similarly, genetically diabetic mice had lower heart rates than the corresponding age-mached controls. Both groups of diabetic mice had significant and comparable decrease in the severity of isoproterenol-induced cardiac necrosis.

Previous studies have demonstrated that the concurrent administration of ICRF-187 protects against anthracycline-induced cardiotoxicity. A study was undertaken to determine whether this protection was exerted on a long-term basis. The results showed that pretreatment with ICRF-187 provided for long protection against the cardiomyopathy, as opposed to the producing only a delay in the appearance of cardiac alteration.

The late (21-90 days) lesions caused in the heart and blood vessels of rats consuming allylamine were studied by light and electron microscopy. These lesions consisted of extensive left ventricular scarring, with the formation of left ventricular aneurysms, endocardial thickening, and focal areas of cartilaginous metaplasia. Vascular lesions were characterized by marked fibromuscular intimal proliferation. These findings indicate that severe myocardial, small vessel, and endocardial injury occurs during the course of chronic allylamine intoxication.

To evaluate the extent of occurrence and the significance of intraluminal fibrosis in interstitial pulmonary disorders, histologic and ultrastructrural studies were made of lung tissues from 373 patients with fibrotic lung disorders of various types. These studies showed that intraluminal fibrosis, i.e., fibrosis involving the lumina of alveoli and alveolar ducts, is more important than is interstitial fibrosis in mediating pulmonary fibrous remodeling in interstitial lung disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03875- 01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Lipid accumulation in venous bypass grafts: influence of arterial pressure.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
E. Rene Rordriguez, Visiting Fellow, Pathology Branch, NHLBI
G. N. Olinger, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI
L. I. Bonchek, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI
I. I. Gunay, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI
A. H. Kissebah, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI

COOPERATING UNITS *(if any)*

Deaprtment of Surgery, Medical College of Wisconsin, Milwaukee, WI

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

Biochemical and morphological studies were made to assess the relative importance of distension at the time of implantation and chronic exposure to arterial pressure on the accumulation of lipids in venous bypass grafts placed in the femoral position in normolipemic stump-tailed macaque monkeys. The effect of chronic exposure to arterial pressure was found to be more important than that of intraoperative distension of the graft.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03876- 01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Allylamine toxicity: late myocardial and vascular lesions.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Paul J. Boor, Department of Pathology, University of Texas Medical Branch, Galveston, Texas.

COOPERATING UNITS *(if any)*

University of Texas Medical Branch, Galveston, Texas.

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NLHBI, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The late (21-90 days) lesions caused in the heart and blood vessels of rats consuming allylamine were studied by light and electron microscopy. These lesions consisted of extensive left ventricular scarring, with the formation of left ventricular aneurysms, and endocardial thickening similar in many respects to that seen in endocardial fibroelastosis, and with focal areas of cartilaginous metaplasia. Vascular lesions were characterized by marked fibromuscular intimal proliferation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03877-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Influence of diabetes mellitus on isoproterenol-induced myocardial necrosis.

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

V. J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
A. N. El-Hage, Division of Drug Biology, Food and Drug Administration, Wash., D.C.
E. H. Herman, Division of Drug Biology, Food and Drug Administration, Wash., D.C.
A. W. Jordan, Division of Drug Biology, Food and Drug Administration, Wash., D.C.

COOPERATING UNITS *(if any)*

Division of Drug Biology, Food and Drug Administration, Washington, D.C.

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

Compared to that in normal control mice, the severity of the myocardial necrosis produced by isoproterenol was markedly decreased in mice with diabetes induced by alloxan and in mice with genetically transmitted diabetes. The administration of insulin restored the sensitivity of the myocardium to isoproterenol-induced necrosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03878- 01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Intraluminal fibrosis in fibrotic lung disorders

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Francoise Basset, Faculte Bichat, Paris, France
Tamiko Takemura, Visiting Scientist, Pathology Branch, NHLBI
Yuh Fukuda, Visiting Expert, Pathology Branch, NHLBI
Ronald G. Crystal, Chief, Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)*

Faculte Bichat, Paris, France
Pulmonary Branch, NHLBI

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

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

Histologic and ultrastructural studies were made of lung tissues from a total of 373 patients with fibrotic lung disorders of various types. These studies showed that intraluminal fibrosis, i.e., fibrosis involving the lumina of alveoli and alveolar ducts, is more important than is interstitial fibrosis in mediating pulmonary fibrous remodeling in interstitial lung disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03879- 01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986.

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

Long-lasting protection by ICRF-187 against doxorubicin-induced cardiotoxicity

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Eugene H. Herman, Division of Drug Biology, Food and Drug Administration, Washington, D.C.

COOPERATING UNITS *(if any)*

Division of Drug Biology, Food and Drug Administration, Washington, D.C.

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

Previous studies have demonstrated that the concurrent administration of ICRF-187 protects against anthracycline-induced cardiotoxicity. The present study was undertaken to determine whether this protection is exerted on a long-term basis. The results obtained show that pretreatment with ICRF-187 provides prolonged protection against the cardiomyopathy, as opposed to producing only a delay in the appearance of cardiac alterations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03880- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Rupture of a Left Ventricular Papillary Muscle During Acute Myocardial .....

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

Deborah J. Barbour, Senior Staff Fellow - Pathology Branch, NHLBI

William C. Roberts, Chief, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch, NHLBI

SECTION

NHLBI/NIH/Bethesda, MD

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Certain clinical and cardiac morphologic findings are described in 22 patients, aged 45 to 80 years (mean 64) (15 men [68%]), in whom rupture of a papillary muscle occurred during acute myocardial infarction. In most, the acute myocardial infarction associated with papillary muscle rupture was their first coronary event (only 18% had myocardial scars consistent with prior infarction and 29% had angina pectoris). The posteromedial papillary muscle, presumably because of its more tenuous blood supply, ruptured almost 3 times more frequently than the anterolateral one (73% and 27%, respectively). Quantitative examination of the amounts of narrowing by atherosclerotic plaque in each of the 4 major epicardial coronary arteries (right, left main, left anterior descending and left circumflex) disclosed less narrowing in the rupture patients than in patients with fatal acute myocardial infarction unassociated with rupture. Of the 519 five-mm sections of coronary artery examined (11 patients), only 68 (13%) were narrowed greater than 75% in cross-sectional area compared to 34% of 1403 sections from 27 patients with fatal myocardial infarction without rupture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03881- 01 PA

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Clinical and Necropsy Observations Early After Simultaneous Replacement .....

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

William C. Roberts	Pathology Branch	NHLBI
Mark F. Sullivan	Pathology Branch	NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pathology Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

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

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

Clinical and necropsy findings are described in 54 patients, aged 25 to 83 years (mean 53), who died within 60 days of simultaneous replacements of both mitral and aortic valves. The patients were divided into 4 groups on the basis of the presence of stenosis (with or without associated regurgitation) or pure regurgitation of each valve: 30 patients (56%) had combined mitral and aortic valve stenosis; 12 patients (22%) had mitral stenosis and pure aortic regurgitation; 8 patients (15%) had pure regurgitation of both valves, and 4 patients (7%) had pure aortic regurgitation and mitral stenosis. Necropsy examination in the 54 patients disclosed a high frequency (48%) of anatomic evidence of interference to poppet or disc movement in either the mitral or aortic valve position or both. Anatomic evidence of interference to movement of a poppet or disc in the aortic valve position was twice as common as anatomic evidence of interference to poppet or disc movement in the mitral position. Interference to poppet movement is attributable to the prosthesis's being too large for the ascending aorta or left ventricular cavity in which it resided. The ascending aorta is infrequently enlarged in patients with combined mitral and aortic valve dysfunction irrespective of whether the aortic valve is stenotic or purely regurgitant. Likewise, the left ventricular cavity is usually not dilated in patients with combined mitral and aortic valve stenosis, the most common indication for replacement of both left sided cardiac valves. Of the 54 patients, 12 (22%) had 1 mechanical and 1 bioprosthesis inserted. In our view, both substitute valves should be mechanical prostheses, or both should bioprostheses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03882- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Mitral Valve Stenosis and Pure Tricuspid Valve Regurgitation: Comparison .....

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

Mark F. Sullivan, Senior Staff Fellow, Pathology Branch, NHLBI

William C. Roberts, Chief, Pathology Branch

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch, NHLBI

SECTION

NHLBI/NIH//Bethesda, MD 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Clinical and morphologic observations are described in 30 patients (23 [77%], all functionally class III or IV), who underwent replacement of the mitral valve for mitral stenosis and either simultaneous replacement (13 patients) (Group I) or anuloplasty (17 patients) (Group II) of the tricuspid valve for pure tricuspid valve regurgitation. Comparison of the 13 patients in group I with the 17 patients in group II disclosed similar mean ages (55 years - vs - 58 years), similar average pre-operative right ventricular systolic pressures (10 mm Hg - vs - 61 mm Hg), similar average right atrial mean pressures (10 mm Hg - vs - 9 mm Hg), similar average left ventricular systolic pressues (126 mm Hg - vs - 120 mm Hg), similar average pulmonary artery wedge - left ventricular mean diastolic pressures (16 mm Hg - vs - 18 mm Hg), similar cardiac indices (2.1 L/min/M^2 - vs - 2.0 L/min/M^2, similar mean heart weights (507 g - vs - 535 g), and similar percents with grossly visible foci of left ventricular necrosis (15% - vs - 12%). Of the 13 patients in group I, 10 (77%) died early ($\leq$ 60 days of tricuspid vave replacement) and 3 (23%) died late (29, 37 and 120 months); of the 17 patients in group II, 14 (82%) died early and (18%) died late (4, 9 and 98 months). The causes of death early in the 2 groups was different: of the 10 patients in group I dying early, the cause was excessive bleeding in 5, low cardiac output of undetermined etiology in 3, dysfunction of both prostheses in 1, and cerebral indult in 1; of the 14 patients dying early in group 2, none died from excessive bleeding, 4 from decreased cardiac output of uncertain cause, 5 from left ventricular inflow obstruction (produced by a Starr-Edwards ball-valve prosthesis in 4 and from a Starr-Edwards disc prosthesis in 1) and 1 from left ventricular outflow.....

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03883- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Combined Mitral Valve Stenosis and Tricuspid Valve Stenosis: Morphologic .....

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

Mark F. Sullivan, Senior Staff Fellow, Pathology Branch, NHLBI

William C. Roberts, Chief, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch, NHLBI

SECTION

NHLBI/NIH/Bethesda, MD 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS: 416	PROFESSIONAL: 416	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.)*

Certain clinical and morphologic findings in 6 patients, all women, who underwent cardiac valve operations for combined mitral stenosis and tricuspid valve stenosis and who died within 60 days of the simultaneous mitral and tricuspid observations were summarized. Two of the 6 patients died from excessive bleeding and other 4 from 3 to 13 days from inadequate cardiac output. In 2 of the latter 4 patients the cause of the diminished cardiac output was anatomic evidence of interference with mitral occluder movement and the cause of the inadequate cardiac output in the other 4 patients was not determined from anatomic study. Combined mitral and tricuspid valve stenosis is a very unusual combination and no previous studies have described morphologic observations after simultaneous valve operations in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03884- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Clinical and Morphologic Observations After Simultaneous Replacement .....

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

Mark F. Sullivan, Senior Staff Fellow, Pathology Branch, NHLBI

William C. Roberts, Chief, Pathology Branch

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch, NHLBI

SECTION

NHLBI/NIH/Bethesda, MD 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS: 416	PROFESSIONAL: 416	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Clinical and morphologic observations are described in 12 patients who underwent simultaneous replacement of the tricuspid, mitral and aortic valves. All 12 patients had mitral stenosis; 10, aortic valve stenosis and 2, pure aortic valve regurgitation; 5 had tricuspid valve stenosis and 7, pure tricuspid valve regurgitation. Of the 10 patients dying within 60 days of triple valve replacement, 7 had the low cardiac output syndrome which in 4, and possibly 5, of the 7 was attributed to prosthetic aortic valve stenosis. In none of the 12 patients was the ascending aorta dilated, and in these 4 (possibly 5) patients with the low cardiac thought, the space between the surface of the caged poppet (4 patients) or margins of the tilting-disc (1 patient) in the aortic valve position and the aortic endothelium appeared inadequate to allow unobstructed flow despite small-sized prostheses in all but 1 patient. Thus, aortic valve replacement in the setting of triple valve dysfunction is hazardous or potentially so. The relative small sizes of the hearts in these patients also makes valve replacement more difficult (and hazardous) compared to hearts with larger sized ventricles and aortas.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 03885- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Major anomalies of coronary arterial origin seen in adulthood

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

William C. Roberts, MD Pathology Branch NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

NHLBI, Bethesda, MD

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 last 25 years many differing types of anomalies of origin of one or more coronary arteries have been observed at necropsy. These various anomalies as observed in adulthood (greater than age 15 years) were examined. The most common major anomaly is origin of one or more coronary arteries from the pulmonary trunk and origin of one or more coronary arteries from the aorta. This anomaly was encountered in 5 patients, only one of whom reached adulthood (44 years). Origin of 1 or 2 coronary arteries from the pulmonary trunk without origin of a coronary artery from the aorta was encountered once, in a newborn. This anomaly has not been observed in adults. The third major category was the anomalous origin of one or more coronary arteries from the aorta without origin of a coronary artery from the pulmonary trunk. The most common of these anomalies is origin of both left main and right coronary arteries from the right aortic sinus. This anomaly is a relatively common cause of sudden death during childhood and this anomaly was encountered in 5 children. The next major anomaly was origin of both left main and right coronary arteries from the left aortic sinus. This anomaly was encountered in 16 individuals at necropsy, all of whom, were adults. In contrast to previous studies of this anomaly, it was shown that it can be a cause of sudden unexpected death. The most common of the anomalies of origin is both right and left circumflex coronary arteries from the right aortic sinus (or origin of the left circumflex from the right coronary artery) and of left anterior descending coronary artery from the left aortic sinus. This anomaly was described initially in 1933.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03886- 01 PA

PERIOD COVERED

October 1, 1985 - September 30, 1986

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

Left Main Coronary Artery Originating from the Right Sinus of Valsalva .....

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

Charles W. Barth III - Senior Staff Fellow - NHLBI
William C. Roberts - Chief, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pathology Branch

SECTION

INSTITUTE AND LOCATION

NHLBI/NIH/Bethesda

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

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

- ☐ (a1) Minors
- ☐ (a2) Interviews

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

Findings are described in five patients who at necropsy were found to have origin of the left main coronary artery from the right sinus of Valsalva and coursing of the anomalously arising artery between aorta and pulmonary trunk to reach the left side of the heart. Three of the five patients were boys and died suddenly at ages 13, 14 and 19 years, respectively: two of them had had one or more episodes of syncope and the third had an abnormal electrocardiogram. The fourth patient, a 64-year old woman, died of chronic congestive heart failure 1 year after an acute myocardial infarction. She had insignificant coronary atherosclerosis. The fifth patient, an 81-year-old man, died of chronic alcoholism, having been free of symptoms of cardiac dysfunction during life.

Additionally, clinical and necropsy findings are summarized in 38 previously reported necropsy patients with the coronary anomaly. Of these 38 (34 male [89%]), 23 (61%) died suddenly in the first two decades of life; death in 6 others (16%) appears to have been related to coronary atherosclerosis and 9 patients (24%) died from non-coronary causes. Thus, this anomaly is life-threatening. Why it frequently causes fatal cardiac arrest in some young individuals and allows a normal life span in others remains unclear.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03887- 01 PA

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Regional Myocyte Hypertrophy, Hyperplasia and Fibrosis in Hypertrophic ....

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

Donald V. Unverferth, MD	Ohio State University College Columbus, Ohio
Peter B. Baker, MD	"
Leesa I. Pearce, B.S.	"
Jeffrey Lautman, B.A.	"
William C. Roberts, MD	Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pathology Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this study is to determine if the thickened cardiac walls of patients with hypertrophic cardiomyopathy (HC) are due to increased size or number of myocytes or increased amounts of fibrous tissue. Eight patients aged 18-42 years, who died from complications of HC and 8 age-matched controls without heart disease were studied. A 1.5 cm^3 of tissue was removed from the left ventricular (LV) free wall, the right ventricular free wall and the ventricular septum (VS). Each region of each wall was evaluated for fibrous tissue by point counting; cell diameter was measured using an ocular micrometer disc. Cell layers were counted across the walls. The results revealed that increased cell size, cell layers and fibrous tissue are characteristic of HC but only in the VS are all 3 significantly increased. The fibrous tissue was most extensive in the VS ($19 \pm 9\%$), but it was greater than in the controls in all 3 walls. Cell diameters were largest in the layers closest to the LV cavity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03888- 01 PA

PERIOD COVERED
October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
QRS Voltage Measurements in Autopsied Men Free of Cardiopulmonary Disease: ....

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

Harrell Odom II	VA Administration Medical Center, Un. of Arkansas
J. Lynn Davis	"
Ha Dinh	"
Bonnie J. Baker	"
William C. Roberts	Pathology Branch, NHLBI
Marvin L. Murphy	VA Administration, Medical Center, Un. of Arkansas

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pathology Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
416	416	

CHECK APPROPRIATE BOX(ES)

- [x] (a) 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 total 12-leads QRS electrocardiographic voltage as a criterion for left ventricular (LV) hypertrophy has been of recent interest. Although the upper and lower limits of QRS voltage for individual electrocardiographic leads have been reported in clinically healthy men and women, the upper limit of total 12-lead QRS voltage has not been established in adults free of cardiopulmonary disease by clinical and necropsy criteria. Therefore, the total QRS voltage from all 12 electrocardiographic leads was determined in 30 autopsied men known to be free of cardiopulmonary disease by clinical assessment and by a special cardiac examination using postmortem coronary angiography and chamber partition determination of LV weight. Gross heart weight, LV weight, and total QRS voltage are reported. Comparisons were made between disease-free patients and previously reported patients with aortic valve stenosis, aortic regurgitation, and cardiac amyloidosis with respect to total QRS voltage and gross heart weight. Total QRS voltage and gross heart weight were significantly greater in patients with severe aortic stenosis (mean 245 mm) and severe aortic regurgitation (mean 274 mm) than in our patients (mean 127 mm). Total QRS voltage was significantly less, while gross heart weight was significantly greater in patients with cardiac amyloidosis (mean 101 mm) than in our normals (mean 127 mm). This data provides a basis for evaluating the total 12-lead QRS voltage as a criterion for LV hypertrophy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03889-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The assembly of myofibrils in the developing heart.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
E. Rene Rodriguez, Visiting Fellow, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A detailed description is given of morphological aspects of the process by which contractile filaments and myofibrils are assembled during embryonic development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03890-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Anatomic changes in right ventricular-pulmonary artery conduits.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Eloisa Arbustini, Guest Worker, Pathology Branch, NHLBI
Elling E. Eidbo, Surgery Branch, NHLBI
Michael Jones, Senior Surgeon, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Surgery Branch, NHLBI

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

Gross anatomic, histologic and ultrastructural studies were made of the changes that developed in right ventricular-pulmonary artery conduits implanted in young baboons for 37 to 61 weeks. These conduits contained either a porcine aortic valve or a bovine pericardial valve. The major changes observed consisted of conduit obstruction by fibrous peel growing on its luminal surface. This fibrous peel tended to involve the bioprosthetic valve, causing cuspal retraction and interfering with valve function, such that many conduits behaved as valveless conduits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03891-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

The Cardiomyopathies

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

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

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

An extensive review was made of the pathologic anatomy of the cardiomyopathies, with emphasis on those which occur in children.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03892-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Activation of DNA synthesis and mitotic events in myocardial injury.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
J. O. Oberprller, Department of Anatomy, University of South Dakota Sch. of Med.
T. J. McDonnell, Department of Anatomy, University of South Dakota Sch. of Med.
J. C. Oberpriller, Department of Anatomy, University of South Dakota Sch. of Med.

