DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27000-30 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

The Mechanism of Muscular Contraction

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

PI:	Richard J. Podolsky	Chief	LPB/NIAMS
	Robert Horowits	Senior Staff Fellow	LPB/NIAMS
	Sergey Malinchik	Visiting Associate	LPB/NIAMS
	Shegiro Chaen	Visiting Scientist	LPB/NIAMS
	Yutaka Sasao	Visiting Associate	LPB/NIAMS

COOPERATING UNITS *(if any)*

Dr. Alasdair Steven, LSBR, NIAMS; Dr. Ellis Kempner, Section on Macromolecular Biophysics, LPB; Dr. Neal Epstein, NHLBI; Dr. Brian Collett, Hamilton College, Clinton, NY.

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Muscle Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

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.)*

1. Titin is required for lateral stability of myosin filament lattice. The spacing of nearest-neighbor myosin filaments in electronmicrographs of cross sections through the A band was measured in irradiated muscle fibers. The irradiation dose was selected to degrade the titin molecules but leave the smaller molecules intact. The average filament spacing was the same in irradiated and control fibers, but the standard deviation of the control fiber spacing distribution was smaller. This result is evidence that the presence of intact titin promotes lateral order in the array of myosin filaments.

2. Crosslinking of the rod portion of the myosin thick filaments interferes with the crosslinking between the myosin head and actin. SDS polyacrylamide gel electrophoresis was carried out to examine the fraction of myosin heads that are crosslinked to actin with EDC. EDC treatment gave 10 times more crosslinking when it followed chymotryptic digestion as opposed to preceding the digestion. The results suggest that only a small fraction of the myosin heads are crosslinked to actin by EDC. This differs from crosslinking assays based on mechanical experiments; these show that essentially every myosin molecule is crosslinked to actin under these conditions (Iwamoto and Podolsky, 1992). Studies to resolve this discrepancy are under way.

3. Properties of mutant myosin in muscle fibers from patients with familial hypertrophic cardiomyopathy (FHC). The contractile force in calcium activated, skinned soleus muscle fibers was measured in control fibers and in mutants 403, 741, and 908. The force was normal in mutants 741 and 908, but half normal in 403. It is concluded that subnormal force is not an essential feature of FHC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27001-18 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Muscle Regulation and Contractility

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

PI: M. Schoenberg, Medical Officer, LPB, NIAMS
Others: A. Ehrlich, Biologist, LPB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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 standerd unreduced type. Do not exceed the space provided.)*

In FY92, our study of the myosin crossbridge interaction with actin in muscle fibers focused mainly on the weakly-binding crossbridge interaction. We examined the changes that occur when alkylating agents such as N-phenyl maleimide (NPM) and para-phenylene diamaleimide (pPDM) bind to muscle fibers and lock the crossbridges in a weakly-binding configuration. We observed that titin and myosin are the two major proteins that NPM and pPDM bind to. The binding of NPM to titin does not appear to have much effect, but binding accompanied by crosslinking of titin by pPDM causes an almost two-fold increase in fiber resting tension. The remaining effects of these agents presumably are due to binding to myosin heavy chain. To support this argument, we have initiated a study designed to measure the stoichiometry of the binding to myosin heavy chain and also to determine the specific binding sites on myosin heavy chain. (These will likely be the SH1 and SH2 reactive sulfhydryls.) Initial results on measuring the stoichiometry showed the efficacy of our techniques, but also suggest that the method we had used satisfactorily for making the sarcolemmas of single fibers permeable, is not adequate for the larger diameter bundles of fibers used in the stoichiometry determination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 27002-14 LSBR

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural Biology of Macromolecular Structure

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

PI: Alasdair C. Steven, Chief, Laboratory, Structure Biology Research

Frank Booy, Visiting Scientist – Mario Cerritelli, IRTA Fellow
Naiqian Cheng, Visiting Associate – James Conway, Visiting Fellow
Donald Heck, Special Volunteer – Eva Kocsis, Visiting Associate
Alexander Makhov, Special Volunteer – Manoj Misra, Visiting Associate
Benes Trus, Guest Worker

COOPERATING UNITS (if any)
Computer Systems Lab, Div. Computer Res. & Tech., NIH; Lab. of Skin Biology, NIAMS (Dr. P. Steinert); Dept. of Biology, Brookhaven Nat'l Lab., (Drs. J. Wall, J. Hainfeld); others as noted.

LAB/BRANCH
Laboratory of Structural Biology Research

SECTION
Section on Structural Biology

INSTITUTE AND LOCATION
NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
10	10	

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

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

This Laboratory aims to elucidate the regulatory mechanisms that govern the assembly of supramolecular complexes and the folding of macromolecules, as well as those that underlie the synthesis of organelles, cells, and tissues. In the past year, we have discovered that three-dimensional reconstruction from cryo-electron micrographs of antibody-labelled virus particles affords a method of mapping epitopes with remarkable, and unexpectedly high, precision. Previous forms of immuno-electron microscopy are indirect, detecting an electron-dense label (ferritin or colloidal gold) which may be 15 - 25nm from the epitope of interest: in contrast, our method directly visualizes the interaction between the Fab fragment and the underlying epitope, and may distinguish between epitopes as close as 1nm apart. It has been applied to three different monoclonal antibodies which bind to the outer surface of the capsid of herpes simplex virus. Two Mabs bind to distinct sites on the hexons, but not to pentons: the third binds to the protruding tips of pentons, but not to hexons. Taking into account our recent biochemical evidence that hexons and pentons are most likely composed of the same viral protein (VP5; 148 kda), these results indicate that there are major conformational differences between the same protein as deployed in pentons (at the capsid's vertices) and hexons (which form the rest of its shell). We have also devised techniques to quantitate the protein compositions of the cell envelopes of cornified epidermal keratinocytes, These structures are covalently cross-linked, rendering them inaccessible to conventional quantitation by gel electrophoresis. Thus we have found that in native epidermal envelopes, the primary constituent is loricrin (70-80%), whereas cultured cell envelopes contain little or no loricrin, but are mainly composed of involucrin, cystatin A and a cysteine-rich protein. We infer that only the early stages of native cornification are induced under these in vitro conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27003-33 LPB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Biophysical Studies of Metabolic Activity and Control

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

PI: Ellis S. Kempner, Ph.D., Physicist and Chief,
Section on Macromolecular Biophysics LPB NIAMS

COOPERATING UNITS *(if any)*

Drs. L. Smith (Baylor Univ.); P. Berger, S. Paul, and S. Kaufman (NIMH); A.C. Ross (Medical Coll. of PA.); M. Parniak (McGill); B. Fleischer (Vanderbilt); R. Salovey (U.S.C.).

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda Maryland 20892

TOTAL STAFF 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.)*

Target analysis of the inactivation of biological activity by ionizing radiation was applied to several major problems including cocaine receptors in brain, various enzymes, and energy transfer between synthetic polymers.

Objectives: 1) An understanding of the nature of active structures in vivo which are involved in biochemical processes, principally by means of the technique of inactivation by ionizing radiation. 2) Detailed knowledge of the molecular damage caused by ionizing radiation and of the mechanisms of the transfer of radiation energy throughout these structures.