COOPERATING UNITS *(if any)*

Department of Anatomy, University of South Dakota School of Medicine, Grand Forks, South Dakota

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.]	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.)*

Atrial myocytes were shown to respond to injury by synthesizing DNA and by undergoing nuclear mitosis in two model systems: ventricular or atrial damage in newts and in ventricular infarction produced by coronary artery ligation in rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03893-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cardiac lesions of selenium-vitamin E deficiency in animals.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch
John F. Van Vleet, Purdue University School of Veterinary Medicine,
West Lafayette, Indiana

COOPERATING UNITS *(if any)*

Purdue University School of Veterinary Medicine, West Lafayette, Indiana

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

This report describes the morphology of cardiac lesions which develop in a number of species of animals as the result of deficiency of selenium and vitamin E. These lesions consist of multifocal areas of cardiac necrosis, which in some species are accompanied by fibrinoid necrosis of blood vessels, and which lead to widespread areas of myocardial fibrosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03894-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Cardiovascular lesions in collagen-vascular diseases.

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
E. Rene Rodriguez, Visiting Fellow, Pathology Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

A detailed review is made of cardiac morphologic changes in the collagen-vascular diseases, including rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, scleroderma, dermatomyositis, polymyositis, polyarteritis nodosa and Wegener's granulomatosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03895-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Histiocytosis X

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Francoise Basset, INSERM U 82, Faculte Bichat, Paris, France
Sylvie Chollet, INSERM U 82, Faculte Bichat, Paris, France
Paul Soler, INSERM U 82, Faculte Bichat, Paris, France

COOPERATING UNITS *(if any)*

INSERM U 82, Hopital Bichat, Paris, France

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The clinical and morphologic findings in histiocytosis X are described in detail, including the characteristic features of the different forms of this disorder. Emphasis is placed on pulmonary histiocytosis X, the most common form in adult patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 03896-01 PA

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Granulomatous inflammation of the heart

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
E. Rene Rodriguez, Visiting Fellow, Pathology Branch, NHLBI
Hugh A. McAllister, Pathology Department, Saint Luke's Hospital, Houston, Texas.

COOPERATING UNITS *(if any)*

Saint Luke's Hospital, Houston, Texas.

LAB/BRANCH

Pathology Branch

SECTION

Ultrastructure Section

INSTITUTE AND LOCATION

NLHBI, NIH, Bethesda, MD 20892

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

A survey was made, based on the combined experience of the Pathology Branch, NHLBI, and the Cardiovascular pathology Department, Armed Forces Institute of Pathology, of the causes and morphological features of granulomatous lesions which occur in the heart.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 03897-01 PA

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of epithelial basement membrane in pulmonary fibrotic remodeling

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

Victor J. Ferrans, Chief, Ultrastructure Section, Pathology Branch, NHLBI
Y. Fukuda, Department of Pathology, Nippon Medical School, Tokyo, Japan
M. Ishizaki, Department of Pathology, Nippon Medical School, Tokyo, Japan
Y. Masuda, Department of Pathology, Nippon Medical School, Tokyo, Japan
O. Kawanami, Department of Pathology, Nippon Medical School, Tokyo, Japan.
K. Aihara , Department of Pathology, Nippon Medical School, Tokyo, Japan.
Y. Masugi, Department of Pathology, Nippon Medical School, Tokyo, Japan.

COOPERATING UNITS *(if any)*

Department of Pathology, Nippon Medical School, Tokyo, Japan.

LAB/BRANCH
Pathology Branch

SECTION
Ultrastructure Section

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20892

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

Ultrastructural and immunohistochemical studies were made to evaluate the role of the alveolar epithelial basement membrane in pulmonary fibrotic remodeling. These studies showed that the basement membrane provides sites of attachment for fibroblasts which migrate from the alveolar interstitium into alveolar lumina and for migrating epithelial cells in early stages of the process of relining the alveolar epithelial surface after injury.

Annual Report of the Pulmonary Branch
National Heart, Lung and Blood Institute
October 1, 1985 through September 30, 1986

The research of the Pulmonary Branch centers on diseases of the alveolar structures, the site in the body in which gas exchange takes place between the air and blood. Three categories of common diseases are investigated: all represent chronic inflammatory disorders of the lower respiratory tract in which the inflammation causes the changes in the lung parenchyma that defines the clinical presentation of each disease.

(1) Disorders characterized by 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", an accumulation of mesenchymal cells and the collagenous matrix produced by these cells. In general, the inflammation of these disorder is dominated by alveolar macrophages, and to a lesser extent, neutrophils and/or eosinophils. Example of these fibrotic disorders include idiopathic pulmonary fibrosis and asbestosis.

(2) Disorders characterized by the accumulation of T-lymphocytes in the lower respiratory tract. These disorders are also a subgroup of the interstitial lung disorders. However, although occasionally associated with progressive fibrosis of the lung parenchyma late in their course, these disorders are universally characterized by large numbers of T-lymphocytes that dominate the inflammation and cause dysfunction by their presence which distorts the alveolar, bronchial, and vascular walls, thus modifying the intimate relationship between air and blood. Examples of the T-lymphocyte disorders include sarcoidosis, berylliosis and hypersensitivity pneumonitis.

(3) Disorders characterized by destruction of the alveolar walls. These disorders are commonly called emphysema. Of the $2x10^6$ individuals in the USA with emphysema, approximately 98% acquire the disease, usually on the basis of cigarette smoking, while 2% have an inherited disorder called alpha-1-antitrypsin deficiency. All forms of emphysema are characterized by a dissolution of the lung parenchyma. The inflammation that causes these changes is dominated by alveolar macrophages together with smaller numbers of neutrophils.

The inflammation of all of these disorders 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, usually 5, 20ml aliquots in 3 sites, is infused into the bronchoscope and then suctioned back, thus sampling the epithelial lining fluid of the lower respiratory tract. Over the past decade, this procedure has been used by the Pulmonary Branch to evaluate several thousand individuals.

I. Disorders Characterized by Progressive Fibrosis of the Lung Parenchyma.

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, primarily neutrophils and/or eosinophils but including macrophages. Second, that there is enhanced proliferation of mesenchymal cells in the alveolar walls driven by growth signals released by alveolar macrophages.

A critical aspect of this process is the mechanisms by which macrophages accumulate in the lungs in these disorders. Recent studies by the Pulmonary Branch have demonstrated that one mechanism by which this occurs is local proliferation of alveolar macrophages. Since transferrin is required for proliferation of mammalian cells, it is a necessary condition that in order to proliferate, the alveolar macrophages must express transferrin receptors. The expression of transferrin receptors by blood monocytes, human alveolar macrophages, and in vitro matured macrophages was evaluated by immunofluorescence, radioligand binding, and Northern analysis, using the monoclonal anti-human transferrin receptor antibody OKT9, [^{125}I]-labeled human transferrin and a [^{32}P]-labeled human transferrin receptor cDNA probe, respectively. By immunofluorescence, the majority of alveolar macrophages expressed transferrin receptors (86 ± 3%). The radioligand binding assay demonstrated the affinity constant (K_a) of the alveolar macrophage transferrin receptor was 4.4 ± 0.7 x $10^8 M^{-1}$, and the number of receptors per cell was 4.4 ± 1.2 x 10^4. In marked contrast, transferrin receptors were not present on the surface or in the cytoplasm of blood monocytes, the precursors of the alveolar macrophages. However, when monocytes were cultured in vitro and allowed to mature, >80% expressed transferrin receptors by day 6, and the receptors could be detected by day 3. Consistent with these observations, a transferrin receptor mRNA with a molecular size of 4.9 kb was demonstrated in alveolar macrophages and in vitro matured macrophages but not in blood monocytes. Thus, although blood monocytes do not express the transferrin receptor gene, it is expressed by mature macrophages, an event that probably occurs relatively early in the process of monocyte differentiation to macrophages.

The progressive fibrosis of the alveolar wall that causes the clinical, roentgenographic, and physiologic features of the fibrotic lung diseases result from chronic inflammation in the local milieu. An extention of this concept is that the inflammation must preceed the fibrosis of these disorders. To evaluate this hypothesis, we evaluated 17 clinically unaffected members of three families with an autosomal dominant form of idiopathic pulmonary fibrosis for evidence of alveolar inflammation. Each person in the study was examined by gallium-67 scanning for a general estimate of pulmonary inflammation, and by bronchoalveolar lavage for characterization of the types of recovered cells and their state of activation. Eight of the 17 subjects had evidence of alveolar inflammation on the lavage studies. Supporting data included increased numbers of neutrophils and activated macrophages that released one or more neutrophil chemoattractants, and growth factors for lung fibroblasts--findings similar to those observed in patients with overt idiopathic pulmonary fibrosis. Four of these eight also had a positive gallium scan; in all the other clinically unaffected subjects the scan was normal. During a follow-up of two to four years in seven of the eight subjects who had evidence of inflammation, no clinical evidence of pulmonary fibrosis has appeared. These results indicate that alveolar inflammation occurs in approximately half the clinically unaffected

family members at risk of inheriting autosomal dominant idiopathic pulmonary fibrosis. Whether these persons with evidence of pulmonary inflammation but no fibrosis will proceed to have clinically evident pulmonary fibrosis is not yet known.

The inorganic dust disorders are characterized, in part, by damage to type I epithelial cells and replacement by cuboidal epithelial cells. Since the damage to the epithelium is mediated primarily by inflammatory cells, we hypothesized that the inflammatory cells present on the epithelial surface of the lower respiratory tract of individuals with these disorders may be spontaneously releasing exaggerated amounts of oxidants such as O_2^- and H_2O_2. To evaluate this concept, inflammatory cells recovered by bronchoalveolar lavage of nonsmoking individuals with asbestosis (n=12), coal workers' pneumoconiosis (n=8) and silicosis (n=3) were compared to unexposed nonsmoking normals (n=12) for spontaneous release of the O_2^- (quantified as nmol of cytochrome C reduced/10^6 cells-hr) and H_2O_2 (quantified using phenol-horseradish peroxidase in nmol/10^6 cells-hr). As a group,the inorganic dust patients had an alveolitis dominated by alveolar macrophages (macrophages 79±3%, lymphocytes 18±3%, neutrophils 3±1%, eosinophils 1±1%). Importantly, on the average, their inflammatory cells released exaggerated amounts of O_2^- (30±3 nmols, normals 16±2, $p<0.01$) and H_2O_2 (7±1 nmols, normals 2±1, $p<0.02$). Superoxide dismutase (0.5 mg/ml) incubated with the cells reduced the amount of O_2^- measured ($p<0.01$), and catalase (4300 units/ml) reduced the amount of H_2O_2 ($p<0.01$). In addition, when the cells were incubated with dehydroepiandrosterone (DHEA, 10^{-4}M), a normally occurring adrenal androgen thought to act as a non-competitive inhibitor limiting substrates for the membrane-bound flavoprotein oxidase that generates O_2^-, the amount of O_2^- spontaneously released by the inorganic dust patients was reduced 59±14% ($p<0.05$). These observations suggest that inflammatory cells may play a role in the epithelial cell damage observed in inorganic dust disorders by virtue of their ability to release exaggerated amounts of oxidants. In this context, drugs such as DHEA that suppress the ability of these inflammatory cells to release oxidants, may be useful in the therapy of these disorders.

Although eosinophils are classically considered to be a "protective" cell for inflammatory and immune processes, there is increasing evidence that the eosinophil can effect damage to normal tissues. To evaluate this concept in the lower respiratory tract in man, it is necessary to find a "pure" eosinophilic inflammatory process. To accomplish this, in conjunction with the Laboratory of Clinical Investigation of the National Institute of Allergy and Infectious Diseases and the Tuberculosis Research Center, Madras, India, and the Indian Council for Medical Research, under the auspices of the Indo-USA joint agreement on technology, members of the Pulmonary Branch evaluated patients with acute tropical pulmonary eosinophilia in Madras, India. These individuals have a pure eosinophil inflammatory process in the lower respiratory tract that includes up to 70% of the total inflammatory cells being eosinophils (normal less than 1%). Following a standard 3 week diethylcarbamazine (DEC) therapy for acute tropical pulmonary eosinophilia (TPE) caused by filarial parasites, there is marked clinical improvement with significant reductions in both blood and lung eosinophilia. Despite this, however, some patients develop a chronic form of TPE that may progress to pulmonary fibrosis. To characterize the inflammatory activity in the lower respiratory tract in such patients, we performed bronchoalveolar lavage on 18 individuals 6 to 12 months post-DEC therapy for acute TPE and 7

untreated normals. A striking persistent increase in total cells in the epithelial lining fluid (ELF) was found in these "chronic TPE" patients ($47\pm5 \times 10^3$ cells/μl ELF; normal $24\pm1 \times 10^3$, $p<0.01$) with an increase in the percent ($5.8\pm.9$ vs $1.7\pm.3$, $p<0.01$) and total lung eosinophils (2830 ± 544 eosinophils/μl ELF vs 400 ± 85 eosinophils/μl ELF, $p<0.01$). Importantly, the inflammatory cells released exaggerated amounts of oxidants including superoxide (50 ± 3 nmols/10^6 cells-hr, normals 16 ± 2, $p<0.01$) and H_2O_2 (16 ± 2 nmols/10^6 cells-hr, normals 2 ± 1, $p<0.001$). In an attempt to suppress the persistent lung inflammation in chronic TPE, 12 individuals were treated with prednisone (1 mg/kg tapering to 0 over 1 wk). Repeat lavage after prednisone showed a significant decline in both lung eosinophilia and spontaneous release of oxidants ($p<0.05$, all parameters). These observations suggest that the chronic fibrosis that develops in some TPE patients after DEC may result from a persistent lung inflammatory process that can be effectively reduced with corticosteroids.

Current concepts of the pathogenesis of wound healing, atherosclerosis, pulmonary fibrosis and hepatic fibrosis, suggest a central role for the mononuclear phagocyte in attracting and/or stimulating mesenchymal cells to proliferate. We have demonstrated that activated human blood monocytes, but not resting monocytes, release a mediator that attracts smooth muscle cells and cooperates with other mediators to stimulate fibroblasts to proliferate. This mediator has a close similarity to platelet-derived growth factor (PDGF) as evidenced by: its chromatographic properties and chemical stability; competition with ^{125}I-PDGF for binding to fibroblasts; and immunoprecipitation with anti-PDGF antibodies. In parallel, stimulated monocytes, but not resting monocytes, express the c-*sis* proto-oncogene, a gene coding for one of the PDGF chains, consistent with the concept that expression of the c-*sis* proto-oncogene may play a role in the ability of mononuclear phagocytes to modulate the accumulation of mesenchymal cells.

Alveolar macrophages from normal individuals and patients with interstitial lung diseases spontaneously expressed a 4.2 kb mRNA complementary to the c-*sis* gene, a proto-oncogene coding for one of the chains of PDGF. Concomitantly, these cells released a mediator with the properties of PDGF, including: (a) chemotactic factor for smooth muscle cells whose activity was resistant to heat and acid, but sensitive to reduction; (b) mitogenic (competence) activity for fibroblasts; (c) ability to compete with PDGF for its receptor; and (d) precipitated by an anti-PDGF antibody. While blood monocytes do not contain c-*sis* mRNA transcripts, monocytes matured *in vitro* expressed c-*sis*, consistent with the concept that expression of c-*sis* occurs during the differentiation of monocytes into alveolar macrophages. Together with the known actions of PDGF, these observations suggest that the c-*sis* proto-oncogene and its PDGF product are part of the armamentarium available to the alveolar macrophages for normal lung defense and participation in lung inflammation.

While normal alveolar macrophages spontaneously release low levels of PDGF, alveolar macrophages of patients with idiopathic pulmonary fibrosis (IPF) spontaneously release high amounts of PDGF and this level is close to the amount of PDGF released by normal alveolar macrophages after *in vitro* stimulation with immune complexes. Furthermore, the PDGF released by the alveolar macrophages of patients with IPF has the same properties as PDGF isolated from platelets and it is biologically relevant since it induces smooth muscle cells to migrate

along a concentration gradient and acts as a "competence" mitogenic factor for fibroblast growth. If one also considers that the mononuclear phagocyte population is increased several-fold in the lungs of patients with IPF, these observations suggest that these patients have a markedly increased burden of active PDGF present in the lower respiratory tract.

In the chronic interstitial lung disorders, alveolar macrophages (AM) are also known to be spontaneously releasing increased amounts of fibronectin (Fn) a mediator that is a chemoattractant for fibroblast and like PDGF, provides "competence" to initiate fibroblast proliferation. Since fibronectin is not produced by blood monocytes but is produced by AM, we hypothesized that the process of maturation of monocytes to AM may involve the expression of the fibronectin gene, thus conveying to the AM the ability to produce a mediator that can aid in recruiting fibroblasts and stimulating them to enter the cell cycle. To evaluate this hypothesis, blood monocytes, *in vitro* matured monocytes and AM from normal individuals were evaluated for the presence of mRNA transcripts for Fn. Monocytes were obtained by Ficoll-hypaque centrifugation of normal blood and adherence (10% serum), *in vitro* matured monocytes were obtained by culture ($4x10^6$ cells/ml, 10% serum for 1,3,7 and 14 days), and AM were obtained by lavage and purified by adherence. RNA was extracted with guanidine hydrochloride, purified by CsCl centrifugation and evaluated by Northern analysis using a ^{32}P-labeled Fn DNA probe. Autoradiograms revealed that fresh blood monocytes did not express detectable mRNA transcripts Fn. In contrast, *in vitro* matured monocytes expressed a 7.8 kb Fn transcript identical to mRNA size for this gene transcript in other cells. Consistent with these observations, AM contained the 7.8 kb Fn transcript. Thus, AM express the Fn gene and likely acquire the ability to express this gene during the process of maturation from monocytes. Furthermore, evaluation of alveolar macrophages of IPF patients for Fn mRNA transcripts have shown a marked increase compared to normal alveolar macrophages, consistent with the knowledge that these cells synthesize and secrete several-fold greater amounts of fibronectin than normal alveolar macrophages.

In addition to PDGF and fibronectin, alveolar macrophages also release the alveolar macrophage derived growth factor (AMDGF), a mediator capable of stimulating competence primed fibroblast to proceed through the cell cycle and proliferate. Macrophages also release Interferon γ (IFN_γ), prostaglandin E_2 (PGE_2), and interleukin-1 (IL-1). To evaluate the importance of these mediators, we examined the effect of each of these other mediators on lung fibroblast replication in response to fibronectin and AMDGF in serum-free, defined medium. IFN_γ had no effect on fibroblast replication. In contrast, PGE_2 resulted in a dosedependent inhibition of fibroblast replication in response to fibronectin and AMDGF with 50% of the maximum inhibition observed at a PGE_2 concentration of <10 ng/ml. IL-1, while not active as a primary growth promoting signal, at concentrations of 4-10 U/ml, augmented fibroblast replication in response to fibronectin and AMDGF by 10 to 15%. Temporarily, the growth augmenting effect of IL-1 occurred early in the G_1 phase of the cell cycle. These date indicate that lung fibroblast replication in response to two of the primary growth promoting signals spontaneously released by alveolar macrophages in the interstitial lung disorders, while uninfluenced by IFN_γ, can be inhibited by PGE_2 and modestly augmented by IL-1.

In collaboration with INSERM, paris, and the Pathology Branch, NHLBI, a recent study of 373 lung specimens of patients with interstitial lung disease has demonstrated that the classic view of the "interstitial" nature of the fibrosis in these diseases is too simplified. Three patterns of intraluminal organization and fibrosis were recognized: 1) intraluminal buds, which partially filled the alveoli, alveolar ducts and/or distal bronchioles; 2) obliterated the lumens of alveoli, alveolar ducts or distal bronchioles, and 3) mural incorporation of previously intraluminal connective tissue masses, which fused with alveolar, alveolar ductal, or bronchiolar structures and frequently became reepithelialized. All three patterns had common morphologic features, suggesting that, regardless of their severity, they resulted from a common pathogenetic mechanism, i.e., the migration of activated connective tissue cells, through defects in the epithelial lining and its basement membrane, from the interstitial into the intraluminal compartment. Intraluminal buds were observed most frequently in hypersensitivity pneumonitis, chronic eosinophilic pneumonia, and organizing pneumonia of unknown cause. Mural incorporation and, to a lesser extent, obliterative changes were observed in most interstitial disorders and were very prominent in idiopathic pulmonary fibrosis. Mural incorporation and obliterative changes play an important role in pulmonary remodeling, especially when several adjacent alveoli and/or other air spaced are involved. Under these circumstances, intraluminal organization can mediate the fuxion of adjacent alveolar structures by intraluminal connective tissue.

In addition to evaluating the mechanisms of fibrosis, the Pulmonary Branch has continued studies relating to the production of collagen, the basic building block of fibrosis, by lung fibroblasts. Type III collagen is one of the major interstitial collagens and, as such, plays an important role in modulating the structure and function of most tissues. To compare the expression of the type III collagen gene to that of the type I collagen $\alpha 1(I)$ and $\alpha 2(I)$ genes, cDNAs encoding the 3' one-third of the human $\alpha 1(III)$ collagen mRNA were obtained by screening a human fetal lung fibroblast cDNA library with a cloned segment of the chicken $\alpha 1(III)$ gene. Northern blot analysis of human fetal lung fibroblast RNA demonstrated two $\alpha 1(III)$-specific mRNAs of sizes 6.6 and 5.8 kilobases, sizes clearly different from those of the type I collagen mRNAs. Analyses of populations of dividing and nondividing human lung fibroblasts revealed that, on a per cell basis, the nondividing population contained twice as much $\alpha 1(I)$ and $\alpha 2(I)$ mRNA transcripts. Similar results were obtained when $\alpha 1(III)$, $\alpha 2(I)$ mRNA transcripts were quantified by using dot blot evaluation of total RNA, Northern analysis of total RNA, and dot blot evaluation of cytoplasmic RNA. Thus, despite the fact that the $\alpha(III)$ collagen gene is located on a chromosome different from the $\alpha 1(I)$ and $\alpha 2(I)$ genes, the expression of these three collagen chains appears to be coordinately controlled during periods of rapid and slow fibroblast growth.

II. Disorders Characterized by the Accumulation of T-Lymphocytes in the Lower Respiratory Tract.

Pulmonary saracoidosis is a disorder of the lower respiratory tract characterized by chronic inflammation, granuloma formation and, in some individuals, parenchymal fibrosis. Together these processes derange the alveoli, airways and blood vessels, consequently impairing the ability of the lung to exchange gas in the normal fashion. As with the other interstitial lung disorders, it

is recognized that the inflammation precedes the other abnormalities that characterize this disorder. The inflammation of active pulmonary sarcoid is dominated by an accumulation of T-helper lymphocytes in the lung parenchyma. These T-cells are thought to play a central role in the pathogenesis of sarcoidosis in two ways. First, the accumulated T-cells distort the architecture of the parenchyma, thus altering the intimate relationships between air and blood. Second, the T-cell populations are activated and spontaneously releasing monocyte chemotactic factor and interferon gamma, mediators that recruit and activate mononuclear phagocytes, respectively, events that are early steps in the process of granuloma formation. In this context, an understanding of the pathogenesis of pulmonary sarcoidosis is intimately linked to the understanding of the process directing the accumulation of T-lymphocytes in the lower respiratory tract of individuals with active disease. Relevant to this question, prior work in this laboratory has demonstrated that the T-lymphocytes recovered from the lungs of these patients are spontaneously proliferating and spontaneously releasing interleukin-2 (IL-2), the T-cell growth factor. Thus, while the stimulus that initiates the process is unknown, the IL-2 releasing lung T-cells are thought to be responsible for maintaining the T-cell inflammation and thus maintaining the disease in an active state.

To identify the lymphocyte subpopulation that is releasing IL-2 in this disorder, lung lymphocytes recovered by bronchoalveolar lavage were characterized using the monoclonal antibodies Leu4 (T-lymphocyte), Leu3 (helper/inducer), Leu2 (suppressor/cytotoxic) and anti-HLA-DR and separated by panning and flow-cytometry. The majority of the IL-2 spontaneously released by T-cells in the sarcoid lung was contributed by the Leu3+ cell population (Leu3+ 65 ± 23 IL-2 units released/10^6 cells-24 hr; Leu2+ 9 ± 8, $p<0.04$). Further characterization of the lung Leu3+ T-cells in sarcoid demonstrated that 30 ± 3% were expressing HLA-DR molecules on their surface compared to 6 ± 1% in normals ($p<0.01$). Importantly, the subpopulation of Leu3+ lung T-lymphocytes expressing a high intensity of HLA-DR molecules on their surface were responsible for the majority of the release of interleukin-2 in the sarcoid lung (Leu3+ high intensity DR 42 ± 17 units/10^6 cells-24 hr, Leu3+ low intensity DR 8 ± 1 units/10^6 cells-24 hr; $p<0.01$). Thus, the spontaneous release of IL-2 in the lung of sarcoid patients appears to be localized to a subset of Leu3+ high intensity DR ("activated" lung helper/ inducer) T-lymphocytes. Since the sarcoid lung is characterized by markedly increased numbers of these cells, it is likely that this compartmentalized T-cell population plays a major role in sustaining the exaggerated localized immune processes of this disorder.

To determine if the IL-2 gene is activated in sarcoidosis T-cells in a systemic fashion or only at sites of disease, cells obtained by broncholaveolar lavage of individuals with active sarcoidosis, inactive sarcoidosis, and normals were evaluated for the spontaneous presence of IL-2 transcripts using a human IL-2 cDNA probe and Northern analysis of extracted RNA. Freshly recovered lung cells of individuals with active pulmonary sarcoidosis contained 0.85 kb IL-2 mRNA transcripts. In contrast, no IL-2 mRNA transcripts could be detected in fresh autologous blood T-cells or in purified autologous blood Leu3+ T-cells, although IL-2 mRNA transcripts were inducible in these cells by PHA/PMA. The sarcoid lung T-cells, however, did not express the IL-2 gene constitutively; when placed in culture with no stimulation and evaluated after 24 hrs, they demonstrated down regulation of the amounts of IL-2 mRNA transcripts despite

the fact that they were capable of reexpressing the IL-2 gene and releasing more IL-2 in response to added activation signals. Thus, the activation of the IL-2 gene in T-cells in active sarcoidosis: (1) occurs at the sites of disease and is not a generalized property of T-cells throughout the body; and (2) is not sustained if the T-cells are removed from the sites of disease. Although the cause of sarcoid is unknown, these observations are consistent with the concept that sarcoid is associated with local stimuli at the site of disease eliciting the Leu3+ T-cell IL-2 gene activation that plays such a critical role in the pathogenesis of this disease.

Since the accumulated T-cells distort the alveolar architectures and thus contribute to lung dysfunction in pulmonary sarcoidosis, suppression of lung T-cell interleukin-2 release should be associated with suppression of lung T-cell proliferation, reduction of lung helper-T-cells numbers, and improvement in lung function. To test this, comparable groups of patients with active sarcoidosis were prospectively evaluated with no therapy or treated with corticosteroids. Over 3.2 ± 0.4 months, the untreated group had no significant change in spontaneous lung T-cell release of interleukin-2, spontaneous proliferation and helper-T-cell relative number or lung function tests ($p>0.2$, all comparisons). In contrast, over the same period, the treated group had marked reduction of spontaneous lung T-cell release of interleukin-2 and proliferation, helper cells relative numbers and marked improvement of lung function tests ($p<0.05$, all comparisons prior to therapy). These observations are consistent with the concept that lung T-lymphocyte interleukin-2 release plays a central role in maintaining the pulmonary inflammation and hence lung dysfunction in active pulmonary sarcoid.

It is known that individuals with sarcoid have circulating anti-T-lymphocyte antibodies, primarily of the IgM class. To evaluate a possible role for these autoantibodies in enhancing lung T-helper processes in pulmonary sarcoid, we isolated the anti-T-cell antibodies from blood and lung of patients with pulmonary sarcoid (n=8) and evaluated them for stimulatory effects on proliferation of T-helper (Leu3+) cells or inhibitory effects on proliferation of T-supressor/cytotoxic (Leu2+) cells. Indirect immunofluorescence with fluorescein conjugated class specific goat antihuman immunoglobulin antibodies demonstrated that sarcoid patients had anti-T-cell antibodies of the IgM type reacting with 39±13% of autologous and 41±11% of normal donor T-cells. IgM recovered in sarcoid lavage fluid also reacted with T-cells, thus demonstrating the auto-antibodies at the site of disease. Two color immunofluorescence (fluorescein antihuman IgM, phycoerythrin anti-Leu2 and anti-Leu3) and flow cytometry demonstrated that these sarcoid autoantibodies bound to 23±9% of Leu2+ T-cells and 6±2% of Leu3+ T-cells. Incubating lymphocytes with sarcoid sera or sarcoid IgM had no stimulatory effect on T-cell proliferation (stimulation indices; control sera 0.4±0.7, sarcoid sera 0.1±0.5, $p>0.5$, sarcoid IgM 0.1±0.3, $p>0.2$). Leu2+ T-cells, purified by negative selection by panning, were stimulated with irradiated allogenic B-cells. Increasing concentrations of sarcoid test sera had no inhibitory effects on the response of Leu2+ T-cells (stimulation indices; control serum 23±1, 1% serum 28±1, 5% serum 30±2, 10% serum 30±3, $p>0.9$). Furthermore, the purified IgM autoantibody had no inhibitory effects on the mitogenic response of Leu2+ T-cells to OKT3 (stimulation indices; control 5±1, sarcoid 5±1, $p>0.8$). Thus the IgM anti-T-cell autoantibodies of sarcoidosis are present at the site of disease and are primarily anti-Leu2+ T-cell antibodies; however, they do not

have an identifiable role in the development of the excess T-helper cell activity of sarcoidosis.

To test the concept that the Leu3+ cell expansion is permitted by functional impairment of suppressor T-cells, Leu2+ (suppressor/cytotoxic) cells from 9 untreated sarcoid patients and 8 normals were compared for: 1) surface markers of maturation; 2) ability to respond to a proliferation signal; 3) ability to suppress antigen specific Leu3+ cell proliferation. First, two color immunofluorescence showed that the expression of VLA1 (an antigen complex expressed on T4+/Leu3+ and T8+/Leu2+ cell lines 2-4 weeks after activation in culture) on sarcoid Leu2+ cells was similar to normal (lung: 48±9% vs 32±6%, $p>0.1$; blood: 5±2% vs 1±1%, $p>0.1$). Second, purified (>85%) sarcoid Leu2+ cells responded normally to the mitogenic antibody OKT3 (3 day stimulation index; 30±17 vs 17 ± 8, $p>0.1$). Finally, using allogeneic antigen activated (6 days) Leu3+ cells to induce in coculture fresh autologous Leu2+ cells (6 days) to suppress proliferation of Leu3+ cells to the same antigen (6 days), sarcoid Leu2+ cells normally suppressed Leu3+ cell proliferation (65±8% suppression vs 75±15%; $p>0.1$). These studies demonstrate that sarcoid Leu2+ cells: 1) in vivo express antigens associated with normal cell maturation; 2) respond to a proliferation signal normally; 3) can be induced to normally suppress Leu3+ cell proliferation. Thus, the expansion of activated Leu3+ cell in pulmonary sarcoidosis is likely not due to a generalized abnormality of suppressor T-cell function.

In order to understand the abnormal accumulation of lymphocytes in sarcoidosis, it is necessary to understand the normal populations of T-cells in the lower respiratory tract. In this context, T-lymphocytes on the epithelial surface of the lower respiratory tract are thought to represent a relatively compartmentalized population of T-cells that exchange slowly with blood. Since the lung is burdened with antigens, "resident" T-cells likely have a history of being activated in the past. To evaluate this concept, we capitalized on the fact that when blood T-cells are antigen activated and maintained 2-4 weeks in culture, they express the VLA1 surface complex of 210kd $\alpha 1$ and 130kd β subunits, i.e., VLA1 indicates T-cells with a history of past stimulation as would be expected from resident lung T-cells. To do so, we evaluated lung lavage and blood T-cells in 29 normal nonsmokers using the monoclonal (Mab) antibodies Leu3 (helper/inducer T-cells), Leu2 (suppressor/ cytotoxic T-cells) anti-Tac (IL-2 receptor) and TS2/7 (α_1 subunit of the VLA1 complex). Blood T-cells rarely express Tac (1±1% Leu3+, 1±1% Leu2+) or TS2/7 (1±1% Leu3+, 2±2% leu2+). In contrast, a subset of lung helper T-cells expressed Tac (6±3% Leu3+) and more expressed TS2/7 (17±8% Leu3+). In comparison, Tac was rarely expressed on lung Leu2+ cells (1±1%) but like Leu3+ cells, lung Leu2+ cells expressed more TS2/7 (38±14%). Furthermore, immunoprecipitation of ^{125}I-surface labeled lung and blood T-cells with A1A5 (another anti-VLA1 Mab), demonstrated that VLA1 proteins are expressed on lung but not blood T-cells. In addition, incubation of lung lavage cells with [3H]thymidine (24 hr, 37°) followed by autoradiography demonstrated that <1% of all lymphocytes were actively proliferating, suggesting that the Leu3+ and Leu2+ cells expressing the VLA1 complex were not an actively dividing population. Thus, a significant proportion of lung T-cells express surface proteins identical to those expressed by blood T-cells maintained for long periods in culture, suggesting that the lung likely represents a site of compartmentalization of resident immunocompetent cells that have been stimulated at multiple times in the past and exchange slowly with blood.

III. Disorders Characterized by Distruction of the Alveolar Walls

Alpha 1-antitrypsin (AAT), a 52,000 dalton serum glycoprotein produced by hepatocytes and mononuclear phagocytes, functions to inhibit neutrophil elastase, a proteolytic enzyme capable of destroying all protein components of connective tissue. The AAT gene is highly pleomorphic; more than 30 different haplotypes have been described. The AAT phenotype, referred to as the Pi (protease inhibitor) type, represents the codominant expression of the two parental AAT haplotypes. The most common AAT haplotypes in the U.S.A. are those of the M-family (M1, M2, M3; combined frequency greater than 0.90), the S type (frequency 0.02-0.04) and the Z type (0.01-0.02). The clinical interest in these AAT haplotypes is based on the knowledge that inheritance of the phenotypes PiZZ and PiSZ is associated with an increased risk for the development of emphysema (in adults) and/or liver disease (in children).

This year we have cloned and sequenced the entire nucleotide sequence of the protein coding region sequence of the α1AT Z gene. Interestingly, we have found that, in addition to known glu^{342} to lys mutation in exon V, there is another amino acid substitution (val^{213} to ala) resulting from a single base substitution (GTG to GCG) in exon III. This mutation was confirmed to be a general finding in Z type α1AT genes by evaluating genomic DNA of 40 Z haplotypes using synthetic oligonucleotide gene probes directed towards the mutated exon III sequences in the Z gene. Furthermore, the exon III val^{213} to ala mutation eliminates a BstEII restriction endonuclease site in the α1AT gene, allowing rapid identification of this val^{213} to ala substitution at the genomic DNA level. Surprisingly, when genomic DNA samples from individuals thought to be M1 homozygotes were evaluated with BstEII, 23% of the M1 haplotypes were BstEII negative, thus identifying a new form of M1 [i.e., M1(ala^{213})], likely identical to M1 but with an isoelectric focusing "silent" amino acid substitution (val to ala) at residue 213. Although the relative importance of the newly identified exon III val^{213} to ala mutation to the pathogenesis of the abnormalities associated with the Z gene is not known, it is likely that the M1(ala^{213}) is a common "normal" polymorphism of the α1AT gene that served as an intermediate in the evolution between the M1(val^{213}) and Z genes.

In contrast to the ZZ and SZ states, in which mutant proteins are found in reduced amounts, the "null-null" state is a rare form of the deficiency in which no α1AT can be found at all. Since α1AT normally provides almost all of the protection against neutrophil elastase in the lower respiratory tract, the lungs of persons with the null-null phenotype are essentially defenseless against a burden of neutrophils, and all those evaluated in early adulthood are found to have emphysema. By evaluating α1AT genes and the cells that produce α1AT in a patient with null-null phenotype,we have demonstrated that the "null" α1AT gene represents a class of mutants different from the Z and S mutants, in that the deficiency of α1AT associated with at least one form of the null gene represents the inability of the gene to direct the synthesis of a detectable mRNA transcript. To identify the molecular defect for this null mutant, a 10 kb EcoRI fragment containing α1AT coding exons (II-V) of the genomic DNA of the null homozygote was cloned into λgtWES. Sequencing demonstrated the α1AT coding region was identical to normal M1-type α1AT except for a single base in exon III (lys^{217} AAG to TAG), causing an amber stop codon. To determine if both parental "null" α1AT alleles had similar abnormalities,

synthetic 19-mer oligonucleotide probes centered at lys^{217} to the normal and amber mutations, respectively, were used to assess genomic DNA from the "null" homozygote and "null" heterozygote and normal family members. The homozygote genomic DNA only hybridized to the amber probe while the parents and affected sibs hybridized to both the amber and normal probes. Thus, this null-type α1AT deficiency results from an amber stop mutation, and inheritance of the identical α1AT mutant from both parents resulted in the null-null state and emphysema. In the context that this null-null individual has no α1AT mRNA, this mutation likely results in α1AT deficiency similar to amber mutations of β^0 thalassemias with no detectable β-globin mRNA.

To accurately identify the SZ phenotype at the level of genomic DNA, four ^{32}P-labeled 19-mer synthetic oligonucleotide probes were prepared, two to identify the M and S difference in exon III, and two to identify the M and Z difference in exon V. These probes were hybridized with various cloned DNAs and genomic DNAs cut with the restriction endonucleases BglI and EcoRI; the genomic DNAs represented all 6 possible phenotype combinations of the M, S, and Z haplotypes (MM, MS, MZ, SS, ZZ, SZ). Using the 4 probes to evaluate 42 samples of genomic DNA, the "at risk" SZ and ZZ phenotypes were correctly identified in all cases as were the "not at risk" phenotypes SS, MS, MM, and MZ, demonstrating that both exon III and exon V directed probes are necessary to properly identify all of the major "at risk" α1AT genes. These observations indicate that oligonucleotide gene probes yielded reliable and accurate assessment of "at risk" α1AT genotypes in almost all situations, but in the context of prenatal diagnosis and genetic counseling this approach must be used with caution and in combination with family studies so as not to misidentify rare genotypes that may be associated with a risk for disease.

To evaluate the contribution of mononuclear phagocytes, and particularly alveolar macrophages, to alpha 1-antitrypsin (α1AT) production in normal and α1AT deficient individuals, Northern analysis with a human α1AT cDNA was used to demonstrate that α1AT mRNA can be detected in liver, blood monocytes, and alveolar macrophages. Quantification of α1AT mRNA expression demonstrated that: (1) type PiMM monocytes and alveolar macrophages expressed respectively, 200-fold less and 70-fold less α1AT mRNA per cell than the liver; (2) the level of expression of the α1AT gene was increased during the in vitro maturation of blood monocytes; (3) blood monocytes and alveolar macrophages levels of expression of the α1AT gene was the same in PiMM and PiZZ individuals. However, the amount of newly synthesized α1AT secreted by ZZ alveolar macrophages was 10 times lower than that by MM alveolar macrophages. Thus, mononuclear phagocytes of PiZZ individuals express a secretory defect in α1AT in a similar fashion to hepatocytes. Not only do mononuclear phagocytes provide a readily accessible cell to evaluate the regulation of α1AT gene expression, but these cells may contribute to the levels of α1AT present in the lower respiratory tract in the normal and ZZ states.