Methods: 1) General biochemical techniques including enzyme reactions, fluorescence, and gel electrophoresis. 2)Ionizing radiation, usually high energy electrons from a linear accelerator, to expose samples under carefully controlled conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27004-23 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

The Dynamic Properties of Cell Membranes and Related Systems

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

PI: Norman L. Gershfeld, Ph.D. Research Chemist, LPB, NIAMS

Maria Tomoaia-Cotisel, Ph.D. Visiting Scientist

Kazuo Tajima, Ph.D. Visiting Scientist

COOPERATING UNITS *(if any)*

Dr. Courtney P. Mudd, ACES, BEIP, NCRR

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland

TOTAL STAFF 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.)*

As part of a continuing effort to test the validity of the critical bilayer theory of assembly as it pertains to membrane instability, we have been examining critical assembly temperatures T* for membrane lipids extracted from normal and diseased neurological tissues. According to the theory if T* is below the physiological temperature, the membranes are unstable and the cells will degenerate. We have previously found this pathogenic mechanism to be applicable in metachromatic leukodystrophy, a disease with a known lipid metabolic defect, and have therefore measured T* for brain tissue with Alzheimer's disease (AD) whose etiology is presently in dispute. For cerebral cortex lipid from three AD brains T* ranged from 19 to 28 degrees, independent of membrane protein composition. In contrast, control cortex lipids and cerebellar lipids from the AD brains yielded a normal value of 37 degrees. Thus, neurodegeneration in AD may be explicable by membrane destabilization due to a lipid defect. Lipid analysis indicates a significant deficit of plasmalogen PE in AD membranes compared to control membranes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27005-10 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Aggregation of Human Platelets Induced by Decompression

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

PI: Makio Murayama Research Chemist LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. K.K. Kumaroo, Biochemist, U.S. Naval Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The main thrust of the laboratory is focussed on the molecular mechanism of platelet aggregation, including both hemostasis and thrombosis. We are investigating the influence of pressure on the hydration layer surrounding the electrically charged amino acid residues involved in platelet aggregation. We have been investigating the molecular mechanism of platelet interactions in DIPA (Decompression-inducible platelet aggregation) for the past several years. We have found that oppositely charged amino acid residues, i.e., positively charged arginyl and negatively charged aspartyl residues interact with their respective receptor sites. We have observed vascular occlusion in the small blood vessels in the web of the frog's foot and in the ear of the mouse. During the current year we have confirmed that the volume of human PRP is increased by 0.3% or 3 ml per liter of packed thrombocyte volume when platelet aggregation is induced by decompression. A similar volume increase has been observed when platelet aggregation is induced by the agonists, epinephrine (adrenalin), ADP, collagen and PAF (platelet activating factor). We theorize that compactly organized water molecules, when randomized into bulk phase, acquire thermal motion which causes a temperature drop; and the human platelet aggregation is an entropy driven process similar to human red cell sickling. To confirm our hypothesis, we are continuing our experiments with a specially designed dilatometer including a thermistor, to measure the volume increase and temperature drop when platelet aggregation is induced by the agonists epinephrine, ADP, PAF. During the current year we have obtained additional confirmation of volume increase of human plasma due to decompression by using the discontinuous, density gradient zonal centrifugation method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27012-08 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Structural and Mechanical Properties of Muscle Fibers

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

PI: Leepo C. Yu, Research Physicist, LPB, NIAMS
Sengen Xu, Visiting Associate, LPB, NIAMS
Daniel Gilroy, Mathematician, LPB, NIAMS

COOPERATING UNITS *(if any)*

University of Ulm, FRG (Drs. B. Brenner and T. Kraft);
East Carolina University Medical School (Dr. J. Chalovich).

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Sections on Muscle Biophyics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

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

The goal of this project is to study molecular structures of actomyosin interaction involved in the process of force generation in skeletal muscle.

The myosin heads, namely the cross-bridges, have shown to generate force in the direction perpendicular to fiber axis, when form crosslinks between the myosin filament and the actin filament. The radial force is a function of the filament separation, i.e. the attached cross-bridges are elastic in the radial direction. Based on our data of cross-bridges in various strong binding states, we have previously proposed that the radial elastic behavior depends on the state of the cross-bridges. In FY92 we obtained data indicating that the radial elasticity of the cross-bridges bound weakly to actin is different from those bound strongly to actin, providing further support to the proposal. The significance of the finding is that the dependence of radial elasticity of the state provides a simple and direct way of differentiating structures of attached cross-bridges.

The effects of strongly bound myosin fragment S1 on regulation of muscle contraction have been investigated. At low Ca^{++} level, S1 increases the Ca^{++} sensitivity while at high Ca^{++} level, the maximal force level is suppressed. The time course of force re-development is affected by the strongly bound S1. The results suggest that strongly bound cross-bridges can activate muscle by modulating kinetics of force production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27013-01 LSBR

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

High-Resolution Structure and Function of Biological Macromolecules

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

PI: C. Craig Hyde, Expert, Laboratory Structural Biology Research
Steven L. Edwards, Staff Fellow
Joseph P. Mack, Special Volunteer

COOPERATING UNITS *(if any)*

Arthritis and Rheumatism Branch, NIAMS (Paul Plotz, Ralph Nichols, Nina Raben); Laboratory of Biochemical Pharmacology, NIDDK (Edith Miles, Nancy Nossal and Reed Wickner); and others as noted.

LAB/BRANCH

Laboratory of Structural Biology Research

SECTION

Hyde Working Group

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

This new group was established this year to study macromolecular assemblies and mutifunctional and multienzyme complexes using x-ray crystallography. Other areas of interest include cytoskeletal proteins and proteins related to HIV and retroviral infection. Most of our effort has been expended planning renovations of space, purchase of new instrumentation, equipment, and supplies and in the recruitment of new post-doctoral associates. The group will be well equipped and is awaiting delivery of a state-of-the-art automated x-ray data collection system based on Fuji image-plate technology, a VAX computer and computer workstations, and an Evans and Sutherland ESV graphics Workstation. Plans are underway to automate much of the routine wet-lab work, particularly in the areas of protein purification and crystallization. The group is adapting a robotic liquid handler to handle the more tedious aspects of experimental screening for crystallization conditions. Research efforts have primarily involved the preparation of large amounts of pure proteins and preliminary attempts to crystallize them. These include the following:

Human histidyl tRNA synthetase, substrate complexes of mutant tryptophan synthase, other tryptophan biosynthetic enzyme including anthranilate synthase and phosphoribosyl transferase from several bacterial sources, the DNA polymerase from T4 phage, and whole LA viral particles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27014-01 LPB

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Molecular Mechanisms of Myofibril Assembly and Function

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

P.I.: Robert Horowits, Senior Staff Fellow, LPB, NIAMS

COOPERATING UNITS *(if any)*

Dr. Bruce Paterson, NCI, Dr. Podolsky, NIAMS, Dr. Neal Epstein, NHLBI

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Muscle Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

As a first step to studying the funcion of extremely large myofibrillar proteins using molecular genetics approaches, genes encoding these proteins must be isolated and cloned. Due to several advantages in the use of Drosophila for genetic manipulations when compared with other organisms, we attempted to identify the gene encoding a homologue of vertebrate nebulin in Drosophila. Several attempts utilizing library screening and polymerase chain reaction technologies failed to identify a gene having sequence similarity to human nebulin. These results suggest that, if a functional analog of nebulin does exist in Drosophila, its nucleotide and amino acid sequence is likely to be quite different from that found in vertebrates.