Despite the overwhelming evidence that the emphysema of PiZZ individuals develops because of a "deficiency" of AAT and hence an insufficient antineutrophil elastase defense of the lung, epidemiologic evidence has shown that levels of AAT of only 80 mg/dl protect the lung from an increased risk of emphysema. With this background, we hypothesized that homozygous inheritance of the Z-type may confer an added risk beyond a simple "deficiency" of AAT by virtue of an

inability of the Z-type AAT molecule to inhibit neutrophil elastase (NE) as effectively as the common M1-type molecule. To evaluate this hypothesis the functional status of AAT from PiZZ individuals (n=7) was compared with that of AAT from PiM1M1 individuals (n=10) for its time independent ability to inhibit NE (% inhibition) as well as its time dependent association rate constant for NE (Kassoc). Plasma AAT concentration, measured by radial immunodiffusion, was 34±3 mg/dl in PiZZ patients vs 237±37 mg/dl for PiM1M1 individuals, a 7-fold difference. When titrated against NE, the % inhibition of PiZZ plasma was significantly less than PiM1M1 plasma (ZZ, 76±5% vs M1M1 93±4% $p<0.001$) as was purified Z-type AAT (ZZ 63±7% vs M1M1 86±5%, $p<0.001$). Furthermore, the Kassoc of purified Z-type AAT was strikingly lower than that of M1-type AAT (ZZ $4.5\pm0.8 \times 10^6$ $M^{-1}sec^{-1}$ vs M1M1 $9.7\pm1.1 \times 10^6$ $M^{-1}sec^{-1}$, $p<0.001$), suggesting that on a molecule for molecule basis, Z-type AAT takes more than twice as long as M1-type AAT to inhibit NE. Consequently, not only is there less total antigenic AAT in PiZZ individuals, but also the proportion that is functional against NE is significantly less and the rate at which it inhibits NE is markedly slower than that in PiM1M1 individuals. This combination of defects suggests that PiZZ individuals have far less functional antielastase protection than suggested by simple total plasma concentration alone, further explaining their profound risk for development of emphysema.

Unlike the Z A1-antitrypsin (AAT) haplotype in which AAT deficiency results from normal synthesis but an aggregation of AAT in the rough endoplasmic reticulum (RER), the mechanism of the serum deficiency associated with S haplotype (glu264 to val) is unknown. One hypothesis to explain the reduced serum levels of AAT associated with S is that the glu264 to val substitution results in intracellular degradation or aggregation of the newly synthesized molecule and thus less AAT available for secretion. To evaluate this hypothesis, blood monocytes, cells that produce AAT, were evaluated from individuals homozygous for M type AAT (n=4), and those homozygous for S type AAT (n=4). Pulse-chase studies using an anti-AAT antibody to immunoprecipitate AAT revealed that 4 hrs after a 1 hr pulse, both SS and MM monocytes secreted mature glycosylated 52 kd AAT, but the SS monocytes secreted significantly less (42±10%, $p<0.01$). To evaluate whether SS monocytes secrete less AAT secondary to altered intracellular metabolism prior to oligosaccharide side-chain addition, pulse chase studies were carried out in the presence of tunicamycin (5 μg/ml), an inhibitor of N-linked oligosaccharide side chain addition. Under these conditions, the MM monocytes secreted 46 kd nonglycosylated AAT. In marked contrast, SS monocytes secreted 100-fold less 46 kd AAT relative to MM monocytes despite identical conditions and overall protein synthesis and secretion. These observations demonstrate that the S haplotype is associated with decreased AAT secretion, likely because this form of AAT is either degraded or aggregated in the RER during the transition from synthesis to glycosylation.

Since the common form of the inherited deficiency (homozygous Z) results from impaired hepatic release of α1-antitrypsin, one therapeutic approach to increase plasma and hence lung α1-antitrypsin levels is to enhance hepatic release and/or production of α1-antitrypsin. In a preliminary trial with 6 α1-antitrypsin deficient individuals we have previously shown that in 1 month, the impeded androgen danazol can augment serum α1-antitrypsin levels by 37%. To evaluate the use of impeded androgens in α1-antitrypsin deficiency on a broader scale, we have treated: (1) 43 homozygous Z patients with danazol

200 mg p.o., t.i.d. x 30 days; (2) 6 homozygous Z patients with a similar danazol dose but for 6 to 18 months; and (3) 7 homozygous Z patients with stanazolol, another synthetic androgen, at 2 mg p.o. t.i.d. x 30 days. Of the 43 patients treated with danazol for 1 month, 23 (53%) responded with a serum α1-antitrypsin level $\geq$20% over baseline, an average increase of 52% over the pretreatment level. Side effects were minimal and reversible but included muscle cramps and hepatic enzyme elevations in 20% of those with $\geq$20% increase in α1-antitrypsin levels. Of the 6 patients treated chronically, all maintained their increased α1-antitrypsin levels and none had significant complications. In contrast to danazol, stanazolol therapy for 1 month produced minimal increases in serum α1-antitrypsin levels above baseline levels. These findings suggest that danazol therapy can increase α1-antitrypsin levels in a significant proportion of Z homozygous α1-antitrypsin deficient patients without major side effects.

Tamoxifen, an agent that binds to intracytoplasmic estrogen receptors, was evaluated as a possible means of increasing alpha 1-antitrypsin (α1AT) synthesis and/or secretion and thus α1AT deficiency. Administration of tamoxifen (10 mg, twice daily) to 30 Z homozygotes over a 30 day period was not asociated with adverse reactions. However, while serum α1AT levels increased significantly ($p<0.03$), the increase was minor (average pretreatment levels 32 ± 1 mg/dl; levels at 30 days of therapy 35 ± 1 mg/dl), and far below the "threshold" level of 80 mg/dl considered "protective" against an increased risk for emphysema. Thus, while the concept that increasing α1AT synthesis and/or secretion is a rational goal for treating the Z homozygous form of α1AT deficiency, tamoxifen will not be useful in this regard.

To evaluate the feasibility, safety, and biochemical efficacy of chronic parenteral infusions of α1AT for this disorder, 21 patients with emphysema secondary to PiZZ type α1AT deficiency were given 60 mg/kg of active plasma-derived α1AT once weekly for up to 6 months. Within a few weeks of beginning therapy, all patients reached a steady state trough serum α1AT level of 126 ± 1 mg/dl, compared to pretherapy level of 30 ± 1 mg/dl and serum anti-neutrophil elastase capacity of 13.3 ± 0.1 μM compared to a pretherapy level of 5.4 ± 0.1 μM. Importantly, while pretherapy lung epithelial lining fluid (ELF) α1AT levels were 0.46 ± 0.16 μM, and anti-neutrophil elastase capacity was 0.81 ± 0.13 μM, on the average, 6 days after infusion, lung ELF α1AT levels (1.89 ± 0.17 μM) and anti-neutrophil elastase capacities were markedly increased (1.65 ± 0.13 μM, $p<0.0001$, both comparisons). In 507 infusions to the 21 individuals, the only significant adverse reactions were 4 self-limited postinfusion fevers. These findings demonstrate that chronic, weekly parenteral infusions of purified plasma-derived α1AT are well tolerated, safe, and can chronically elevate both serum and lung epithelial lining fluid α1AT levels and anti-neutrophil elastase capacities, suggesting that chronic, weekly infusions represent logical therapy for this disorder.

To evaluate the potential use of recombinant DNA produced α1-antitrypsin (AAT) to reestablish the lung antineutrophil elastase defenses absent in AAT deficiency, we compared the kinetics of recombinant produced AAT (rAAT) and purified normal human plasma AAT (pAAT) in the blood and lung of rhesus monkeys. The rAAT was produced in yeast transformed with an expressing plasmid containing a full length human AAT cDNA and purified to >99% homogeneity (Cooper Biomedical). The rAAT

had a MW of 46,000 daltons, no carbohydrates and was identical in sequence to normal plasma AAT except for an additional N-terminal methionine. Rhesus monkeys were infused with 120 mg/kg of rAAT (n=7) or pAAT (n=5) and the serum, lung lavage, and urine human AAT concentrations quantified at various intervals. At 30 min the serum AAT values were comparable (rAAT 43±8 μM, pAAT 69±10 μM) but at 2 hr the rAAT was lower (15±3 μM, pAAT 59±7 μM) and at 24 hr the serum concentration of rAAT was undetectable through pAAT was still present (37±10 μM). rAAT was found in the urine 30 min following infusion; by 2 hr, 44±4% of the total dose had been excreted as a functional anti-elastase, while no AAT was detected in the urine of those receiving pAAT. Despite the serum differences, at 2 hr the concentration of pAAT in the epithelial lining fluid (ELF) of the lower respiratory tract was 1.2±0.3 μM compared to 2.8±1.8 μM for rAAT. At 24 hr the lung ELF concentration of pAAT was 4.3±3.0 μM and despite its absence in serum, the concentration of rAAT in the ELF was 4.2±1.7 μM. Consistent with these observations, at 24 hr the antineutrophil elastase capacity of the ELF was 2.6±0.6 μM for pAAT and 2.6±1.6 μM for rAAT, a five-fold increase from endogenous antielastase capacity. Thus, despite the rapid disappearance of rAAT from blood, the concentration of rAAT in the ELF at 2 and 24 hr is equivalent to that achieved with pAAT and results in similar augmentation in the anti-elastase screen. These observations suggest that while the plasma kinetics of rAAT differ from pAAT, its transfer into the lung may be sufficient to consider its potential use in reestablishing the anti-elastase defense in the lower respiratory tract of AAT deficient patients.

Oxidative processes occuring in the lower respiratory tract in cigarette smokers may play a central role in the pathogenesis of emphysema by inactivating α1-antitrypsin thus upsetting the neutrophil elastase-antineutrophil elastase balance within the lung. One such source of oxidants is alveolar macrophages; in cigarette smokers alveolar macrophages release exaggerated amounts of O_2^- and H_2O_2 and can inactivate normal α1-antitrypsin by oxidative mechanisms. One approach to this problem is to augment the antineutrophil elastase protection in the lung with a genetically engineered recombinant α1-antitrypsin (rAAT) variant containing an active site amino acid substitution ($MET^{358} \rightarrow VAL^{358}$) which retains effective inhibition of neutrophil elastase but is resistant to oxidation. To evaluate this concept, we compared the ability of VAL^{358}rAAT to withstand oxidative inactivation to that of genetically engineered MET^{358}rAAT and human plasma M1M1 α1-antitrypsin. Each type of α1-antitrypsin was exposed to H_2O_2 or to smokers alveolar macrophages and was then titrated against neutrophil elastase and its % activity determined. After dialysis against 5mM H_2O_2 (2 hr, 23°) VAL^{358}rAAT retained 91±3% activity, M1M1 α1-antitrypsin 17±9%, and MET^{358}rAAT only 9±7% ($p<0.001$ VAL^{358}rAAT vs M1M1 α1-antitrypsin or MET^{358}rAAT). Following incubation (18 hr, air, 37°) with 2×10^6 alveolar macrophage from cigarette smokers (n=5) in chambers which separated cells from α1-antitrypsin by a dialysis membrane, % activity of VAL^{358}rAAT was 83±11%, M1M1 α1-antitrypsin 33±22%, and MET^{358}rAAT only 3±3% ($p< 0.01$ VAL^{358}rAAT vs M1M1 α1-antitrypsin or MET^{358}rAAT). Thus, VAL^{358}rAAT is more resistant to inactivation by cigarette smokers alveolar macrophages than either M1M1 α1-antitrypsin or MET^{358}rAAT, suggesting that VAL^{358}rAAT is a potentially superior agent for emphysema preventive therapy in the oxidative milieu of the lower respiratory tract in cigarette smokers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02407-12 PB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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:	Ronald G. Crystal, M.D.	Chief	Pulmonary Branch, NHLBI
Others:	Mark Brantly	Senior Staff Fellow	Pulmonary Branch, NHLBI
	Anthony Casolaro	Senior Staff Fellow	Pulmonary Branch, NHLBI
	David Curiel	Guest Researcher	Pulmonary Branch, NHLBI
	Robert Garver	Senior Staff Fellow	Pulmonary Branch, NHLBI
	Richard Hubbard	Senior Staff Fellow	Pulmonary Branch, NHLBI
	Toshihiro Nukiwa	Visiting Associate	Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)*

Michael Courtney, Jean Pierre LeCoq - Transgene, Strasbourg, France

LAB/BRANCH

Pulmonary Branch

SECTION

INSTITUTE AND LOCATION

NHLBI:NIH: Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12.7	8.7	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 2 million individuals in the U.S.A. with emphysema. Two percent develop the disease because of inheritance of a deficiency of alpha 1-antitrypsin (AAT), an antiprotease that protects the lower respiratory tract from destruction mediated by elastase released by neutrophils. Cloning, sequencing and oligionucleotides have been used to detect specific mutations in the AAT gene. The "null" AAT state is associated with an intact gene but no detectable AAT mRNA. Alveolar macrophages produce AAT, thus providing the protein at the site of disease. Site directed mutagenesis has been used to produce a recombinant AAT molecules in E.coli that is oxidation resistant. Therapy of AAT deficiency with AAT purified from pooled plasma has demonstrated that the anti-neutrophil-elastase defenses of the lung can be re-established with intermittent intravenous administration of 60 mg/kg AAT.

Others: (Cont.)

Fumitaka Ogushi	Guest Research	Pulmonary Branch, NHLBI
Ken Satoh	Visiting Fellow	Pulmonary Branch, NHLBI
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02533-02 PB

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Mechanism of Fibrosis

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

PI:	Ronald G. Crystal, M.D.,	Chief	Pulmonary Branch, NHLBI
Others:	Paul Basset	Visiting Fellow	Pulmonary Branch, NHLBI
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	Kohei Yamauchi	Visiting Associate	Pulmonary Branch, NHLBI

COOPERATING UNITS *(if any)* Pathology Branch, ODIR, NHLBI, NIH, Victor Ferrans, Kyo Adachi, Jean-Francois Bernaudin; Hopital Bichat, INSERM, Paris, Francoise Basset; Laboratory of Clinical Investigation, NIAID, Eric Ottesen; Laboratory of Developmental Biology and Anomalies, NIDR, George Martin.

LAB/BRANCH

Pulmonary Branch

SECTION

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
11	7	4

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- [X] (a) Human subjects
- [X] (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 often progressive and can be 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 mediators are platelet-derived growth factor, fibronectin and alveolar macrophage derived growth factor. Other mediators include interleukin-1. With knowledge of the specific processes involved, strategies can be developed to modulate these mediators as therapy for these disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02534-02 PB

PERIOD COVERED
October 1, 1985 through September 30, 1986

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

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

PI:	Ronald G. Crystal, M.D.,	Chief	Pulmonary Branch, NHLBI
Others:	Bruno Balbi	Visiting Fellow	Pulmonary Branch, NHLBI
	Kazuki Konishi	Guest Researcher	Pulmonary Branch, NHLBI
	Kenji Mizoguchi	Visiting Fellow	Pulmonary Branch, NHLBI
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	Joachim Muller-Quernheim	Guest Worker	Pulmonary Branch, NHLBI
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LAB/BRANCH
Pulmonary Branch

SECTION

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10.5	6.5	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 T-lymphocyte lung disorders occur in 20 to 50 per 100,000 of the U.S.A. population. The "model" disorder of this group is sarcoidosis, a disease characterized by the accumulation of activated helper T-lymphocytes at the sites of disease. These T-cells spontaneously express the interleukin-2 gene, thus driving T-cells in the local milieu to proliferate. Treatment of these individuals with corticosteroids results in suppression of interleukin-2 gene expression, cessation of lung T-cell proliferation, and improvement in lung function.

ANNUAL REPORT OF THE SURGERY BRANCH
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
OCTOBER 1, 1985 THROUGH SEPTEMBER 30, 1986

The clinical and laboratory research performed by the Surgery Branch is focused on elucidating new solutions to long-standing, difficult problems in cardiac surgery. The major programs are: 1) mechanisms of amelioration of intrinsic failure of bioprosthetic heart valves; 2) assessment of new ultrasonic technologies for prosthetic devices and native pathologies; 3) new surgical approaches for the treatment of hypertrophic cardiomyopathy; 4) basic studies of pulmonary hypertension in the young and 5) new surgical procedures for palliation of ischemic cardiomyopathies.

STUDIES OF PROSTHETIC HEART VALVES

Although more than 20 years have passed since the first prosthetic cardiac valves were implanted in humans, the development of an ideal cardiac valve substitute remains a major problem in cardiac surgery. Bioprosthetic cardiac valves have become the valves of choice at many institutions and for certain subgroups of patients, primarily because they do not require chronic anticoagulant therapy. However, it is now apparent that the long-term durability of bioprosthetic cardiac valves is the major complication of long-term implantation. It is estimated that more than one-half million bioprosthetic valves have been implanted in human patients to date. Of additional concern is the fact that somewhere between 50-100,000 valve replacement operations continue to be performed annually world wide.

Hemodynamic, Ultrasonic and Pathologic Evaluations of Prosthetic Heart Valves

Previous work by M. Jones has led to a standarized animal model of accelerated mineralization and fatigue of bioprosthetic heart valves implanted into the tricuspid and mitral positions of juvenile sheep. The early hemodynamic data demonstrated a consistent 10-15% improvement in pressure difference and calculated flow area for valves made from bovine pericardium compared to those made from porcine aortic valve tissue. The long-term findings showed that these prostheses increased in calcium content 82-232 fold over a five month interval. The pathologic alterations were similar to those found in bioprostheses explanted from patients. Two types of bioprostheses (bovine pericardium and porcine aortic) treated by two different processes prior to implant have been studied. Treated porcine aortic demonstrated 4-10 fold decrease in calcium content for the porcine valves only after five months in vivo. Hemodynamic and pathologic changes at this time interval were similarly improved compared to the non-treated prostheses. Valves made from bovine pericardium and treated with the mineralization mitigation processes were similar to controls, thus showing no effect of the new processing. Similar implantation and long-term studies have been performed on six types of synthetic trileaflet heart valves which demonstrated superior velocity and pressure difference characteristics compared to similar sizes of bioprosthetic and mechanical valves. Polyurethane and polytetrafluorethylene valves have had durability problems in the animal model. These data are highly important to new efforts to improve bioprostheses and to the development of synthetic leaflet valves which offer hope for improvement in the long-term freedom from complications of an intracardiac device.

Use of Ultrasonic Technologies for Assessment of Prosthetic Valve Function, Mitral Regurgitation and Right Ventricular to Pulmonary Artery Conduit

An ultrasonic device with multiple frequencies and integrated with continuous, pulsed wave, and two-dimensional color-encoded Doppler, combined with two dimensional and M-mode imaging has been used to evaluate prosthetic heart valves, mitral regurgitation and right ventricular to pulmonary conduits. Seventy studies of 23 different types of heart valves were performed after implantation in the mitral position in sheep. Normal mitral valves had no in-orifice velocity disturbances. Bioprosthetic valves had a 2 1/2 - 3 fold increase in high velocity jets directed toward the septum during ventricular diastole. Bovine pericardial valves had less maximal velocities. Flow areas for both types of bioprostheses occupied only 50-75% of the orifice inflow area compared to 100% for normal mitral valves. New important data was obtained demonstrating hemodynamically important differences in velocity patterns as a function of orientation of 5 types of tilting disc valves. Large areas of reversed velocities while the prosthetic valve was open were found along the left ventricular free wall when the major orifice was oriented toward the septum. These findings have important implications for the clinical surgeon and suggest that orientation of eccentric flow orifice valves may play an important role in the incidence of thromboembolic phenomena and blood element destruction.