In an attempt to isolate a clone carrying large portions of the coding sequence for mouse nebulin, a cDNA library was constructed using conditions designed to optimize the chances of cloning very large cDNAs. Several putative nebulin cDNA clones have been isolated from this library, and we are currently in the process of characterizing them.

Mutations in the cardiac myosin heavy chain gene are a major cause of familial hypertrophic (FHC), a serious genetic disease of the heart. By studying skeletal muscle fibers which utilize the same form of myosin as found in the heart, we have determined that missense mutations in the myosin heavy chain gene sometimes result in abnormal myofiber mechanics, but that isometric force output need not be affected to cause FHC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41020-25 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Pathogenesis of Autoimmunity in Mice with SLE-like Illness

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

PI:	Alfred D. Steinberg, M.D.	Chief, Cellular Immunology Section	ARB/NIAMS
Others:	*Henry Metzger, M.D.	P.I. effective 5/1/1992 for CIS	ARB/NIAMS
	Mark Gourley, M.D.	Special Volunteer	ARB/NIAMS
	Dorothy Scott, M.D.	Special Volunteer	ARB/NIAMS
	William Schwieterman, M.D.	Senior Staff Fellow	ARB/NIAMS
	Wendy Kisch	Biologist	ARB/NIAMS
	Geryl Wood	Biologist	ARB/NIAMS

COOPERATING UNITS (if any)

LAB/BRANCH: Arthritis and Rheumatism Branch

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS: 5.8	PROFESSIONAL: 4.0	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In lupus-prone strains of mice, bone marrow derived pluripotent stem cells and/or their immediate progeny demonstrate excessive proliferation. They have increased numbers of both endogenous and exogenous stem cell derived B cell production of IgM and anti-DNA relative to other strains. The results were confirmed and extended using sorter purified stem cells. In addition, such sorter purified stem cells transfer the NZB phenotype to normal mice. These studies suggest a fundamental abnormality in stem cell activity.

Retrovirus studies demonstrate excess Mpmv RNA in thymuses of lupus-prone mice. The abnormality is transferable with bone marrow stem cells. The basis for this abnormal expression has been studied. First, a genomic library from NZB mice was probed with Mpmv specific probes and a full-length clone isolated. The LTR was sequenced, demonstrating a mutation in a negative regulatory region. Gel retardation studies confirm that the mutation alters binding of a regulatory DNA binding protein.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41023-18 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Studies of Patients with Immune-mediated Diseases

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

PI:	Alfred D. Steinberg, M.D.	Chief, Cellular Immunology Section	ARB/NIAMS
Others:	*Henry Metzger, M.D.	P.I. effective 5/1/1992 for CIS	ARB/NIAMS
	Sahar Dawisha, M.D.	Clinical Associate	ARB/NIAMS
	Mark Gourley, M.D.	Special Volunteer	ARB/NIAMS
	Geryl Wood	Biologist	ARB/NIAMS
	Collaborative: Dr. Dennis Klinman	FDA	FDA

COOPERATING UNITS (if any)

LAB/BRANCH: Arthritis and Rheumatism Branch

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.6	OTHER: 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.)

Patients with systemic lupus were found to have an increase in mutations in their T cells as defined by their ability to grow in the presence of 6-thioguanine. The extent to which this results from a primary abnormality and the extent to which drugs contribute remains to be determined.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41025-21 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Studies of the cell surface receptor for IgE

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

PI:	Henry Metzger, M.D.	Chief, Chemical Immunology Section	ARB/NIAMS
Others:	Sue Mao, Ph.D.	Visiting Associate	ARB/NIAMS
	Gottfried Alber, DVM	Visiting Associate	ARB/NIAMS
	Carole Jelsema, Ph.D.	Senior Staff Fellow	ARB/NIAMS
	Ute Kent, Ph.D.	IRTA Fellow	ARB/NIAMS
	Victor Pribluda, Ph.D.	Visiting Associate	ARB/NIAMS
	Lisa Rider, M.D.	Clinical Associate	ARB/NIAMS
	John Rivera, Ph.D.	Senior Staff Fellow	ARB/NIAMS
	Patrizia Germano, M.D.	Visiting Fellow	ARB/NIAMS
	George Poy	Biologist	ARB/NIAMS

COOPERATING UNITS (if any)

Dr. Janet Oliver - University of New Mexico

LAB/BRANCH: Arthritis and Rheumatism Branch

SECTION: Chemical Immunology Section

INSTITUTE AND LOCATION: NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 10	PROFESSIONAL: 10	OTHER:

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.)

The high affinity receptor for IgE on mast cells and basophils (FcεRI) plays a central role in immediate hypersensitivity reactions. Aggregation of receptor-bound IgE by polyvalent antigen leads to aggregation of the receptors and cellular secretion of both preformed and newly synthesized mediators of inflammation. The molecular mechanisms by which aggregation of the receptors generate these cellular responses are the central focus of these studies. During the past year we continued to employ mutated receptors in order to establish which portions of the receptor participate in its principal functions. Our new results show: 1) No single cytoplasmic domain is required for receptors to localize to coated pits and thereafter to become internalized after aggregation. However, the lipid-anchored ectodomain of the alpha subunit fails to participate in this process. 2) Previous results with transfected P815 mastocytoma cell suggested a critical role for the gamma subunit. Because the effects on signaling by the endogenous Fc receptors for IgG closely paralled the effects on FcεRI, we postulated that of the three types of Fc receptors for IgG on these cells, only type III would be capable on initiating a variety of biochemical changes. This was confirmed by studying rat mucosal mast cell tumor line (RBL) transfected with individual Fc receptor isoforms. 3) One of the earliest consequences of receptor aggregation (but not of stimulation of the cells with phorbol esters) is phosphorylation of tyrosines on the beta and gamma subunits of the receptor. We have been successful in retaining some of this activity on broken cell preparations of RBL cells. This should greatly assist our efforts to elucidate the molecular events that result from aggregation of the receptor.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41040-20 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Study of Various Cytotoxic Drug Programs in Diffuse Lupus Nephritis

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

PI:	Alfred D. Steinberg, M.D.	Chief, Cellular Immunology Section	ARB/NIAMS
Others:	*Henry Metzger, M.D.	P.I. effective 5/1/1992 for CIS	ARB/NIAMS
	Mark Gourley, M.D.	Special Volunteer	ARB/NIAMS
	Dorothy Scott, M.D.	Special Volunteer	ARB/NIAMS
	Sahar Dawisha, M.D.	Clinical Associate	ARB/NIAMS
	William Schwieterman, M.D.	Senior Staff Fellow	ARB/NIAMS

COOPERATING UNITS (if any)

James E. Balow, Senior Investigator, NIDDK

Howard A. Austin, Attending Nephrologist, Clinical Center

Foreign: NONE

LAB/BRANCH: Arthritis and Rheumatism Branch

SECTION: Cellular Immunology Section

INSTITUTE AND LOCATION: NIAMS, Building 10, Room 9N218, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Patients were randomized to receive (i) monthly IV-cyclophosphamide, (ii) monthly IV methylprednisolone, or (iii) both. The study is now closed to new entrants. Rate of progression to renal failure, requirements for re-treatment, and drug toxicities are being assessed. Clear-cut differences have yet to emerge for the entire group of patients completing 3 years of study.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41048-13 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Bacterial cell wall-induced arthritis and hepatic granuloma formation in the rat

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

R.L. Wilder	Sr. Investigator	H. Sano	Visiting Associate
Y. Du	Visiting Fellow	B. Mittleman	Med. Staff Fellow
L. Crofford	Med. Staff Fellow		
E. Goldmuntz	Med. Staff Fellow		
J. Cash	Med. Staff Fellow	All ARB/NIAMS	
E. Remmers	Sr. Staff Fellow		
K. Kalogeras	Sr. Staff Fellow		
P. Mathern	Visiting Fellow		

COOPERATING UNITS *(if any)*

Clinical Neurosciences Branch, NIMH
Developmental Endocrinology Branch, NICHD
Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

Streptococcal cell wall arthritis in rats is an experimental model that closely resembles rheumatoid arthritis in humans. Investigations in progress are focused on defining the pathogenesis of this model disease by characterizing arthritis-susceptible and arthritis-resistant rat strains, specifically Lewis (LEW/N) and Fischer (F344/N) inbred rats. During the past year, new data were generated directly implicating platelet derived growth factor, fibroblast growth factor and corticotropin releasing hormone in arthritic process. Interestingly, we observed that LEW/N rats produce abundant CRH in the joints but fail to upregulate CRH production in the hypothalamus. F344 rats were just the opposite. In addition, we observed that inflammatory disease-prone, hypothalamic CRH deficient LEW rats, produced high levels of hypothalamic arginine vasopressin. Again, the F344 rats were just the opposite. These data support are view that the neuroendocrine, immune and inflammatory systems are closely intertwined and may play a role in the susceptibility to autoimmune diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066-10 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Characterization of Synovial Tissues from Patients with RA and Related Conditions

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

R. Wilder	Sr. Investigator	H. Sano	Visiting Associate
B. Mittleman	Med. Staff Fellow		
L. Crofford	Med. Staff Fellow		
E. Goldmuntz	Med. Staff Fellow		
P. Mathern	Visiting Fellow	All ARB/NIAMS	
E. Remmers	Sr. Staff Fellow		

COOPERATING UNITS *(if any)*

Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

The single feature most characteristic of rheumatoid arthritis is tumorlike proliferation of the synovium. During the past year, we have obtained further evidence implicating platelet-derived growth factor and fibroblast growth factor-1 in the disease process. In brief, we have demonstrated high level expression of tyrosine phosphorylated proteins in RA synovia that colocalized with PDGF and FGF-1. Upregulation in synovia from patients with osteoarthritis was minimal. In addition, further evidence was also obtained implicating upregulated expression of cyclooxygenase, uteroglobin and corticotropin releasing hormone in the pathobiology of RA synovitis.

The data showing secretion of corticotropin releasing hormone in rheumatoid joint fluids and tissues further support our view that complex interactions between the neuroendocrine system and the immune system are involved in regulating synovitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41074-05 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Studies on etiology and pathogenesis of idiopathic inflammatory myopathy in humans

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

PI: Paul H. Plotz, M.D., Chief Connective Tissue Diseases Section, ARB, NIAMS
F.W. Miller & Lori Love, Special Volunteers, ARB, NIAMS
Richard Leff, Senior Staff Fellow, ARB, NIAMS

Others: Nina Raben, Visiting Scientist, ARB, NIAMS
Ralph Nichols, Staff Fellow, ARB, NIAMS
Catherine Nicastri, Biologist, ARE, NIAMS Jay Amin, ARB, NIAMS
Ashish Jain, Biologist, ARB, NIAMS
Jeffrey Sherman, Senior Staff Fellow, ARB, NIAMS

COOPERATING UNITS *(if any)*

Craig Hyde, LSBR, NIAMS, Dennis Klinman, FDA

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.50	5.50	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.)*

Idiopathic inflammatory myopathy (polymyositis, dermatomyositis, and related disorders) is a family of inflammatory diseases in which disease-specific autoantibodies occur and for which there is considerable indirect evidence pointing to a viral etiology. We have over the past several years, seen and studied and collected serum, blood, and muscle specimens from well over 375 patients suspected of having myositis. We have collected epidemiologic information on many patients. We have cloned, sequenced, and expressed histidyl-tRNA synthetase HRS, the principal target autoantigen in idiopathic polymyositis and dermatomyositis and are analyzing its structure and promoter. We have extended the analysis of HLA antigens in the sets of myositis patients defined by autoantibodies using the sequence specific oligonucleotide hybridization/PCR method. We have analyzed promoter activity of the HRS gene and are currently investigating translational control of its synthesis. We have successfully obtained high level expression of HRS, purified it, and are attempting to crystallize it (with and without substrate) so as to obtain x-ray crystallographics structure. We have successfully cloned a mutant HRS with the first two exons removed in order to probe antigenic structure and tRNA binding.

We have made substantial progress in attempts to clone isoleucyl and leucyl tRNA syntheses.

Using recombinant HRS, we have developed a technique to identify individual B cell producing anti-HRS autoantibodies from patients with myositis. We are in the midst of an analysis of the V region used by individual cells making these autoantibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41076-05 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Therapeutic trials in idiopathic inflammatory myopathies

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

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB, NIAMS

Others: Richard Leff, M.D., Senior Staff Fellow, ARB, NIAMS
Sarah Cochran, M.D., Clinical Associate, ARB, NIAMS
David Fraser, M.D., Clinical Associate, ARB, NIAMS
Jeffrey Sherman, M.D., Senior Staff Fellow, ARB, NIAMS
Frederick Miller, M.D., Ph.D., Special Volunteer, ARB, NIAMS

COOPERATING UNITS *(if any)*

Jeanne Hicks, M.D., CC Rehabilitation

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Analysis of a double-blind, randomized, controlled therapeutic trial of apheresis in the treatment of polymyositis or dermatomyositis was completed. Neither plasmapheresis nor lymphapheresis led to a better outcome than a sham procedure.

In an attempt to find a better way to treat steroid-resistant myositis (other than inclusion body myositis), we have carried out a randomized crossover trial of intravenous methotrexate with leukovorin rescue and a combination of methotrexate and azathioprine. Accrual of patients is about two-thirds complete.