Mitral regurgitation was studied in sheep and two-real time Doppler color flow mapping systems were compared using interrogation frequencies of 2.5, 3.75 and 5.0 MHz. Other variables tested were gains, pulse repetition frequencies, flow states and regurgitant orifice sizes. Differences between instruments were demonstrated although both systems over-estimated regurgitant orifice diameters. For each system, there was a linear relation between imaged area of the regurgitant jet and the calculated regurgitant volume.

Simultaneous hemodynamic and Doppler studies were performed at different time intervals in 12 baboons which had right ventricular to pulmonary artery conduits. Peak systolic pressure differences across the conduit system had a high correlation ($r = 0.96$) to ultrasonic maximal velocities. Progression of obstruction was demonstrated over a 24 month interval. These data show that non-invasive technology can supplant traditional serial cardiac catheterizations in following children after Rastelli types of operations.

In vitro investigations of prosthetic heart valves have continued to develop with the acquisition of various valve testing systems and sophisticated instrumentation. The initial studies focused on the correlation of laser Doppler anemometry and continuous wave and two-dimensional color-encoded Doppler ultrasonic data through a collaborative study with biomedical engineers at the Georgia Institute of Technology. The new systems will be used in the forthcoming year to study freshly explanted valves and new prototypes including a new trileaflet system and to study the effect of endothelial cell coatings of bioprosthetic valves.

The Influence of the Mitral Valvular Apparatus in the Setting of Acute and Chronic Mitral Regurgitation and Mitral Valve Replacement

Previous data from this Institute and the literature demonstrate that pure or predominant mitral regurgitation and mitral valve replacement has a high perioperative mortality (10-15%) compared to the rate for this operation

for mitral stenosis (5-8%). Previous studies have shown that all survivors had early depression of left ventricular function and only those without significant impairment preoperatively developed improved function after many months to several years. Mitral valve replacement has, in the past, involved excision of the mitral valve leaflets and subtended chordae tendinae. The hypothesis tested was that the mitral valvular apparatus (annulus, leaflets, chordae tendinae and papillary muscles) provides clinically important support for the dilated left ventricle. The studies involved the development of the first known animal model of chronic mitral regurgitation, implantation of a prosthetic bioprostheses, and later external transection of the chordal apparatus. An ultrasonic global coordinate microcrystal system for detection of wall thickness, regional and global wall motion and geometry, and hemodynamic and biochemical analyses were employed. The data demonstrate significant changes in diastolic geometry and systolic function when the chordal apparatus is transected in animals with chronic mitral regurgitation but not those with acute mitral regurgitation. These data show that left ventricular function is enhanced by preserving the mitral valvular apparatus in the setting of chronic mitral insufficiency. Five patients have had the posterior mitral leaflet and chordae preserved and two patients have had no resection. All have survived the perioperative intervals. The clinical and laboratory studies continue.

Clinical Studies of Children and Adults with Mitral and Aortic Prosthetic Valves

Three retrospective studies were completed which evaluated the palliative worth of treatment of valvular heart disease with prosthetic valve replacement.

Six of nine patients with prosthetic heart valves were located who had mitral valve replacement as a child 15 or more years before study. Four had received a caged-ball valve and all had positive CAT brain scans for one or more cerebral thromboembolic events, three of whom had no history for CNS problems. Two patients with bioprosthesis, 12 and 13 year postoperatively, had no history or CAT scan data for an embolic event. None of the six patients had evidence for emboli to the kidneys. Exercise capacity was diminished in all. These data demonstrate a high incidence of silent cerebral emboli in young patients receiving mechanical prosthetic heart valves and an unsuspected diminution in exercise tolerace and ventricular function.

The use of 17 and 19 mm diameter prosthesis without aortic root enlargement has been criticized in the literature. Fifty-two patients were evaluated six years after implantation of small diameter valves without root enlargement. There was no correlation of symptoms to hemodynamic data. Effective orifice areas and pressure differences across the valves were constant with time. It was concluded that root enlargement and its concomittant potential complications were not warranted in most patients requiring a small diameter aortic valve prosthesis.

The long-term durability of bioprosthetic heart valves in the mitral position was assessed by review of the first 100 surviving patients who had this prosthesis inserted from 1970-1974. Intrinsic valve failure in the absence of infection occurred in 23 patients and patient survival was low at 10 and 15 years at 51 ± 5 and 30 ± 6 % respectively. The actuarial freedom from intrinsic valve failure was only 75 ± 6 and 40 ± 12 % at 10 and 14 years. These data demonstrate an unacceptable long-term durability and

emphasize the need for caution in using these devices except for highly selected patients. Further, the urgent need for a new process for mitigation of the mineralization of bioprosthetic valves is demonstrated.

SURGICAL PALLIATION OF SEVERE STATES OF HYPERTROPHIC CARDIOMYOPATHY

Mitral Valve Replacement

The Surgery Branch has had 26 years experience with surgical palliation of severe states of hypertrophic cardiomyopathy. For the first two decades, patients with asymmetric hypertrophy of the cephalad portion of the interventricular septum were selected for surgical treatment and had a subtotal ventricular septal myectomy performed through the aortic valve (Morrow operation). During the most recent half decade, however, an increasing number of severely symptomatic patients have had symmetric septal hypertrophy of less than classic proportions. Patients with these so-called 'thin' hypertrophied septums are poor candidates for the standard operation as determined by both retrospective and prospective studies. Unknown are the etiologic factors in this change in the spectrum of pathologic anatomy, although change in genetic factors and/or the use of beta and calcium blocking agents have been proposed. Accordingly, 36 months ago a prospective study of the effect of mitral valve replacement (MVR) alone was initiated for patients with cephalad septal thickness of 18 mm or less, those with highly atypical septal morphology as determined by intraoperative 2-D echocardiography, those with persistent obstruction after a septal myectomy procedure and those with severe mitral regurgitation and minimal outflow tract gradients. Forty-six patients have had mitral valve replacement of whom 24 have been restudied by cardiac catheterization, echocardiography, radionuclide angiography and exercise testing. There have been only two perioperative deaths (4 %) and all but 3 patients had improved at least one NYHA functional class at the six month follow-up interval. There have been 3 long-term deaths the causes for which were sudden in two and one had respiratory failure. Complications have been few and the palliation achieved is equal hemodynamically to that achieved by the classic operation.

Septal Myectomy - Prospective Group

The standard operation continues in the hands of Dr. C.L. McIntosh. His personal series now numbers 102 patients of whom 22 had the procedure in the past year. The total series now has accumulated 431 patients. The perioperative mortality was 0% for the past year and overall is 7%. The late mortality was 5%. Nine patients have had an intraoperative ventricular septal defect, all of whom had concommittant coronary artery disease. Postoperative catheterization data show good relief of the resting and provokable pressure gradients, 12 and 52 mm Hg respectively which did not correlate to the degree of symptomatic relief. The operation now carries a low operative risk with a high probability of palliation of symptoms.

Septal Myectomy in the Elderly

A retrospective review of those patients 65 yrs and older (81 yr max) who had septal myectomy was performed to determine the palliative worth in this increased age-risk group. Fifty-two patients had complete follow-up at 4.5 yrs.

No patient has come to reoperation. Eighty-five percent and 78% showed symptomatic and functional improvement, respectively. The resting mean gradient after operation was 12 mm Hg with a mean reduction of 85%. The actuarial survival was 82% at five years demonstrating that surgical palliation in elderly patients with hypertrophic cardiomyopathy is worthwhile in terms of both quality and prolongation of life.

MECHANISMS OF INDUCEMENT, REGRESSION AND PESISTENCE OF PULMONARY HYPERTENSION

Neonatal pulmonary hypertension is the most common pathohemodynamic accompaniment of anatomic disorders associated with pulmonary hyperemia. A host of etiologic mechanisms have been proposed including threshold flow, kinetic energy, platelet deposition, intrinsic hyper-reactive vasculature as well as a variety of local and systemic humoral agents. Recent investigations have shown that vascular endothelium produces a protein which is different than endothelial growth factor and causes marked vascular smooth muscle relaxation. Removal of both systemic and pulmonary arterial endothelium results in increased vascular tone and lack of responsiveness to factors known to increase production of endothelial derived relaxing factor (EDRF).

The first study in the Surgery Branch's new initiative in this important area of pulmonary hypertension was to investigate the early consequences of creating pulmonary hypertension, and hyperperfusion without increased pressure in relation to endothelial cell sensitivity to EDRF stimulating agents. The hypothesis tested was that hypertension, but not high flow without hypertension, would result in decreased vasodilator response to EDRF stimulating agents. Hyperperfusion and localized pulmonary hypertension were created in the same animal by a two staged procedure involving use of an aortic to left pulmonary artery conduit which delivered arterial blood to the left lower lobe and the entire cardiac output passed through the right lung. Adult dogs and weanling swine were used and studied 3 - 6 months later. Platelet aggregation studies and arachadonic acid metabolite analyses from samples from various vascular beds were performed. Using an *in situ* isolated organ perfusion system, pressure-flow data were generated. The pulmonary vasculature was preconstricted with prostaglandin PGF_a and endothelial cell metabolism was inhibited with indomethacin. The isolated pulmonary vasculature was then tested using four known EDRF stimulants bradykinin, acetylcholine, a calcium ionophore and adenosine triphosphate. If relaxation was achieved, enzymatic inhibitors of cyclooxygenase and lipoxygenase were used to block the relaxation response. The vasculature was barium-gel cast and histologic morphometric studies were performed. The results demonstrated that the hyperperfused and the normal pulmonary vasculature were similar in all aspects, e.g. vascular resistances and responses to contrictor and dilator agents. Maximal pressure decrease and change in vascular resistance in response to bradykinin was diminished in the hypertensive vasculature. Further, use of a lipoxygenase-cyclooxygenase inhibitor attenuated the EDRF stimulant response. A pure cyclooxygenase inhibitor failed to attenuate the relaxant response. These data suggest that endothelial cell metabolism is altered in the hypertensive-hyperperfused pulmonary vasculature and that the smooth muscle relaxant factor produced by endothelial cells is mediated through the lipoxygenase pathway and is diminished in early pulmonary hypertension.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 - HL 02714-6 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Evaluation of prosthetic cardiac valve failure in an animal model system

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

Michael Jones, M.D., Senior Surgeon, Surgery Branch, NHLBI

Victor J. Ferrans, M.D., Ph.D., Pathology Branch, NHLBI
Yoshimui Tomita, M.D., Ph.D., Guest Worker, Surgery Branch, NHLBI
Elling E. Eidbo, B.A., Research Assistant, Surgery Branch, NHLBI
Jesse L. Sandlin, M.S., Research Assistant, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Pathology Branch

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	5	5

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.)*

The purpose of this project is to develop an animal model of bioprosthetic cardiac valve failure and to utilize this animal model system to evaluate the pathologic alterations and hemodynamic dysfunction which develops in the valves. We have shown that bioprosthetic valves implanted in juvenile sheep demonstrate the same pathologic alterations of degeneration and calcification as those implanted in humans; however, the development of these alterations is accelerated in sheep as compared to humans. Nearly 600 porcine aortic or bovine pericardial bioprosthetic valves from ten different sources have been implanted in the animal model system to assess the characteristics of the pathologic changes, to compare the alterations in different types of valves, to compare the alterations occurring in valves implanted in the mitral versus the tricuspid positions, and to evaluate valves treated prior to implantation with processes to retard or to eliminate the calcification process. Due to these types of observations, clinical trials have been initiated with one new type of bioprosthetic valve. Equally important, if not more so, at least five types of valves have been rejected from consideration for clinical use. The model is additionally being utilized to validate ultrasonic techniques, including color-encoded 2-D Doppler, for the characterization of bioprosthetic, mechanical, and synthetic leaflet valve velocity/flow profiles and for the noninvasive detection of valve failure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02731-04 SU

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Operative Assessment and Results of Left Ventriculomyotomy and Myectomy

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

Charles L. McIntosh, M.D., Ph.D., Senior Surgeon, Surgery Branch, NHLBI

Barry Maron, M.D., Senior Investigator, Head, Echo Lab, Cardiology Branch, NHLBI

OOPERATING UNITS *(if any)*

Cardiology Branch

.B/BRANCH

Surgery Branch

CTION

STITUTE AND LOCATION

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

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

HECK APPROPRIATE BOX(ES)

[X] (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

A standard left ventriculomyotomy and myectomy (LVMM) has been performed for relief of left ventricular outflow tract obstruction secondary to idiopathic hypertrophic subaortic stenosis (IHSS) in 431 patients. This report summarizes 201 patients undergoing an LVM&M since January 1982. An attempt has been made to define criteria for choice of operation, LVM&M vs mitral valve replacement (MVR) based upon septal thickness, distribution of hypertrophy, level of systolic anterior motion (SAM) contact of septum, and concomitant coronary artery disease. Intraoperative 2-D and M-mode echos have been performed on a number of these patients providing precise data utilized intraoperatively. Patients with concomitant CAD are at greater risk for an iatrogenic VSD creation which may be avoided by a modified LVM&M or MVR. Operative mortality is 8.4% and late mortality 4.0%. Results are presented based on preoperative resting gradients < 50 mm Hg and > 50 mm Hg. Postoperative hemodynamic studies reveal good relief of resting gradient in most patients but significant provokable gradients remain in some patients. Two patients have demonstrated significant RVOT obstruction (> 50 mm Hg) and underwent concomitant LVM&M and resection of RVOT obstruction. One patient developed a late VSD which was hemodynamically insignificant (QP:QS = 1.1:1). Reoperation has been performed in some patients with persistent symptoms and gradients. Medical therapy is continued in patients with significant gradients regardless of symptomatic status.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02733-03 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Mitral Valve Replacement in Selected Patients Having IHSS

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

Charles L. McIntosh, M.D., Ph.D., Senior Surgeon, Surgery Branch, NHLBI

Barry Maron, M.D., Cardiology Branch, NHLBI

COOPERATING UNITS *(if any)*

Cardiology 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:
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.)*

Mitral valve replacement (MVR) has been performed on 46 patients as primary or secondary treatment of severely symptomatic patients with resting and/or provokable pressure gradients across the left ventricular outflow tract (LVOT) secondary to idiopathic hypertrophic subaortic stenosis (IHSS). Indications for MVR include: 1) septal thickness < 18 mm; 2) persistent LVOT obstruction after a prior adequate left ventriculomyotomy and myectomy (LVM&M); 3) atypical septal morphology; and 4) severe mitral regurgitation secondary to ruptured chordae tendinae or papillary muscle. Intraoperative echocardiography has provided definition of septal morphology allowing selection for MVR. There have been 2 (4%) perioperative deaths, one a result of hepatic failure and the other was suspected to be caused by prosthetic valve malfunction. Three patients (7%) died after hospital discharge, two suddenly and one of congestive heart and respiratory failure. One patient had a late central embolus. Symptomatic improvement to NYHA functional class I or II has occurred in 80% of 27 patients returning for postoperative evaluation. Excellent relief of both resting and provokable gradients has been demonstrated. Three patients continue to be symptomatic (FC III) and have been shown to have abnormal coronary vascular resistances with no reserve indicating the presence of severe small vessel disease. Thus relief of LVOT obstruction does not always relieve symptoms of chest pain and fatigue. Long-term follow-up will be necessary to assess late mortality and morbidity which will be compared to the well-known results of LVM&M used for palliation in IHSS for the past 26 years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02735-03 SU

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Mitral Valve Replacement With and Without Chordal Excision

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

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

Charles L. McIntosh, M.D., Senior Surgeon, Surgery Branch, NHLBI
Michael Jones, M.D., Senior Surgeon, Surgery Branch, NHLBI

OOPERATING UNITS *(if any)*

AB/BRANCH

Surgery Branch

ECTION

STITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

he purpose of this clinical trial is to test the hypothesis that the mitral alve apparatus (leaflet, chordae tendineae and papillary muscles), if left ntact at the time of valve replacement, will prevent the nearly uniform ostreperfusion left ventricular dilation and low cardiac output associated ith surgical treatment of mitral insufficiency of long duration.

he specific aims are: (1) measure LV hemodynamics and dimensions with ltrasound prior to and after cardiopulmonary bypass in the OR; (2) replace the itral valve in patients with pure mitral regurgitation with and without esection of the entire mitral valve apparatus; (3) determine immediate ostoperative hemodynamic characteristics of each group; (4) analyze 6 month nd 3 year results in terms of exercise capacity, LV dimensions, and emodynamic criteria.

he rationale of this investigation is that if the immediate postbypass course f patients receiving mitral valve replacement for long- standing mitral nsufficiency can be altered by the mechanism of preventing left ventricular ilation by maintaining the innate physical structures of the left heart, perative mortality and support measures may be reduced and long-term benefits ay accrue.

he results in five patients have been excellent. Two patients have required ostoperative support. There have been no perio-operative deaths associated ith the procedure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02740-03 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Coronary Vascular Tone After Coronary Bypass Operations

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

Robert March, M.D., Clinical Associate, Surgery Branch, NHLBI

Charles L. McIntosh, M.D., Senior Surgeon, Surgery Branch, NHLBI
Michael Jones, M.D., Senior Surgeon, Surgery Branch, NHLBI
Richard Cannon, M.D., Senior Investigator, Cardiology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Cardiology Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

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.)*

This clinical study tests the hypothesis that coronary blood flow dynamics of patients may be altered in the immediate interval after a coronary bypass procedure which may be deleterious to the patient's future course if unrecognized. A thermal dilution catheter is placed into the coronary sinus percutaneously and threaded to the great cardiac vein. Ports located at the tip allow sampling of the anterior myocardium and those more proximal sample the entire left ventricle. Blood samples are used to determine regional oxygen consumption, acid base balance, lactic acid, pyruvate, creatine kinase, and lactic dehydrogenase isoenzyme concentrations. Complete hemodynamic evaluations are performed preoperatively and in serial fashion for 6-8 hours after operation. Six patients have had complete studies. In some, no significant changes in coronary vascular resistance, cardiac output, or any of the biochemical variables occur. Other patients show changes which can be related to less than optimal contractility and systemic flow. Control of heart rate through atrial pacing and preload may eliminate some of this variability. Animal studies indicate that the use of a fiberoptic, thermodilution catheter will allow on-line documentation of myocardial metabolic trends. Such continuous measurement will allow better assessment of coronary flow dynamics in future patients studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02742-03 SU

PERIOD COVERED

October 1985 through September 30, 1986

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

Assessment and use of new ultrasonic technologies

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

Michael Jones, M.D., Senior Investigator and Surgeon, Surgery Branch, NHLBI

Elling E. Eidbo, B.A., Research Assistant, Surgery Branch, NHLBI
Jesse L. Sandlin, M.S., Research Assistant, Surgery Branch, NHLBI
Scott T. McMillan, Ph.D., Post Doctoral Fellow, Georgia Institute of Technology
Ajit P. Yoganathan, Ph.D., Assistant Professor, Georgia Institute of Technology
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Bio Fluid Dynamics Laboratory, School of Chemical Engineering, Georgia Institute of Technology

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

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

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

CHECK APPROPRIATE BOX(ES)

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

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

New modes of Doppler ultrasound and new signal conditioning of the received sonic spectra permit accurate assessments of velocities of intracardiac blood flow. Color-encoded, two-dimensional Doppler permits qualitative and quantitative evaluations of entire flow field velocity patterns. Studies utilizing these technologies include the following: 1) Doppler velocity/flow mapping in vivo and in vitro of clinical and preclinical prosthetic mitral valves; 2) comparison of in vivo Doppler ultrasound with in vitro Doppler ultrasound and laser Doppler anemometry; 3) assessment of mitral regurgitation; 4) quantitation of obstruction and regurgitation in right ventricular to pulmonary artery conduits. Studies in patients, both intraoperatively and postoperatively, have been initiated for assessment of operations for valvular lesions.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02743-3 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