We have carried out two extensive retrospective analyses of the therapy of myositis. One, of well over 100 patients on whom thorough records of responses were available, was analyzed for the factors that influence the responses to prednisone, methotrexate, and azathioprine. The other was a close analysis of the responses to steroid and cytotoxic therapy in almost 30 patients with inclusion body myositis, including an analysis of the first ever controlled therapeutic trial in that condition which we carried out over the past several years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41080-04 ARB

PERIOD COVERED

October 1, 1992 to September 30, 1992

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

The use of MRS to detect inflammation in muscle of patients with myositis

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

PI:	Paul H. Plotz, M.D.	Chief, Connective Tissue Disease Section	ARB/NIAMS
	David Fraser, M.D.	Clinical Associate	ARB/NIAMS

Others:

To be discontinued this year.

COOPERATING UNITS (if any)

Dr. Joseph Frank Radiology Clinical Center

LAB/BRANCH Arthritis and Rheumatism Branch

SECTION Connective Tissue Diseases Section

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: .25	PROFESSIONAL: .25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

In an attempt to provide an improved assessment of muscle inflammation in patients with myositis, we have evaluated MRS (Magnetic Resonance Spectroscopy). In a group of myositis patients, muscle biopsy, laboratory tests, and the "STIR" image of the thighs were compared to an assessment of clinical disease activity. MRI was more sensitive than biopsy in detecting inflammation in these patients. The extent of muscle inflammatory changes could be assessed in patients since the entire muscle group is imaged. Magnetic resonance spectroscopy allows measurement of the metabolic state of muscle cells. Profound abnormalities of inorganic phosphate/creatine phosphate have been found in inclusion body myositis and in the tryptophan-induced eosinophilia-myalgia syndrome, and lesser abnormalities in polymyositis and dermatomyositis.

U S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41083-03 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Genetics of Familial Mediterranean Fever

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

P.I. Daniel L. Kastner, Senior Investigator, Cellular Immunology Section, ARB/NIAMS
Ivona Aksentijevich, IRTA Fellow, Cellular Immunology Section, ARB/NIAMS
Elon Pras, Visiting Associate, Cellular Immunology Section, ARB/NIAMS
Leandrea Prosen, Biologist, Cellular Immunology Section, ARB/NIAMS

COOPERATING UNITS *(if any)*

Heller Institute for Medical Research
Sheba Medical Center
Tel-Hashomer 52621 Israel

Dept Cytogenetics/Molecular Genetics
Adelaide Children's Hospital
North Adelaide, South Australia 5006

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIAMS - Building 6, Room 112, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Familial Mediterranean fever (FMF) is a rheumatic disease characterized by intermittent bouts of fever with abdominal pain, pleurisy, and/or arthritis; some patients also develop systemic amyloidosis, leading eventually to renal failure. FMF is inherited as a single autosomal recessive gene. The biochemical lesion of FMF, as well as the chromosomal location of the FMF gene, was unknown at the outset of this project. The purpose of this project has been to identify the FMF gene by the method of positional cloning ("reverse genetics").

During the first two years of this project, we prepared DNA samples from Israeli FMF families and tested for genetic linkage with a panel of polymorphic DNA markers. By this approach, we excluded more than one third of the human genome as the site for the FMF gene. We had also obtained preliminary evidence linking the FMF gene to an area on the long arm of chromosome 17 for some, but not all, families.

To clarify the situation, we continued to screen genetic markers. Within the past year we have obtained unequivocal evidence that the FMF gene resides on the short arm of chromosome 16. For a panel of 31 non-Ashkenazi Jewish families, we obtained a maximal pairwise lod score of 22.00, 2 centiMorgans from the PCR marker D16S283. We have identified 8 additional markers in this region of chromosome 16 that confirm linkage. Analyses of linkage heterogeneity and disease severity in subsets of families indicate that an earlier suggestion of linkage to chromosome 17 was a Type I error.

By multipoint linkage analysis and the study of recombinant families, we have identified D16S94 and D16S80 as flanking markers for the FMF gene. Moreover, we have demonstrated linkage disequilibrium between the FMF gene and chromosome 16 markers among Moroccan families. A specific haplotype defined by alleles at D16S291, D16S283, and D16S94, was present in 18/27 Moroccan carrier chromosomes, but 0/27 noncarrier chromosomes. This association was not observed in two other non-Ashkenazi Jewish populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41084-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Structural features of keratin and related intermediate filaments

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	John G. Compton, Ph.D.	Senior Staff Fellow	LSB/NIAMS
	Robert (Bruce) D.B. Fraser, Ph.D.	Fogarty International Center Scholar-in-Residence	
	Robert D. Goldman, Ph.D.	Prof. & Chrm., Dept of Cell, Mol. & Structural BioI..	Northwestern Univ. Med School
	Kathleen Green, Ph.D.	Assit. Prof., Dept of Patholgy	Cancer Ctr of Northwestern U.
	William W. Idler	Chemist	LSB/NIAMS
	Bernhard P. Korge, M.D.	Visiting Associate	LSB/NIAMS
	James W. Mack, Ph.D.	Assistant Prof., Dept. of Biochemistry	Howard U. Med. Sch.
	Dietmar Mischke, Ph.D.	Staff Scientist	Free Univ. of Berlin, Germany
	David A.D. Perry, Ph.D.	Prof., Dept. of Physics & Biophysics	Massey Univ., New Zealand

COOPERATING UNITS (if any)

Howard University, Washington, DC; Northwestern University, Chicago, IL; Free University of Berlin, Germany; and Massey University, New Zealand

LAB/BRANCH

Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.5	OTHER: .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 structure, function and expression of the keratin intermediate filaments of human and mouse skin, and the related intermediate filament proteins of other cell types, are being investigated. These studies are designed to understand the structural features that determine how the rod domains of the chains pack to form the filament core. Current models are being tested using electron microscopic methods as well as by analysis of the products generated on limited proteolytic digestion of intact filaments or subfilamentous forms of them. The glycine-rich end domains of especially the keratin 1/10 filaments of epidermal cells are unique in biology. We believe these organize into a glycine-loop configuration. Current studies are designed to determine how these are packed and how they might interact with other macromolecules co-expressed in epidermal tissues. The glycine loop sequences on the human keratin 10 chain are extraordinarily polymorphic in size and sequence. Using genomic clones to the human keratin chains 1 and 10, transgenic mice have been constructed to examine the expression characteristics of the genes as well as to probe in vivo the likely functions of the various portions of the chains, such as rod domain segments and glycine-rich end domains.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41085-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Expression, structure and function of filaggrin

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	Constantin C. Chipev, Ph.D.	Visiting Associate	LSB/NIAMS
	John G. Compton, Ph.D.	Senior Staff Fellow	LSB/NIAMS
	Song Qing Gan, M.D.	Visiting Associate	LSB/NIAMS
	Bernhard P. Korge, M.D.	Visiting Associate	LSB/NIAMS
	James W. Mack, Ph.D.	Assistant Prof. , Dept of Biochemistry	Howard U. Med. School
	Lyuben Marekov, Ph.D.	Visiting Scientist	LSB/NIAMS
	Nedialka Markova, Ph.D.	Visiting Associate	LSB/NIAMS
	Dietmar Mischke, Ph.D.	Staff Scientist	Free Univ of Berlin, Germany

COOPERATING UNITS (if any)

Howard University, Washington, DC; Free University, Berlin, Germany

LAB/BRANCH

Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Filaggrin is a major differentiation product of terminally differentiating mammalian epidermal cells, that is thought to be involved in the aggregation and specific alignment of keratin intermediate filaments during the final stages of differentiation. Thus filaggrin is an important example of an intermediate filament-associated protein. We have isolated both cDNA and genomic clones which show that filaggrin is initially expressed as a large polyprotein precursor, profilaggrin, which is subsequently proteolytically processed into individual functional filaggrin molecules. The structure of the gene for human profilaggrin has now been settled: it consists of 3 exons separated by 2 introns, the first of which is huge (about 10 kbp). The amino-terminal end of human and mouse profilaggrins possess two functional calcium binding domains of the EF-hand type. There is evidence for multiple alternate splicing of introns and exons, giving rise to a series of other calcium binding proteins of as yet unknown function. We have constructed genomic clones for the production of transgenic mice. We have begun a systemic analysis of regulatory sequences that control the expression of the profilaggrin gene system. We have determined that filaggrin aggregates keratin and vimentin intermediate filaments by ionic interactions.

U.S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41086-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Expression, structure and function of loricrin, a major cell envelope protein

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	Kozo Yoneda, M.D.	Visiting Fellow	LSB/NIAMS
	John G. Compton, Ph.D.	Senior Staff Fellow	LSB/NIAMS
	Alasdair C. Steven, Ph.D.	Chief, LSBR	LSBR/NIAMS
	O.Wesley McBride, M.D.	Senior Investigator	LB/DCBDC/NCI
	Kerstin Cehrs	Electron microscopist	DB/DCBDC/NCI
	James W. Mack, Ph.D.	Assistant Prof., Dept. of Biochemistry	Howard University
	Gennaro Melino, M.D., Ph.D.	Assoc. Prof., Dept. of Pathology	Univ. Tor Vergata, Italy

COOPERATING UNITS (if any)

LSBR/NIAMS; LB/DCBDC/NCI; DB/DCBDC/NCI; Howard University, Washington, DC; and University Tor Vergata, Rome

LAB/BRANCH Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 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 cell envelope constitutes a thick layer of highly insoluble protein on the inside of the plasma membrane of cornified epidermal cells and of other stratified squamous epithelial cells. Of several putative protein components, none has hitherto proven to be a major component because of major differences in amino acid composition, levels of mRNA or of expressed protein. We have recently identified a new protein, termed loricrin, that fulfills all of the requirements of a major constituent of the cell envelope. Loricrin clones were first identified in a mouse epidermis, and now in more detail, from human epidermis. Loricrins are glycine-rich proteins that contain the highly flexible glycine loop motif. They are crosslinked in cell envelopes by isodipeptide N epsilon-(gamma-glutamyl)lysine bonds. By use of Northern slot blotting techniques and mathematical modeling estimates, we have determined that loricrin constitutes up to 70-80% of the total mass of mature cell envelopes of mouse and human epidermis, while other components are much less than this. We postulate that the cell envelope is first assembled by addition of certain soluble proteins such as involucrin, cystatin A, etc, followed by massive subsequent deposition of loricrin.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41087-03 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Epidermal transglutaminases

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	In-Gyu Kim, M.D., Ph.D.	Visiting Associate	LSB/NIAMS
	Soo-You Kim, Ph.D.	Visiting Fellow	LSB/NIAMS
	O. Wesley McBride, M.D.	Senior Investigator	LB/DCBDC/NCI
	Soo-Il Chung, Ph.D.	Senior Investigator	LCDO/NIDR
	Sang Chul Park, M.D., Ph.D.	Prof. Dept. of Biochemistry	Seoul Natl U Med Ctr Korea
	Jeffery J. Gorman, Ph.D.	Principal Investigator	CSIRO, Melbourne, Australia

COOPERATING UNITS (if any)

LB/DCBDC/NCI; LCDO/NIDR; CSIRO, Melbourne, Australia; and SNU, Seoul, Korea

LAB/BRANCH Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Transglutaminases form isodipeptide crosslinks between acceptor amide groups of glutaminyl residues and donor epsilon-NH2 groups of lysines. In the epidermis, and other stratified squamous epithelial tissues, these enzymes are thought to be involved in the crosslinking of putative protein components to form the insoluble cell envelope. Using molecular biology approaches, we have found that there are 3 different transglutaminase activities in normal human and mouse epidermis. These are known as the K (TGase1), C (TGase2) and E (Tgase3) enzymes. We have isolated and characterized cDNA clones encoding full-length Tgase1 and TGase3 systems. The complete genomic structure (14.1kbp) of the TGase1 system has been completed. Work is in progress on the genomic structure of the TGase3 gene. Full-length and deletion constructs of the TGase 1 enzyme have been expressed in E. coli and in mammalian cells in an effort to understand the structural/functional domains of this enzyme. The aim of all of these studies is to determine the likely functions of these different activities in normal epidermis, and whether or how these may be involved in pathology.

U.S GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41088-02 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Genetic and Neuroendocrine Factors in the Autoimmune Diseases

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

R. Wilder	Sr. Investigator	
B. Mittleman	Med. Staff Fellow	
L. Crofford	Med. Staff Fellow	
P. Mathern	Visiting Fellow	All ARB/NIAMS
K. Kalogeras	Sr. Staff Fellow	
J. Cash	Med. Staff Fellow	

COOPERATING UNITS *(if any)*

Clinical Neurosciences Branch, NIMH
Developmental Endocrinology Branch, NICHD

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

Recent studies form animal models have rekindled our interest in the role of the hypothalamic-pituitary-adrenal (HPA) axis and other neuroendocrine factors in rheumatoid arthritis and other autoimmune diseases. During the past year, we have continued our evaluation of the HPA axis in rheumatoid arthritis and have initiated studies addressing the question of familial aggregation. The data, although still preliminary, are consisted with the hypothesis that some rheumatoid arthritis patients have inappropriately blunted HPA axis responses to inflammation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41089-01 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Genetic Studies of Hereditary Skin Disorders

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

P.I.: S Bale, Acting Chief, GSS, LSB

Others:

C Amos, Staff Fellow, GSS,LSB,NIAMS
K Kearns, Biologist, GSS,LSB,NIAMS
OW McBride, Section Chief, LB,DCBDC,NCI
P Steinert, Branch Chief, LSB,NIAMS
B Korge, Special Volunteer, LSB,NIAMS
N Markova, Visiting Assoc, LSB,NIAMS
S Doyle, Research Nurse,GSS,LSB,NIAMS
A Revell, Biologist, GSS,LSB,NIAMS
J DiGiovanna, Sr. Investigator, DB,DCBDC,NCI
J Compton, Sr. Staff Fellow, LSB,NIAMS
C Chipev, Visiting Assoc, LSB,NIAMS
A Goldstein, Staff Fellow, EEB,DCE,NCI