The development of a specific immune tolerance model in rhesus monkeys

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

Robert D. Moses, M.D., Senior Staff Fellow, Surgery Branch, NHLBI

Ronald E. Gress, M.D., Senior Investigator, Immunology Branch, NCI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
David H. Sachs, M.D., Chief, Immunology Branch, NCI
Eli Glatstein, M.D., Chief, Radiation Oncology Branch, NCI
Martin L. Morin, D.V.M., Chief, Primate Research Unit, DRS

COOPERATING UNITS *(if any)*

Immunology Branch, NCI
Radiation Oncology 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:
5	4	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.)*

It has been shown both experimentally by Medawar and in nature by Owen that exposure of various mammalian species to foreign antigens during fetal or neonatal life can result in permanent specific immunological tolerance to those antigens. Burnet proposed the clonal selection theory of acquire immunity, which explains the tolerance phenomena by the deletion of foreign antigen-specific lymphocytes when they are exposed to their respective antigens while the lymphocytes are in an immature state. These observations led to the hypothesis that an adult animal, modified so as to have a fetal-like immune system and subjected to a foreign graft transplant, would develop permanent specific immunological to a graft. A juvenile rhesus monkey model has developed over the past three years to test the hypothesis. Briefly, the interventions are: 1) recipient bone marrow harvest, 2) T lymphocyte removal from the marrow by physical (E-rosette) and immunological (antibody plus complement) methods, 3) total body irradiation of the recipient with a myelo-ablative dose to eliminate all immunologically competent cells, 4) reinfusion into the recipient of the T lymphocyte-depleted marrow to salvage the recipient from the radiation, and 5) transplantation of an antigenically-mismatched heterotopic heart allograft. Each experiment includes a treated recipient paired with an appropriate control animal. The major endpoint is time to graft rejection, determined by loss of electrocardiographic activity and confirmed by histopathological examination. Other responses being followed are the time course of return of immunological function, and tests of specific immunological tolerance. Only recently have preliminary results become available.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HL 02752-03 SU

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Augmentation of Bloodflow to Ischemic Myocardium

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Joseph T. Dodd, M.D., Clinical Associate, Surgery Branch, NHLBI

Robert J. March, M.D., Clinical Associate, Surgery Branch, NHLBI
Ellis Unger, M.D., Clinical Associate, Cardiology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*
Cardiology Branch

LAB/BRANCH
Surgery Branch

SECTION

INSTITUTE AND LOCATION
National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) 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 determine the efficacy and safety of augmenting blood flow to the ischemic myocardium. The rationale is that both endstage coronary disease and hypertrophic cardiomyopathy result in global or regional ischemia at rest which is severely exacerabated by any increase in cardiac work. The clinical manifestations are angina pectoris, fatigue and malignant forms of ventricular tachycardias. Over the past two years more than 60 dogs have had applications of one to two ameroid contrictors to the left anterior descending coronary artery and the circumflex coronary artery and an internal mammary artery (IMA) implanted into the zone(s) of ischemia. New efforts to develop a series of standard ameroid constrictors has been made so that closure times are reproducible at 30, 60 and 90 days. Previous data have demonstrated that: 1) an IMA implant is protective to the development of myocardial infarction; 2) all IMA implants remained open and collateralized to native vessels; and 3) blood flow through the IMA represented 15 - 20% of normal resting flow per gram of tissue. Extracardiac myocardial blood flow was augmented by catecholamine administration as determined by serial radioactive microsphere injections but not by the addition of an omental overlay. New data demonstrate that the entire left ventricle can be made slowly ischemic by two ameroid contrictors and be totally supported by two IMA implants. Studies performed at 9 or more months after the implants demonstrate: 1) complete closure of the native vessels of the left ventricle; 2) marginal aerobic metabolism at rest with evidence of ischemia as shown by increased coronary sinus lactate concentrations with minimal increase in heart rate; and 3) diminished left ventricular wall motion. These data suggest that dual internal mammary implants alone are insufficient to supply the metabolic requirements of the left ventricle of the dog and that collateral enrichment techniques, additional sources of blood supply must be sought to totally support the left ventricle under basal conditions and with exercise.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02753-03 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Development and Evaluation of a Synthetic Trileaflet Valve

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

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

Michael Jones, M.D., Senior Surgeon, Surgery Branch, NHLBI

Victor Ferrans, M.D., Ph.D., Chief, Ultrastructure Section, Pathology Branch, NHLBI

Francisco A. Arabia, M.D., Clinical Associate, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Pathology Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

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

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.)*

During a 16 year program, a synthetic trileaflet valve had been developed. Prototype clinical valves use a narrow soft sewing collar of knitted polyster, a flexible coronal shaped stent and a micro-woven fabric which is highly flexible and has stiffness and anisotropic properties similar to normal aortic leaflet tissue. Extensive durability and soft tissue implant studies were performed prior to mitral valve replacement in sheep. The in vivo data showed that valvular insufficiency occurred early (24 hours) which has subsequently been traced to alteration of filament geometry from stress compaction. New fabric designs have been completed and a new prototype has been produced. Valves are inserted into a hydraulic testing device which characterizes forward and regurgitant power losses. Accelerated fatigue testing is performed at cyclic rates of 1000-1200/min. Implant studies consist of subcutaneous placement of materials in rabbits, conduit insertions in the arterial and venous systems of dogs and baboons and valve insertion in juvenile sheep.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02762-02 SU

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Chronic Mitral Insufficiency

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

Robert J. March, M.D., Clinical Associate, Surgery Branch, NHLBI

Michael Jones, M.D., Senior Surgeon, Surgery Branch, NHLBI
Marc E. Visner, M.D., Asst. Professor, Dept of Surgery, Georgetown University
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

OOPERATING UNITS *(if any)*

Department of Surgery, Georgetown University

AB/BRANCH

Surgery Branch

ECTION

STITUTE AND LOCATION

National Heart, Lung, and Blood Institute

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

HECK APPROPRIATE BOX(ES)

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

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

This laboratory study tested the hypothesis that left ventricular function following mitral valve replacement may be better preserved by retaining the tethering effect of the mitral valve apparatus in chronic mitral regurgitation. A chronic model of mitral regurgitation has been developed an extensively studies in sheep. Due to the progressive enlargement of an anterior mitral leaflet defect, there were significant increases in left ventricular mass, wall thickness, LV mass to body weight ratio, end-diastolic volume, stroke volume and ejection fraction over a 7-8 month period compared to controls. Mitral annular - papillary muscle discontinuity following mitral valve replacement in chronic, volume overloaded ventricles, led to a significant increase in the end-diastolic volume thus creating an increased "effective" preload. This resulted in an increased stroke volume secondary to increased equatorial, minor axis shortening. Despite this increase in forward flow, the reserve of the left ventricle with chornic mitral regurgitation to perform pressure-volume work was severely impaired with dramatic, significant decreases in maximum systolic blood pressure and max dp/dt at matched heart rates and preload after all chordae tendinae were severed. These data show that maintenance of mitral annular-papillary muscle continuity following mitral valve replacement preserves left ventricular function in setting of chronic volume overload secondary to mitral regurgitation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02764-01 SU

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Hemodynamic and Clinical Analysis of Small Diameter Aortic Valve Prostheses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
Andrew H. Foster, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Charles L. McIntosh, M.D., Senior Staff, Surgery Branch, NHLBI
Cynthia M. Tracy, M.D., Staff, Cardiology Branch, NHLBI

COOPERATING UNITS (if any)

Cardiology Branch, NHLBI

LAB/BRANCH
Surgery Branch

SECTION

INSTITUTE AND LOCATION
National Heart, Lung and Blood Institute, Bethesda, Md. 20891

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.)

Aortic valvular prostheses exhibit greatest hemodynamic limitation, viz., inherent obstruction to flow or so-called prosthetic stenosis, when the smallest sizes are employed. Complex annular reconstructive procedures have been devised to make possible the insertion of larger size prostheses in patients with small aortic roots.

We correlated long-term clinical data with serial hemodynamics in 52 patients who received small diameter (17 mm or 19 mm) aortic valvular prostheses without annulus enlargement.

No predictors for significant prosthetic stenosis were found for 17 mm Bjork-Shiley valves. Patients with 17 mm Bjork-Shiley valves tended to have greater transprosthetic gradients at rest but effective orifice areas were similar to those found in patients with 19 mm prostheses. Greater transprosthetic gradients did occur in those patients who received 19 mm Bjork-Shiley valves and had greater resting blood flows. Progressive prosthetic stenosis was not observed in patients who underwent multiple postoperative catheterizations over intervals of 2-12 years. It was concluded that acceptable palliation was provided by aortic valve replacement with small diameter prostheses over long periods and that resting hemodynamic studies had limited predictive value for long-term prognosis.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02765-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Intrinsic failure of Hancock mitral bioprostheses

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

Andrew H. Foster, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Michael Jones, M.D., Senior Staff, Surgery Branch, NHLBI
Charles L. McIntosh, M.D., Ph.D., Senior Staff, Surgery Branch, NHLBI
David J. Underhill, M.D., Clinical Associate, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

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

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 advantage of low thrombogenicity without anticoagulation therapy may be outweighed by diminished durability in the second decade following implantation of glutaraldehyde preserved porcine bioprostheses. Hancock mitral valve bioprostheses have been implanted at the National Institutes of Health since July 1970. Eight porcine models were placed in 111 patients during a 41 month period ending December 1974. These patients have been followed annually by the NHLBI Surgery Clinic by annual examination and serial hemodynamic studies. Intrinsic valve failure, defined as structural degeneration of valve tissue and/or stent geometry alteration, in the absence of a history of infection, occurred in 23 patients. The linearized and actuarial incidence of valve failure increased markedly in the ten-fifteen year interval since implantation. The incidence and rate of failure were not related to model type. No predictors of valve failure were found on early postoperative catheterization. Catheterization prior to reoperation demonstrated significant prosthetic stenosis and valvular regurgitation. Explants showed gross leaflet disruption and/or perforation, with variable mixtures of calcification, stent creep, and intracuspal hematomae. Mortality at reoperation was high, subduing our initial enthusiasm for the Hancock mitral bioprosthesis in younger patients, in those who might require eventual anticoagulation, and in those who would present prohibitive operative risks in 8-12 years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02766-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Mitral Valve Replacement in Children: The Adult Prosthetic Valve

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

Andrew H. Foster, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Cynthia M. Tracy, M.D., Senior Staff Fellow, Cardiology Branch, NHLBI
John Schwankhaus, M.D., Medical Staff Fellow, Neurology Branch, NINCDS

COOPERATING UNITS *(if any)*

Cardiology Branch, NHLBI
Neurology Branch, NINCDS

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

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 first artificial mitral valve was implanted in children at this institute over 25 years ago. Annual examinations at the NHLBI Surgery Clinic over a period of 15 to 20 years has permitted description of a unique patient population of children who have now entered adulthood in their second decade of artificial valve function. All surviving children (N=6) with mitral valve prosthesis in place for at least 15 years, were reevaluated at the NHLBI Surgery Branch clinic. The presence of previous multiple cerebral infarctions was found by computerized axial tomography in three patients who had exhibited no clinical history suggestive of central thromboembolism and all four patients with a mechanical prosthesis. A complete neurological examination failed to demonstrate any residua of these multiple cerebrovascular accidents in all patients. A consistent discrepancy between the history of exercise tolerance elicited from the patient interview and objective data from the tread mill exercise capacity was also demonstated. Echocardiogram confirmed tread mill evidence for reduced left ventricular function in these long term survivors. No kidney scan was positive for emboli. We conclude that the frequent presence of subclinical central thromboemboli and the inability of patient history to confirm exercise capacity in these young adults mandates the use of more objective modalities in the long term follow up of prosthetic valve complications in children.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HL 02767-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Operation for Hypertrophic Subaortic Stenosis in the Elderly

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

Matthew M. Cooper, M.D., Clinical Associate, Surgery Branch, NHLBI

Charles L. McIntosh, M.D., Senior Staff, 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

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.)*

A retrospective review of 52 patients 65 years and older with hypertrophic cardiomyopathy who had a left ventricular septal myectomy performed. The mean follow up interval was 54 months. No patient has required reoperation. A reduction in left ventricular outflow tract obstruction of 85% was achieved Symptomatic and functional improvement rates were 85 and 78% respectively an average of 1.3 classes (NYHA). The actuarial survival was 82% at five years. It was concluded that age greater than 65 years is not a contraindication to the operation which significantly improved both quality and duration of life.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02768-01 SU

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Analysis of the Interaction of Heparin with Endothelial Cell Growth Factor (ECGF)

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

Todd K. Rosengart, M.D., Clinical Associate, Surgery Branch, NHLBI

Robert Friesel, Division Cell Biotechnology Research Center, Rockville, MD.
Tevie Mehlman, Division Cell Biotechnology Research Center, Rockville, MD.
Wilson H. Burgess, Ph.D., Division Cell Biotechnology Center, Rockville, MD.
Thomas Maciag, Ph.D., Division Cell Biotechnology Center, Rockville, MD.
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

OOPERATING UNITS *(if any)*

Division Cell Biotechnology Center, Rockville, MD.

AB/BRANCH

Surgery Branch

ECTION

ISTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

OTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

e have demonstrated using I^{125}-ECGF autoradiography that heparin markedly nhibits proteolytic digestion of ECGF by trypsin and other proteases. This roperty was lost after thermal denaturation of ECGF, suggesting a heparin: CGF structural interaction rather than a heparin:trypsin interaction is esponsible for the trypsin resistance of ECGF. Heparin was better able to rotect ECGF from subsequent trypsin digestion after thermal denaturation in he presence of heparin as compared with thermal denaturation in the absence of eparin suggesting that heparin ameliorates ECGF denaturation and provides onformational stability to the polypeptide growth factor. The stabilizing ffect of heparin was dependent upon the concentration of heparin as well as emperature and duration. Autoradiography of ^{125}I-ECGF incubated with human mbilical vein endothelial cells demonstrated near complete inhibition of roteolytic digestion of ECGF when the incubation was performed in the presence f heparin. These data suggest that the mechanism of the heparin-induced human ndothelial cell phenotype involves the protection of ECGF by heparin against nactivation by endothelial cell-derived proteolytic enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02769-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Heparin Fragments & Endothelial Cell Growth Factor to Prevent Myointimal Hyperplasia

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

Todd K. Rosengart, M.D., Clinical Associate, Surgery Branch, NHLBI

John Kupferschmid, M.D., Clinical Associate, Surgery Branch, NHLBI
Victor Ferrans, M.D., Senior Staff, Pathology Branch, NHLBI
Ward Cassells, M.D., Senior Staff, Cardiology Branch, NHLBI
Ellis Unger, M.D., Medical Staff Fellow, Cardiology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Cardiology Branch, NHLBI
Pathology Branch, NHLBI

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A multiphased analysis in an animal model of the effects of non-anticoagulant heparin fragments and endothelial cell growth factor (ECGF) on myointimal hyperplasia (MIH) is planned to: a) develop a model of MIH using vein to artery transplant that simulates the lesion occurring in clinical practice; b) examine the ability of the non-anticoagulant heparin fragments to inhibit MIH; c) study the pharmacokinetics of ECGF in a small animal model and then; d) examine the ability of ECGF to accelerate endothelial regeneration and act synergistically with heparin in retarding MIH. Heparin fragments have been demonstrated to cause inhibition of smooth muscle cell hyperplasia, thought to be the primary mechanism of MIH. ECGF causes acceleration of endothelial cell growth in vitro. Endothelial injury an important component in the development of MIH, should be ameliorated by use of ECGF and accelerate re-endothelialization. A return of endothelium to a quiescent state should retard or prevent the progression of MIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02770-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Natural History of Repaired Diffuse Supravalvular Aortic Stenosis

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

Andrew H. Foster, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Charles L. McIntosh, M.D., Senior Staff, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Eight children, 8-15 years old, underwent patch aortoplasty across the supravalvular constriction between 1961 and 1974. The clinical course and hemodynamic alterations of these patients over the ensuing years is described.

Changes in progression of left ventricular outflow tract obstruction, in aortic morphology and in valvular function occurred with the growth of these children. These new findings dictate strict, life-time follow up in patients undergoing patch aortoplasty for diffuse supravalvular aortic stenosis in childhood.

800

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02771-01 SU

ERIOD COVERED

October 1, 1985 through September 30, 1986

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

Intra-aortic Balloon Counterpulsation and Acute Aortic Insufficiency

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

Andrew H. Foster, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Julia A. Swain, M.D., Senior Staff, Surgery Branch, NHLBI
Francisco A. Arabia, M.D., Clinical Associate, Surgery Branch, NHLBI
Richard H. Koehler, M.D., Clinical Associate, Surgery Branch, NHLBI
Benjamin Schneider, CCRT, Clinical Perfusionist, Clinical Center
Dennis Coyne, CCRT, Clinical Perfusionist, Clinical Center
~~Ram Paul, B.S., Physical Seience Technologist, Technical Development Lab, NHLBI~~

OOPERATING UNITS *(If any)*

Technical Development Laboratory, NHLBI

B/BRANCH

Surgery Branch

CTION

STITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

HECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The use of intra-aortic balloon counterpulsation (IACP) for low cardiac output complicating acute aortic insufficiency is controversial. Beneficial effects of diastolic augmentation are offset by conflicting and poorly documented reports of altered coronary blood flow and theoretical increases in regurgitant volume and left ventricular wall tension during IACP use. We have found that regurgitation is not significantly increased by IABP during acute severe AI in dogs. Preliminary data have not demonstated deleterious effects of IACP on myocardial metabolism, coronary blood flow or ventricular function during aortic insufficiency.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 HL 02772-01 SU

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Fenoldopam in Postcardiotomy Low Output Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Todd K. Rosengart, M.D., Clinical Associate, Surgery Branch, NHLBI

Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI
Charles L. McIntosh, M.D., Ph.D., Senior Staff, Surgery Branch, NHLBI
David E. Webb, M.D., Expert Consultant, Office of the Director, CC

COOPERATING UNITS *(if any)*
Nephrology 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	1	0

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

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

The effect of the selective dopamine receptor agonist fenoldopam on renal perfusion, renal function and cardiac function and cardiac hemodynamics will be assessed in 30 patients who demonstrate low cardiac output syndrome (LCOS). Ten patients without low cardiac output syndrome following open heart operation will serve as controls.