COOPERATING UNITS *(if any)*

DB,DCBDC,NCI
LB,DCBDC,NCI — Yale University, Depts of Dermatology & Genetics, New Haven CT
EEB,DCE,NCI — Erfurt University, Germany

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	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 studying the genetic basis of a variety of skin disorders, including six disorders of cornification (epidermolytic hyperkeratosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, Darier's disease, Hailey-Hailey disease, and ichthyosis vulgaris) and two disorders predisposing to skin cancer (basal cell nevus syndrome, familial malignant melanoma). Patients and their families are recruited for study. They travel to the NIH clinics (or rarely we travel to them) to enable our research group to perform detailed family and medical histories, skin examinations, skin biopsies, phlebotomy, and clinical photography. DNA is extracted from patient's blood and permanent cell lines established. Skin samples are used to confirm diagnoses and to investigate ultrastructural abnormalities specific to each disease. Clinical heterogeneity (different clinical appearances of the "same" disease) is investigated using the information collected. DNA-based polymorphisms (i.e. RFLPs, PCR) are used for linkage studies to determine the chromosomal location of the skin disease locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41090-01 LSB

PERIOD COVERED

November 4, 1991 to September 30, 1992

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

Development of New Statistical Methods for Genetic Analysis

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

P.I.: C Amos, Staff Fellow, GSS,LSB,NIAMS
Others:
SJ Bale, Acting Chief, GSS,LSB,NIAMS

COOPERATING UNITS (if any)

Howard University, Washington DC

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF 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.)

New statistical methods for analysis of the genetic component of complex familial diseases are needed in order to determine the relative contribution of environment and hereditary to a variety of traits, and to elucidate the specifics of these determinants. We have been focussing on the problem of genetic linkage studies in complex diorders (e.g. arthritis, other autoimmune diseases, mitochondrial diseases) when certain usual conditions of analysis are not met (e.g. known mendelian model, large sample size, non-normally-distributed quantitative trait measurements).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41091-01 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Consultation for Genetic Analyses

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

Co-P.I.: SJ Bale, Acting Chief, GSS,LSB,NIAMS
Co-P.I.: C Amos, Staff Fellow, GSS,LSB,NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF 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 provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases. We have worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41092-01 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Mapping of Genes and Genetic Polymorphisms in Rats

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

P.I.: R. L. Wilder, Senior Investigator, ARB, NIAMS

Others: Y. Du, Visiting Fellow, ARB, NIAMS
E. Goldmuntz, Medical Staff Fellow, ARB, NIAMS
E. Remmers, Senior Staff Fellow, ARB, NIAMS
P. Mathern, Visiting Fellow, ARB, NIAMS
L. Crofford, Medical Staff Fellow, ARB, NIAMS
J. Cash, Medical Staff Fellow, ARB, NIAMS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	3	0

CHECK APPROPRIATE BOX(ES)

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

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

Rats are an important experimental model for many human diseases, many of which have a genetic component. As followup of our previous work demonstrating impressive differences in the phenotypic responses of LEW and F344 rats to various proinflammatory and noninflammatory stimuli, we have begun to develop a genetic linkage map for rats, specifically applicable to these rat strains. We have now identified about 190 polymorphisms that differ in these rat strains and have mapped most of these to specific chromosomes or linkage groups. We have observed impressive conservation of synteny between rat, mouse and human chromosomes.

In addition to beginning a study of cosegregation of inflammatory arthritis with the various polymorphisms in F2 intercross progeny of LEW and F344 rats, we studied the segregation of the athymic nude trait in F2 intercross progeny of athymic nude LEW x euthymic F344 rats. We mapped the locus that controls the nude phenotype to rat chromosome 19m approximately 4 cM from the myosin heavy chain locus.

Moreover, through extensive genotyping of 11 additional rat strains, we demonstrated that the LER rat arose as an outcross between LEW and BUF rats, and not, as originally reported, as a spontaneous mutation in LEW rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41093-01 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Expression, structure and function of trichohyalin

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	Seung-Chul Lee, M.D.	Visiting Fellow	LSB/NIAMS
	In-Gyu Kim, Ph.D.	Visiting Associate	LSB/NIAMS
	Lyuben Marekov, Ph.D.	Visiting Scientist	LSB/NIAMS
	O.Wesley McBride, M.D.	Sr. Investigator	LB/DCBDC/NCI
	Edward O'Keefe, M.D., Ph.D.	Chairman, Dept. of Dermatology	Univ. of N.C. Med. School Chapel Hill, NC

COOPERATING UNITS (if any)

LB/DCBDC/NCI; University of North Carolina

LAB/BRANCH

Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Trichohyalin is a major differentiation product of the inner root sheath cells of the hair follicle, where it is initially deposited in large dense granules, termed trichohyalin granules. Subsequently, during terminal differentiation, it is dispersed and becomes associated with the keratin intermediate filaments of the inner root sheath cells. Thus it seems to serve largely as an intermediate filaments associated protein in this tissue. It is also expressed in the medulla of coarser hairs where it does not interact with filaments, but rather changes into a loose amorphous product. Trichohyalin is of interest because it s a major substrate for transglutaminases, and it also undergoes postsynthetic modifications of certain of its arginine residues to citrullines. Of especial interest is the recent observation that trichohyalin is also expressed in the epidermis, but its role in epidermal differentiation and role in pathology remain to be elucidated. We have obtained a cDNA clone by PCR analysis of genomic DNA and have used this as a probe to isolate the human trichohyalin gene. The human trichohaylin protein consists largely of a series of quasi-repeating peptides, and interestingly like profilaggrin, its amino terminus contains two functional calcium binding domains of the EF-hand type. Our interest in this system is to explore its role as a transglutaminase substrate, as a major calcium binding protein, as an important new intermediate filament associated protein, and its role, if any, pathology.

U.S GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41094-01 LSB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

The molecular bases of hereditary skin disorders

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

PI:	Peter M. Steinert, Ph.D.	Chief, Laboratory of Skin Biology	LSB/NIAMS
Others:	Sherri J. Bale, Ph.D.	Acting Chief, Genetics Studies Section	LSB/NIAMS
	Constantin C. Chipev, Ph.D.	Visiting Associate	LSB/NIAMS
	John G. Compton, Ph.D.	Senior Staff Fellow	LSB/NIAMS
	John D. DiGiovanna, M.D.	Expert	DB/DCBCD/NCI
	William W. Idler	Chemist	LSB/NIAMS
	Bernhard P. Korge, M.D.	Visiting Associate	LSB/NIAMS
	Nedialka Markova, Ph.D.	Visiting Associate	LSB/NIAMS
	O. Wesley McBride, M.D.	Senior Investigator	LB/DCBDC/NCI
	Gabrielle Robert, M.D.	Staff Physician, Dept. of Dermatology	Efurt Univ. Med. Sch. -Germany
	Jun-Mo Yang, M.D.	Special Volunteer	LSB/NIAMS

COOPERATING UNITS (if any)