Fenoldopam is an investigational agent that is 6x as potent as dopamine in causing renal vasodilatation. It should be an ideal agent in reversing the renal hypoperfusion thought to play a significant role in acute renal failure following post-cardiotomy low output syndrome. Fenoldopam also causes systemic vasodilatation and should provide effective afterload reduction.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02773-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Pulmonary Hypertension - Study of Chronic Models by Isolated Lobar Perfusion

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

Lawrence I. Schmetterer, M.D., Clinical Associate, Surgery Branch, NHLBI

Allan R. Milewicz, M.D., Clinical Associate, Surgery Branch, NHLBI

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

COOPERATING UNITS *(if any)*

Department of Physiology and Biophysics, Georgetown University Medical Center

Department of Pathology, Armed Forces Institute of Pathology

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung and Blood Institute, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The mechanisms involved in the development of irreversible pulmonary vascular pathology associated with pulmonary hypertension in the young have not been elucidated. A recent hypothesis was that the pathologic and hemodynamic alterations are regulated by endothelial cell derived relaxing factor (EDRF) and that changes in EDRF production may be a major contributor. This hypothesis was tested in dogs and weanling swine who had 6-8 mm polytetrafluorethylene conduits anastamosed to the left pulmonary artery and aorta. At a second operation, flows were diverted such that the right lung was hyperperfused and normotensive and the left lower lobe was made hypertensive and hyperperfused. Three to six months later isolated lobar perfusion was used to provide a wide variety of flow-pressure conditions. EDRF stimulants; bradykinin, acetylcholine a calcium ionophore and adenosine triphosphate were tested as vasodilators after preconstuction with prostaglandin PGF_{2_α} and inhibition of cyclooxygenase metabolism with indomethacin. The results demonstrated no diminution in vascular response to EDRF stimulants in normal and hyperperfused lungs. A lipoxygenase inhibitor was shown to completely inhibit vasodilatory responses to bradykinin but the responses were not inhibited by an enzymatic cyclooxygenase inhibitor. Species differences were also identified. These data suggest that endothelial derived relaxant factor can play a major role in the development of irreversible pulmonary hypertension and that the pathway of expression is probably through lipoxygenase derived metabolites of arachodonic acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 02774-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Hydraulic and Ultrasonic Studies of Prosthetic Heart Valves in Vitro

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

Francisco A. Arabia, M.D., Clinical Associate, Surgery Branch, NHLBI

Michael Jones, M.D., Senior Invesigator, Surgery Branch, NHLBI
Elling E. Eidbo, B.A., Research Assistant, Surgery Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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.)*

Since the development and use of prosthetic heart valves, numerous in vitro and in vivo techniques have been used to determine the performance of these valves. In vitro circulatory systems have been used to determine flow patterns, regurgitation, and pressure differences for a given valve. In the past years, Doppler echocardiography has become a useful, noninvasive, diagnostic method in the study of cardiac anatomy and physiology. An in vitro circulatory system capable of accepting heart valves in the aortic and mitral positions is utilized. This system is capable of reproducing physiologic flow rates and pressures. Doppler echocardiography will be used to determine the fluid velocity patterns of various prosthetic valves placed in the in vitro circulatory system. Pressure differences will be calculated from these velocites and compared to those pressures obtained from pressure transducers. Doppler color flow mapping data obtained from Doppler echocardiography will also be used to compare flow patterns obtained in vivo to those obtained for the same explanted valves in vitro.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 02775-01 SU

PERIOD COVERED

October 1, 1985 through September 30, 1986

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

Creation of Transmural Myocardial Microchannels with Lasers

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

Joseph T. Dodd, M.D., Clinical Associate, Surgery Branch, NHLBI

Paul Smith, Ph.D., Physicist, EEES, BEIB
Robert Bonner, Ph.D., Physicist, EEES, BEIB
Ellis Unger, M.D., Medical Staff Fellow, Cardiology Branch, NHLBI
Richard E. Clark, M.D., Chief, Surgery Branch, NHLBI

COOPERATING UNITS *(if any)*

Cardiology Branch, NHLBI
EEES, BEIB

LAB/BRANCH

Surgery Branch

SECTION

INSTITUTE AND LOCATION

National Heart, Lung, and Blood Institute

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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.)*

This pilot feasibility study was designed to test the hypothesis that laser-induced transmyocardial channels could be created and provide augmentation of blood flow to an ischemic zone. Three laser systems were tested; carbon dioxide, argon and excimer. The first had too little power to penetrate the full thickness of the myocardium and created charred lesions. Argon passed through 1 mm fibers penetrated but charred. The excimer system was reliable and produced clean channels. Five dogs had the application of an ameroid constrictor to the left anterior descending coronary artery. Excimer lasing was performed. Collateral flow will be measured with the radioactive microspheres technique. Biochemical and morphologic studies will be performed.

ANNUAL REPORT OF THE
LABORATORY OF TECHNICAL DEVELOPMENT
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

OCTOBER 1, 1985 TO SEPTEMBER 30, 1986

Separation Science Instrumentation

Development of the countercurrent chromatography (CCC) technology has been continued in various directions. A new centrifuge, called the improved angle rotor coil planet centrifuge, was designed for performing CCC. The acceleration produced by this planetary motion was mathematically analyzed. A set of general formulae derived for computation of the acceleration field facilitated comparative studies of all types of the synchronous planetary motion used for performing CCC. These studies indicated that the new system can yield an efficient mixing of the two solvent phases in the coiled column by a three dimensional fluctuation of the centrifugal force vectors in addition to the asymmetrical distribution of the radial force field characteristic of the existing high-speed CCC centrifuge. The apparatus was successfully constructed and evaluated for performance in terms of retention of the stationary phase and partition efficiency using a standard set of DNP amino acid samples. The results indicated that the new scheme can yield a high performance in CCC comparable to that produced by the most refined high-speed CCC apparatus.

The foam CCC method developed last year has been applied to a broad spectrum of samples ranging from small molecules to macromolecules and cells. Acidic and basic dyes showed expected foam affinity and separated according to the electric charge of the molecule. Addition of NaCl at a 0.1M concentration substantially altered the elution profile of the samples indicating that foam affinity of these samples can be conveniently adjusted with salt concentration in the surfactant solution. Experiments have further demonstrated that foam CCC can be applied to more useful samples such as macromolecules and cells without the use of surfactants. By adjusting ionic strength and pH of sodium phosphate solution, various proteins were separated with minimum evidence of denaturation. The fact that the protein sample, which produce a single peak in electrophoresis, is completely separated into two fractions suggests that the foam CCC method will provide valuable means of protein separation for biomedical research. Using isotonic saline containing bovine serum albumin as a foam-producing agent, blood cells were also separated according to the foam affinity. The preliminary studies indicated that the platelets are collected with the foam and erythrocytes or their membranes are eluted with the liquid, while the hemolysis is completely prevented by choosing the proper concentration of bovine serum albumin at 0.05-0.1g%.

In addition to the above experimental works, efforts were made to develop a hypothesis on the mechanism of unilateral hydrodynamic distribution of two immiscible solvent phases in the rotating coil. Establishment of reliable theory on this complex hydrodynamic phenomenon will greatly contribute to future development of the high-speed CCC technology.

Pulmonary and Cardiac Assist Devices

It has previously been shown that mechanical pulmonary ventilation in a healthy population of young sheep at pressures from 30-50 cm H_2O can rapidly lead to a deterioration in lung function, a worsening in arterial blood gases, and death within a few days. Our studies suggested that mechanical ventilation in and of itself is injurious, that it can delay and impair healing of the lungs, and that it could be the major cause of morbidity and mortality in patients with pre-existing pulmonary disease.

This year, we have induced respiratory insufficiency in a group of healthy sheep following the application of high pressure mechanical pulmonary ventilation, to explore the effects of various treatment protocols. When respiratory failure was very severe, no form of ventilator management lead to survival. Similarly, we have been unsuccessful in obtaining any survival when so assisted with an extracorporeal membrane lung. Death invariably was preceded by CNS, cardiovascular, hepatic, renal and pulmonary failure. Importantly, this animal model suggested that survival from primary lung failure can progress to a stage beyond which no form of treatment known today would succeed.

In another group of 22 sheep with similarly induced acute respiratory failure except that injury from high pressure mechanical ventilation was stopped some 6-12 hours sooner. Ten of these sheep were then randomized to best state of the art mechanical pulmonary care, and only one such animal survived. The rest succumbing to progressive respiratory failure with multiorgan system disease. The remaining 12 sheep with induced respiratory failure were placed on continuous positive airway pressure (CPAP) and an extracorporeal membrane lung bypass. In eleven out of 12 sheep there was a progressive improvement in lung function following some 24 hours of bypass, leading to weaning off bypass.

Results to date suggest significant danger from continuing mechanical pulmonary ventilation at elevated peak airway pressures. Such injury process is cumulative, and predictable. Recovery from this injury process can be uncertain in the face of progressive involvement of multiorgan systems.

During 1986, some 30 medical centers will be applying neonatal ECMO (extracorporeal membrane oxygenation) to newborns with acute lung failure of various causes, with a predicted survival rate rising from the expected 10%, to 80% with this new treatment. The key to lung recovery is reducing ventilator settings to "normal", i.e. peak airway pressures less than 20 cm H_2O. Such pulmonary management is in accord with our previous publications in the management of highly preterm fetal lambs, and in lambs with induced meconium aspiration. Similar management of the lungs when applied to the adult patient population is likely to succeed.

Biophysical Instrumentation

The Section on Biophysical Instrumentation has continued to develop a series of instruments for the study of protein and cellular reactions. The ability to detect minute amounts of protein is of interest both in protein chemistry and in immunology. With our pulsed fluorometer we are exploring a possible replacement for radioimmuno assay (RIA) by tagging proteins with a caged molecule of Europium. By taking advantage of the very long fluorescence lifetime of Europium (500 microseconds), we are able to achieve levels of sensitivity equal to or greater than those of RIA. A pulsed nitrogen laser has been used to examine a simple Europium tagged system. Detection of 10^{-14} molar Europium was readily achieved in a 100 microliter sample. Efforts to extend this to 10^{-16} M are presently being pursued along with techniques for greatly improving the reliability of the phototube, counting electronics, and the laser light source. A new circuit under development will allow the data from each flash to be entered in the computer individually. This improvement will allow the kinetics of fast reactions to be studied at very high dilutions. Such a system could potentially be used in the detection of very low levels of retrovirus in studies on AIDS, slow virus diseases, etc.

Batch microcalorimetry has been used for many years for the determination of heats of reaction of various biochemicals, cell growth, bacteria identification, and heat capacity measurements. Very little use has been made of its potential for analytical determination because of an overall lack of sensitivity and a very low sample rate -- i.e. one can run only two to six experiments per day, depending on sensitivity. In order to overcome these problems, we have developed two types of stopped-flow microcalorimeters which operate in a time range of seconds to minutes. The first system consists of 2 polypropylene flow cells fitted into the batch calorimeter. These cells have been made by a special molding process developed by Kolobow (LTD) and Biele (BEIB). They are then coated with black diamond-like carbon so that they are impervious to water vapor. Electrical and chemical tests using a 50 microliter sample and 200 microjoules (48 microcalories) are in close agreement. Sample insertion, data collection, and analysis are all done by an online microcomputer. Since a two second electrical pulse can be accurately deconvoluted, chemical kinetics can be followed with this instrument for all reactions whose half-lives are greater than 1 second. Amino acid detection at nanomolar levels using the enzyme decarboxalase are being quantified. Work on several nucleic acid reactions will be resumed with this system. In addition, studies of the assembly of the phospholipid membrane with our older batch calorimeter operating in a titration mode are presently underway with Gershfeld (NIADDKD).

A number of years ago we reported the development of an isoionic hemoglobin in Berger, et al. Anal. Letters, Vol. 16, 125-138 (1973). The isoionic point, as defined by Sorensen, Linderstrom-Lang and Lund: Compt. Trav. Lab. Carlsberg, Vol. 16, No. 5 (1926) is the pH at which the number of protons combined on the basic groups is equal to the number of protons dissociated

from the acidic groups. A group refers to unchanged moieties such as COOH, NH_2, etc. We improved this preparation by chromatography and ultra centrifugation so that as presently prepared, it is 99.99% pure human hemoglobin Ao. Heavy metals, except for iron, are excluded by this method to less than 1 part per 10 million. The need to study such highly purified material is demonstrated by the fact that the level of oxygen needed to half saturate hemoglobin is only .8mm of Hg for isoionic hemoglobin, 4 mm of Hg if 1 mM Cl^- is present, and 10 mm of Hg for .1MCl^-. This compares with 26 mm of Hg for normal hemoglobin in the red cell. Thus an understanding of the electrostatic interaction of proteins and other molecules must start with an isoionic preparation. This preparation, moreover, has recently had an interesting industrial application to the production of stroma-free hemoglobin on a large scale to be used as a blood substitute. Various factors in the preparations now being produced by commercial firms produce toxic effects, large P_{50} shifts, etc. The Army Medical Research Institute has therefore recently adopted our isoionic hemoglobin as the "gold standard" for purity. In the future, commercial firms will be able to test the purity of their products against our standard.

A new inertial-drive flow system has been developed for exploring quench flow reaction kinetics in the investigation of the mechanism of reaction of Sacroplasmic Reticulum, ATPase, and other transport enzyme systems which cannot be followed by optical means. In order to use this instrument for Sacroplasmic reticulum investigations, it is necessary to work in what is called the push-push mode: the instrument pushes the syringes, waits 50 to 500 milliseconds, and pushes again. To achieve this a cam driven lever was developed to allow the hitch feed to advance automatically after each firing. The repetition rate at present is 100 milliseconds. A new hitch feed using a tongue and groove lever action has proven very effective for use with the inertial drive thermal-optical stopped flow system. It provides a positive stop as well as drive, and has greatly improved the operation of the flow system. Pressure, velocity, and thermal measurements have provided information needed for the understanding of the mixing process in the ball mixer. The results to date demonstrate that for a viscosity of one centipoise, a pressure differential of less than 4 psi is sufficient to produce better than 99% mixing at 3M/sec with thermal fluctuations of only .50 microdegrees C. The flow system developed here will be useful for work on cells, membranes, and proteins. The combined thermo-optical flow system offers a new dimension in our ability to study suspension reactions.

Luminescence Instrumentation

Modern spectroscopic methods have been applied to problems of biomedical interest: The anomalous green fluorescence of serotonin and other 5-hydroxy-indoles has now been shown to arise by protonation during the excited state lifetime. Using the picosecond tunable dye laser system, we have directly observed the growth and decay of the green fluorescence. Other observations such as the chloride ion enhancement and the different rates of quenching of the ultraviolet fluorescence support these findings.

The fact that light causes these compounds to have a different reactivity than in the ground state may be of significance: melatonin (N-acetyl-5-methoxygryptamine) exhibits excited state protonation and is involved in physiological response to light.

An investigation was carried out on tryptophan dipeptides in order to define the chemical groups which enhance or quench tryptophan fluorescence. Such information is relevant to how protein tryptophan is influenced by nearby structures. About 16 peptides have been characterized by measuring quantum yields, decay kinetics, pH dependence of fluorescence, binding to copper ion, and absorption spectra. It was concluded that the combination of groups which cause quenching, such as the amino, carboxyl, and peptide groups, cause a much greater quenching than would be expected by a simple sum of their individual effects.

A method for determining the association constant for 1:1 complexes of metal ions with tryptophan or tyrosine derivatives has been worked out, based on the fluorescence quenching due to the metal ions. An equation was worked out to describe the binding, and then the data were fit to the equation by computer. Collisional quenching can cause artifacts, but the conditions where this occurs were defined and usually can be avoided.

A series of NBS standards was left with us by Dr. Mavrodineanu, who developed them after several consultations with us over the years. These inorganic phosphors embedded and sintered in a matrix of polytetrafluoroethylene are mounted as translucent strips in cells that fit into spectrofluorometers. Testing has begun on the accuracy and convenience of using these standards to calibrate spectrofluorometers for corrected spectra and quantum yield measurements and to correct for instrumental instabilities during assay runs.

Laser Fluorescence Instrumentation

Our new time resolved fluorescence spectrophotometer was used to study a variety of protein and membrane systems. The unique advantages of this instrument were exploited to obtain structural data that cannot be obtained from other fluorometers. In particular, this instrument was designed with the goal of obtaining precise time-resolved fluorescence polarization and decay signals within minutes, while other instruments may require hours. For example, we were able to study oligomerization of actin (under non-polymerizing conditions), then analyze the data "on line". This gave us an immediate picture of the size changes that are occurring within a few hours. Ironically, this was done at a laser wavelength where our system is weakest and without use of our T-format routing system (under repair). We expect to do an order of magnitude better when both wavelength and router modifications are complete. Meanwhile, we have begun to study the changes in size that occur when various small proteins bind and sequester actin.

The uniqueness of our facility is exemplified by our study of thio redoxin during sulfhydryl reduction/oxidation, folding and unfolding. The intrinsic tryptophyl fluorescence of this protein is quite sensitive to these conformational changes, but the signal is both weak and short-lived. Our powerful laser-based excitation provides ample signal, and our detectors are already capable of ~200 picosecond resolution (another aspect slated for fivefold improvement soon). We are now busy extending these studies to genetically engineered proteins that contain single replacements for each of the tryptophan reporter groups. This will help us track the conformational data to individual sites.

We also continued to study the basic photochemical mechanisms that provide our signals from proteins. Mechanisms for protonation (in the excited state) and the quenching by metal and halogen ions of tryptophyl-like indoles and derivatives were studied, as were selected tryptophyl dipeptides. An understanding of these processes will help us evaluate the site-specific decay data presented to us by Trp residues inside proteins.

We also studied the complicated fluorescence of "self quenched" systems (such as VSV spike proteins) as they aggregate to promote capsid fusion, a key to viral infection.

Effective molecular "sizing" was the goal of our studies with enzyme I of the PTS system. EI dimerizes as temperature is raised above 6°C, and its phosphorylating activity is thought to be confined to the dimeric form. We gathered as much information in a period of two days as had been garnered in four weeks of instrument time elsewhere. Further, some of our results were obtained under conditions where the protein denatured during the period of measurement elsewhere.

The instrument is designed to evolve as our needs change, so we have recently tested automated scan programs to give us high resolution decay data surfaces. These are exactly the sort of data needed for our programs that utilize "global least-squares". We have also improved the T-format capability for polarization measurements and the online data analysis capabilities of the system. In particular, we have continued to develop the widely heralded "global" approach, extending it to systems so complex that only a lifetime distribution can adequately describe the microenvironment. We also completed and published accounts of how our "spectral association" methods in fluorescence can be used to study macromolecules of different size or conformation, or how they apply to the detection (and quantitation) of lipid "domains". We also published the first (globally analyzed) protein axial ratio from fluorescence (we adsorbed several dyes to the same protein, then used the variety of bound orientations to overdetermine the host shape).

In addition to the focus on exploiting our existing instrument, we have expanded our interests into novel fluorescence instrumentation. In particular, we have established a collaboration with Walter Reed to combine high power (military radar) microwave sources with our instrument. We are

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seeking an entirely new type of biophysical measurement: electric vector induced resonant anisotropy. Basically, we hope to see the oscillating electric field couple into the internal molecular dynamics of enzymes and lipids. We are combining state-of-art RF circuitry with the most sensitive optical instrument for this phenomenon. We have also developed ideas for novel fluorescence instruments based on optical cross-correlation and new photomultiplier technologies.

In all, the time resolved fluorescence project has advanced well on both the applied and basic research fronts.

Cell Measurements Systems

The major role played by calcium in the regulation of many cellular processes has prompted us to improve methods for measuring free Ca activity in cell systems. In addition to developing a method for making micropipettes from fused quartz for intracellular Ca^{++} measurements, a 2 mm electrode has been designed and made for the measurement of Ca^{++} activity in small amounts of solution similar to the cytoplasm of cells. Such solutions are often made for biochemical studies on the basis of calculations of a calcium buffer system without experimental verification. The 2 mm electrode developed in this laboratory has rapid response, low resistance, 2 weeks life and negligible equilibration time. Thus it should be useful for determination of Ca^{++} activities using existing pH meters.

The porous bottom culture dishes (PBCDs) and related devices developed in this laboratory for the sterile measurement of electrophysiological parameters of cell layers are used in nearly 100 laboratories. Two companies are making commercial versions of the PBCDs using cellulose ester membranes (Millipore Corp.) and polycarbonate membranes with and without cell culture treatment (Costar Corp.). Most are used for epithelial cell layers.

Recently, we have grown endothelial and smooth muscle cell layers on our PBCDs made with our transparent collagen membranes. These hold promise of being good models of several types of blood vessels.

Clinical Devices

Reports that a heated tip can be used instead of a laser radiation to ablate atherosclerotic plaque suggested the need for a simple method of making a hot tip catheter but the size limitation, long leads and power demand require a special effort. A polarized electric-arc-heated tip has been designed, fabricated, and tested in cooperation with the Cardiology Branch. In addition a second method has also been demonstrated to have possibilities. This method utilizes the catalytic combustion of a stoichiometric mixture of hydrogen and oxygen on palladium sponge. The heating capacity is adequate and control seems practical.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01404-18 LTD

RIOD COVERED

October 1, 1985 to September 30, 1986

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

Membrane Lung System for Long Term Respiratory Support

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

P. I.	T. Kolobow	Medical Officer	LTD:NHLBI
	M. Borelli	Visiting Fellow	LTD:NHLBI
	R. Spatola	Guest Worker	LTD:NHLBI

OPERATING UNITS *(if any)*

B/BRANCH

Laboratory of Technical Development

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Section on Pulmonary and Cardiac Assist Devices

TITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2	0.5

ECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have applied high peak airway pressure (50 cm H_2O) positive pressure ventilation to healthy anesthetized and sedated sheep while monitoring changes in total static lung compliance (TSLC), functional residual capacity (FRC), and arterial blood gases. Following some 12-48 hours of continuous mechanical ventilation, there was marked reduction in TSLC, FRC, and a severe deterioration in arterial blood gases. One group of animals was then randomized to a state of the art mechanical pulmonary ventilation management group, with best efforts made to wean to room air. All but one animal so managed died of progressive respiratory failure.

In the remaining animals, high pressure mechanical pulmonary ventilation was discontinued and they were placed on continuous positive airway pressure (CPAP), and on an extracorporeal membrane lung perfusion system. Following some 24 hours of such treatment, all but one animal showed progressive improvement in arterial blood gases, and in lung function, and could be weaned from bypass.

We believe mechanical pulmonary ventilation at elevated peak airway pressures is a major cause of clinical morbidity and mortality. The novel application of the extracorporeal perfusion system with the membrane artificial lung is likely to find rapid clinical application.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01407-23 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Luminescence Spectroscopy in Biomedical Research

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

P. I.	R. F. Chen	Senior Investigator	LTD:NHLBI
	C. H. Scott	Biologist	LTD:NHLBI
	J. R. Knutson	Sr. Staff Fellow	LTD:NHLBI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Technical Development

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INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The excited-state protonation of serotonin and other 5-hydroxy indoles has been studied with a picosecond laser excitation system. It was confirmed that anomalous green emission of these compounds when excited at 280 nm arose from protonation of the excited molecule. Data supporting this conclusion included direct observation of the rise of the green emission after laser excitation, Stern-Volmer plots showing quenching of the normal ultraviolet fluorescence by protons, and chloride ion enhancement of protonation.

A systematic characterization of tryptophan dipeptides has been performed with the aim of defining factors which influence the fluorescence of the indole group, which is the major group governing protein fluorescence. It was found that several properties of peptides with N-terminal tryptophan were distinct from those of peptides where tryptophan was C-terminal. Quantum yields, lifetimes, ionization constants, copper binding constants, and absorption spectra supported the idea that there are several non-interconverting forms of these compounds in solution whose relative populations are influenced by indole-protonated amino group interaction.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01408-21 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Methods in Fluorescence Spectroscopy

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

P. I. R. F. Chen Senior Investigator LTD:NHLBI

COOPERATING UNITS *(if any)*

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NHLBI NIH, Bethesda, Maryland 20892

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1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A method for determining the association constant of tryptophan-containing peptides with the ions of copper, nickel, and zinc has been developed. The technique measures the quenching of tryptophan fluorescence upon binding. The quenching is complete when Cu^{++} is bound, and partial when Ni^{++} binds. Zn^{++} does not quench the fluorescence of these peptides, but its association constants can be obtained by competition with either copper or nickel. As part of these binding studies, it was necessary to measure the ionization constants for the amino group. This was done by following fluorescence as a function of pH, coupled with curve fitting.

A series of fluorescence standards being developed by the National Bureau of Standards was evaluated. These new standards consist of sintered mixtures of inorganic phosphors and polytetrafluoroethylene resin. The standards are solid samples which emit over the wavelength region 400 to 700 nm. Because the corrected spectra of these standards are known, it is in theory possible to calibrate detector systems with the standards. Use of the standards for this purpose, their stability, and their usefulness as standards to eliminate instrumental nonlinearities are being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01413-24 LTD

RIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Biophysical Methods for Study of Bio-Macromolecular Reactions

INCIPAL 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

OPERATING UNITS *(if any)*

NIA, Lab. Molecular Biology (J. Froehlich), Univ. of Pennsylvania (L. Thiebault), Biomedical Engineering & Instrumentation Branch (H. Casio, and Commonwealth Technology, Alexandria, Virginia.

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TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

ECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A new inertial drive flow system has been developed for doing quench flow reaction kinetics in the investigation of the mechanism of reaction of Sacroplasmic Reticulum, ATPase, and other enzyme systems which cannot be followed by optical means. In order to use this instrument for Sacroplasmic reticulum investigations, it is necessary to work in what is called the push-push mode. Thus one does a push of the syringes, waits 50 to 500 milliseconds, and pushes again. To achieve this a cam driven lever was developed to allow the hitch feed to advance automatically after each firing. The limit at present is 100 milliseconds, but the system works well at 2 mm i.d. tubing diameter. At 0.5 mm i.d. diameter a materials failure was experienced in the drive mechanism and this is presently being repaired and greatly strengthened. The new hitch feed using a tongue and groove lever action has proven very effective when moved from position to position by hand. Because it provides a positive step as well as drive it has greatly improved the operation of the flow system. A series of test reactions have been run on the thermo stopped-flow giving a dead time of 1.5 msec, a thermistor time resolution of 7.5 msec. and a sensitivity of 100 microdegrees C. Pressure, velocity, and thermal measurements underway over a series of viscosities have provided information needed for the support of the ball mixer theory, i.e. that mixing is mainly the result of changing linear momentum to angular momentum. The results to date demonstrate that for a viscosity of one centipoise a pressure differential of less than 4 psi is sufficient to produce better than 99% mixing at 3 M/sec with thermal fluctuations of only .2 millidegrees C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01414-14 LTD

RIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Biocalorimeters for Solution and Cell Biochemical Studies

INCIPAL 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
	T. Kolobow	Medical Officer	LTD:NHLBI

OPERATING UNITS *(if any)* H. Edelhock, Endocrinology Branch, NIAAD, N. Gershfeld, Lab. Physical Biology, NIAAD, C. Mudd, Biomedical Eng. & Instrum. Branch, DRS, M. Marini, Northwestern University, N. Davids, Penn State Univ., Commonwealth Scientific, Alexandria, VA, and Commonwealth Technology, Inc.,Alexandria, VA.

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TAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

ECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Black diamond coated polypropylene flow cells have been used to produce a stopped flow batch microcalorimeter. The instrument is under complete computer control for sample insertion, data collection, and analysis.

Several biochemical reactions are presently being explored using the instrument. These include clatherin assembly, tRNA-transferase reactions and ATCase reactions with various substrates. Protein concentrations of 10^{-6}M can be used with only 50 µl needed per reaction. Four experiments per hour can be run at this level of sensitivity.

The significance of the project lies in the possibility of detecting new reaction pathways as revealed by the thermal reactions and in the ability to make basic thermodynamic measurements on biological reactions where no other detection system is available. In addition, the direct measurement of the reaction enthalpy is a much more precise method than the determination using the Van't Hoff method, i.e. measuring of the reaction equilibrium at different temperatures. When the Van't Hoff method is used, the range of temperature, 4°C to 40°C, is too small for an accurate determination of the enthalpy. Thus to predict the change in reaction equilibrium in, for example, drug metabolism, a direct calorimetric measurement is much to be preferred.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01421-11 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Electrochemical and Physiological Methods for Cell Research

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

P. I.	R. E. Steele	Physical Science Investigator	LTD:NHLBI
Others	R.L. Bowman	Chief, LTD	LTD:NHLBI
	H. S. Kruth	Senior Investigator	EA:NHLBI
	J. W. Handler	Section Chief	KE:NHLBI

COOPERATING UNITS *(if any)*

Laboratory of Experimental Atherosclerosis, NHLBI
Laboratory of Kidney and Electrolyte Metabolism, NHLBI

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NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

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

The porous bottom culture dishes (PBCDs) and related devices developed in this laboratory for the sterile measurement of electrophysiological parameters of cell layers are used in nearly 100 laboratories. Two companies are making commercial versions of the PBCDs using cellulose ester membranes (Millipore Corp.) and polycarbonate membranes with and without cell culture treatment (Costar Corp.). Most are used for epithelia cell layers.

Recently, we have grown endothelial and smooth muscle cell layers on our PBCDs made with our transparent collagen membranes. These show promise of being good models of several types of blood vessels.

The major role played by Ca in the regulation of many cellular processes has prompted us to improve methods for measuring free Ca activity in cell systems. We have developed a 2 mm diameter electrode using a hydrophobic porous membrane and neutral carrier Ca exchanger which has a response time of 10 seconds and a resistance of 10 megohms. It is useful for measuring small quantities of solutions representative of cell interiors for many biochemical studies.

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NOTICE OF WITHHELD RESEARCH RESULT

[illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01451-03 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Electron Spin Resonance Development for Medical and Biological Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Neme, title, leboretory, and institute affiliation)*

P. I.	J. Zweier	Visiting Scientist	LTD:NHLBI
Others:	R.L. Bowman	Chief, LTD	LTD:NHLBI

COOPERATING UNITS *(if any)*

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of this project is to develop and adapt electron spin resonance spectroscopy to study the biochemistry, physiology, and pathology of cells and tissues in order to answer problems of medical and biological importance. To accomplish this goal we are working on approaches to increase the sensitivity and developing cavity design suitable for different problems ranging from microsamples, to cultured cells to whole tissues. By utilizing different frequency microwave sources it is possible to optimize resonator design for each type of biological sample. Initially we assembled an X-band, 9 GHz spectrometer. Various resonators were designed and tested at X-band including several loop gap resonators. In order to accommodate large aqueous samples such as living perfused organs work was started on development of L band and S band spectrometers. S and L band loop gap resonator were designed and built to enable the study of free radical generation in living perfused hearts.

Over the past year we have focused on 2 important cardiovascular applications (1) the mechanism of the adriamycin cardiomyopathy (2) the mechanism of ischemic and reperfusion heart damage. We demonstrated that Fe(III) binds to adriamycin and that these complexes cycle to reduce oxygen. This mechanism explains the formation of reduced oxygen and drug radicals which are thought to mediate adriamycin's therapeutic and toxic effects. Free radicals are thought to be generated in the ischemic and post-ischemic reperfused heart and to mediate cellular damage. We developed a direct ESR technique to measure free radical generation in the ischemic and post ischemic heart. This technique was used to quantitate and characterize free radical generation in the post-ischemic heart. In addition the molecular mechanisms of free radical generation were studied as well as approaches to prevent the free radical mediated cell damage which occurs.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01452-03 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 chereters 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.) (Neme, title, laboretory, and institute affiliation)*

P. I.	J. R. Knutson	Sr. Staff Fellow	LTD:NHLBI
Others	R. F. Chen	Sr. Investigator	LTD:NHLBI
	J. L. White	Engineer	BEIB:DRS

COOPERATING UNITS *(if any)*

P. Lambooy and E. Korn (NHLBI:LC); M. Han, L. Brand and C. Anfinsen (Johns Hopkins Univ.); C. N. Rafferty (WRAIR); J. M. Beechem (Illinois/Urbana); L. Davenport (CUNY); and P. Neyroz (U. Diparma).

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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 new time-resolved fluorescence spectrophotometer was developed to provide rapid collection and analysis of macromolecular size, decay lifetimes, and spectra. The instrument was exploited to study protein associations (eg; active oligomerization with P. Lambooy and E. Korn, NHLBI:LC; enzyme I dimerization with M. Han, L. Brand, S. Roseman, JHU; VSV spike protein/G aggregation with A. Walter, P. Blumenthal, NCI:). The system was modified to speed studies of conformational change in proteins (eg; thioredoxin sulfhydryl reduction/oxidation and folding, with M. Han, C. Anfinsen, L. Brand, JHU). Model tryptophyl-related systems (melatonin, serotonin, trp peptides, copper and nickel quenchings) were examined with Dr. Chen. The latter will provide us a better understanding of the origins of heterogeneous protein decay signals.

Fluorescence data analysis methods were developed and published that associate spectra with macromolecular size, lipid domain viscosity, and surface proton transfer. "Global analysis" methods were also developed to quantitate macromolecule axial ratios and to study proteins and lipids exhibiting distributed decay.

The instrument was modified to provide emission scanning under computer control, so we can revisit glutamine synthetase and other proteins (see 1985 report).

The laser-based fluorescence instrument was also combined with state-of-the-art microwave instrumentation (with Dr. Rafferty, WRAIR) to provide an entirely new measurement capability: dielectric resonant motions in macromolecules. Several other new laser fluorescence measuring instruments were designed and are being prototyped.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01454-02 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Disintegration of Intravascular Atherosclerotic Plaque

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

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Technical Development

SECTION

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.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.)*

No work was done on this project during 84-85 in favor of other projects. In view of the new project of hot tip catheter methods for disintegration of atherosclerotic plaque some additional experiments were done to compare microexplosives, hot tips and laser effects on atherosclerotic plaque.

The 83-84 work established that silver azide explosive charges that produce energy equivalent to that delivered by lasers could be detonated at the end of a intravascular catheter and positioned to direct the energy to the target plaque. In addition it was shown that the azide charge could be shaped to concentrate the energy to a specific localized point equivalent to that produced by laser beams. It was further noted that the use of exploding oxyhydrogen gas in the catheter to initiate the azide detonation produced a partial vacuum following the explostion that aspirated the debris of the explosion into the catheter. The additional work done this year perfected the production of "shape charges" by pressing the silver azide in a hard steel die against a .062" steel ball. The focused discharges were tested against artherosclerotic plaque in human post mortum aortic specimens and the lesions produced were examined histologically and compared to those produced by the hot tip catheter or laser shots by various types. The principle drawback of the method remains the inability to repeat the shots without reloading the azide on the catheter tip.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01455-02 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of Foam Countercurrent Chromatography (CCC)

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

P. I.	Y. Ito	Senior Investigator	LTD:NHLBI
	M. Bhatnagar	Summer Student	LTD:NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Technical Development

SECTION

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	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.)*

The present studies were focused on foam affinity of various samples including small molecules, macromolecules, and cells as follows:

1. Various pigments were separated with three different types of surfactants: SDS (sodium dodecyl sulfate, anionic), POE-23-LE (polyoxyethylene-23-lauryl ether, neutral) and CPC (cetyl pyridinium chloride, cationic). The results show that basic dyes were collected with SDS foam and acid dyes with CPC foam while none collected with POE-23-LE foam. Addition of NaCl (0.1M) to the surfactant solution shifted the solute peaks toward the liquid outlet in the SDS group and toward the foam outlet in the CPC group whereas addition of methanol (10%) showed no significant effect.
2. The above studies were extended to various non-colored samples including nucleotides and related compounds, peptides and proteins, catecholamines, plant hormones, etc. Among those abscisic acid and indole-3-acetic acid were collected with the CPC foam and bovine insulin with the SDS foam.
3. Various proteins were subjected to foam CCC with 0.2M Na_2HPO_4 solution containing no surfactant. Bovine serum albumin (BSA) was almost entirely collected with foam while most of other proteins were eluted through both foam and liquid lines with various ratios.
4. With an isotonic saline solution containing BSA as a foam-producing agent, blood cells were subjected to foam separation. Our preliminary studies indicated that platelets were collected with foam while erythrocytes and their membranes were eluted with the liquid stream.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HL 01456-02 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Development of a Pulsed Light Source, Single Photon

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
	W. Friauf	Chief, Section Electrical Engineering	BEIB:DRS
	C. Reimer	Chief, Section Immunochemistry	DHR:CDC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Technical Development

SECTION

Biophysical Instrumentation Section

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	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 ability to detect minute amounts of protein is of interest both in protein chemistry and immunology. A possible replacement for radioimmuno assay (RIA) is the tagging of the protein by a caged molecule of Europium. By taking advantage of the very long fluorescence lifetime of Europium (500 microsceconds) extreme sensitivities are possible. A pulsed nitrogen laser operating at 337 nm at 15 pps and power output of 200 microjoules has been used with a ½ inch end on PET photomultiplier tube to examine a simple Europium tagged system. Detection of 10^{-14} molar Europium was readily achieved in a 100 microliter sample. Efforts to extend this to 10^{-16} M are presently being pursued along with techniques for greatly improving reliability of both the phototube, counting electronics, and the laser light source. One of the possible uses of such a system is in the detection of very low levels of retrovirus.

8-65

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 01031-07 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

A New Angle Rotor Synchronous Coil Planet Centrifuge for Countercurrent Chromatography.

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

P. I.	Y. Ito	Senior Investigator	LTD/NHLBI

COOPERATING UNITS (If any)

None

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)

A new angle rotor coil planet centrifuge was constructed and examined in its performance in countercurrent chromatography. Analysis of acceleration produced by the synchronous planetary motion of the holder revealed three dimensional fluctuation of the centrifugal force vectors to produce efficient mixing of the two solvent phases in the coiled column. Studies on phase distribution diagrams obtained from various two-phase solvent systems indicated that the present system can be adapted to a wide variety of solvent systems by adjusting the centrifugal conditions. Excellent partition capability of the apparatus was successfully demonstrated in separations of dinitrophenyl (DNP) amino acid samples with chloroform/acetic acid/0.1N hydrochloric acid (2:2:1).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01459-01 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Mechanism of Unilateral Distribution of Two Solvent Phases in the Rotating Coil

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

P. I. Y. Ito Senior Investigator LTD:NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Technical Development

SECTION

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Proposed hypothesis is based on the interplay between two force components acting on the rotating coil. The tangential force component generates the Archimedean screw effect to move two solvent phases toward the head of the coil whereas the radial force component acts against the Archimedean screw force to establish hydrostatic distribution of the two phases throughout the coil. The unilateral hydrodynamic distribution of the two phases is governed by the degree of asymmetry in the radial force field on the coil in both simple rotation and synchronous planetary motion. The present hypothesis successfully explains all the observed hydrodynamic phenomena reported in the past.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 01460-01 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Versatile Coil Planet Centrifuge for Countercurrent Chromatography (CCC)

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

P. I.	J. Sandlin	Biologist	LTD:NHLBI
	Y. Ito	Senior Investigator	LTD:NHLBI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Technical Development

SECTION

INSTITUTE AND LOCATION

NHLBI NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.2	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.)*

A versatile coil planet centrifuge was developed for performing countercurrent chromatography (CCC). The apparatus can accommodate three different types of coiled column for comparative studies. The performance of each column was examined with a set of peptide samples and two-phase solvent systems. The multilayer coil coaxially mounted around the holder produced most efficient separations while it necessitated raising the column temperature for application of viscous sec.-butanol solvent system. The eccentric coiled column consisting of eight coil units arranged around the holder produced efficient separations under room temperature. The third column called the toroidal coil with a coiled helix configuration produced least efficient separations among three types of columns.

810

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HL 01461-01 LTD

PERIOD COVERED

October 1, 1985 to September 30, 1986

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

Hot Tip Catheter for Percutaneous Removal of Atherosclerotic Plaque

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
	D. Y. Lu	Staff Associate	CB:NHLBI
	M. Leon	Sr. Investigator	CB: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	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 are exploring methods of delivering a heated metal tip via a percutaneous catheter to disintegrate arterial atherosclerotic plaque that is obstructing coronary or other vessels. While a metal tip hot tip heated by absorbtion of laser light has been shown effective in disintegration of plaque other methods of heating the tip may be more convenient and much less expensive. In our work we are exploring the use of an electric arc to provide an intense concentrated heat inside of a metalic tip on the catheter. Problems involve the safe delivery of power to a very small area without jeopardizing the flexibility of the catheter. The arc offers concentrated heat that can be powered by relatively high voltage low current lead which retain the flexibility of the catheters. Other electric and chemical heating schemes are also being investigated.