DB and LB/DCBDC/NCI; University of Erfurt, Germany

LAB/BRANCH

Laboratory of Skin Biology

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

There are a number of heritable diseases of cornification of human epidermis, which affect the suprabasal cell layers of the epidermis. These include the various ichthyoses (such as ichthyosis vulgaris, lamella ichthyosis, bullous- and non-bullous congenital ichthyosiform erythroderma) and others including Darier's Disease, Hailey-Hailey Disease, etc. In all cases, these diseases follow simple or relatively simple genetic expression characteristics implying they involve simple mutations in one (or more) proteins expressed in epidermal cells committed to terminal differentiation. Although some morphological and biochemical analsyes have been performed on several of these, in no case has the underlying genetic defect been identified. This project involves a collaboration with the primary genetic studies work of Dr. Bale (see project number Z01 AR 41089-01). Following identification of suitable families, linkage analyses will be performed using PCR mapping of polymorphisms of candidate and other gene products of known location, to be followed by identification of the specific mutations in the genes that cause these occurrences of the disease. To date, we have characterized the genetic (a leucine-to-proline mutation in the keratin 1 chain) in one family of the autosomal dominant disorder BCIE.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41095-01 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Translational & rotational power terms in 6 degree-of-freedom joint modeling

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

PI:	F.L. Buczek	Staff Fellow, Biomechanics Laboratory	ARB/NIAMS
	S.J. Stanhope	Dir., Biomechanics Lab. (BML)	DRM/CC
Others:	T.M. Keppel	Programmer Specialist, BML	DRM/CC
	K.L. Siegel	Senior Staff Therapist, BML	DRM/CC

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH: Biomechanics Laboratory

SECTION: Biomechanics Section, DRM

INSTITUTE AND LOCATION: NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 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.)

Joint powers have been used to evaluate mechanical energy flows for individual joints. If added for all major joints of the body during gait, the sum represents a mechanical energy cost for movement, an important parameter for the estimation of mechanical efficiency. Traditionally, joint models have allowed only rotations at joints and did not account for translations such as sliding, compression, and distraction. Our immediate objective is to implement full six degree-of-freedom (DOF) joint models for the lower extrimity, i.e., allow for three rotational and three translational movements (DOFs). We believe this detailed model will provide more robust calculations for joint powers, improving reliability and accuracy in total mechanical power estimates. An engineering methodology called "inverse dynamics" provides us with resultant forces and moments at major anatomical joints during walking. These "joint reactions" perform mechanical work, and consequently have power associated with them. Traditional models assumed that translational powers canceled each other across a joint. Our new methods no longer force this relationship, but account for joint powers associated with three translational DOFs. Lower extremity data are calculated over full stride cycles for multiple walking trails. Ensemble averages and coefficients of variability are determined for each DOF.

Preliminary ankle results for inter-subject data (n=25) have shown that X-axis rotational powers (associated with ankle dorsi/plantarflexion) predominate, with peaks (normalized by body mass) approaching 4.5 W/kg. Peaks for the remaining five DOFs were below 10% of the predominant DOF. When all six DOFs were added to provide total power at the ankle complex, the combined effect of the smaller power terms was to attenuate the peak in dorsi/plantarflexion related powers. Similar relative magnitudes were found for intra-subject calculations (n=5). Joint powers at the knee and possibly the hip are likely to be studied to complete the major joints of the lower extremity. A separate but related project may be undertaken to better understand the source of the measured joint translations.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 AR 41096-01 ARB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Nonequilibrium in the translational equations of motion

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

PI:	S.J. Stanhope	Dir., Biomechanics Lab. (BML)	DRM/CC
Others:	M.R. Whetstone	Special Volunteer, BML	DRM/CC
	T.M. Kepple	Programmer Specialist, BML	DRM/CC
	F.L. Buczek	Staff Fellow	ARB/CC
	K.L. Siegel	Senior Staff Therapist, BML	DRM/CC

COOPERATING UNITS (if any)
Department of Rehabilitation Medicine, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH: Biomechanics Laboratory

SECTION: Biomechanics Section, DRM

INSTITUTE AND LOCATION: NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.6 | PROFESSIONAL: 0.4 | OTHER: 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.)

The basis of many biomechanical analyses has long been inverse dynamics through the use of rigid body mechanics. Equations of translational motion stipulate that the sum of all external forces acting on a system of rigid bodies must equal the sum of each body's mass/acceleration product. The extent to which this relationship is maintained depends upon the validity of the geometric and segment mass distribution model, the assumptions regarding segment stiffness, and the accuracy of the individual segment acceleration estimates. Our immediate objective is to evaluate the error associated with the implementation of translational equations of motion in a fifteen segment rigid body model of the human form during gait. Our goal is to improve the accuracy of our three-dimensional full-body model. We plan to use measured ground reaction forces (GRF) as criteria against which to compare the sum of segmental mass/acceleration products, calculated using various data collection and analysis techniques. The full-body model is comprised of hand, forearm, foot, shank, thigh, pelvis, trunk/abdomen, and head segments, all modeled as regular geometric shapes. Anthropometric measurements are made to individualize segments for each subject. A passive infra-red motion analysis system is used to collect segmental kinematics, with GRF obtained using two strain gauge force plates. Residual errors are calculated at each sampled instant as the total external force on the body minus the sum of all segmental mass/acceleration products, expressed as percentages of body weight. Seventeen trials were collected on 5 normal males.

The mean RMS residual was 6.1 +/- 4.4% for the vertical axis, 3.1 +/- 1.3% for the anterior/posterior axis, and 3.0 +/- 4.8% for the medial/lateral axis. Masked by the RMS and ensemble averaging process, residuals in the vertical direction reached magnitudes as large as 43% of body weight near foot strike. These data suggest that our geometric model produced acceptable levels of "nonequilibrium," except at foot strike. Additional study will be made of the effect of kinematic data processing on the minimization of residuals.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41097-01 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Changes in ankle function after forefoot arthroplasty in rheumatoid arthritis

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

PI:	F.L. Buczek	Staff Fellow	ARB/NIAMS
Others:	S.J. Stanhope	Dir., Biomechanics Lab. (BML)	DRM/CC
	K.L. Siegel	Senior Staff Therapist, BML	DRM/CC
	T.M. Kepple	Programmer Specialist, BML	DRM/CC
	L.H. Gerber	Chairman, DRM	DRM/CC
	J.E. Hicks	Dep. Chairman, DRM	DRM/CC
	P.G. O'Connell	Physiatrist	DRM/CC

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH Biomechanics Laboratory

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 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.)

Mechanical powers have been used to study energy flows in human movement. By indicating whether muscles at a joint are used to generate or absorb power, these parameters can identify the function of joint activity during gait. Our immediate objective is to use joint powers (rotational) and segmental powers (translational) to evaluate changes in ankle function before and after forefoot arthroplasty for rheumatoid arthritis. Our goal is to develop an objective measure of improved push-off capability following surgery. We plan to analyze patient gait pre-operatively, and at six and twelve months post-operatively. Energy flows into the foot will indicate non-propulsive lifting of that segment, while energy flows out of the foot will indicate the use of the foot to push the body forward. Lower extremity kinematics are collected using a passive infra-red motion analysis system. Ground reaction forces (GRF) are collected using two strain gauge force plates. Inverse dynamics based upon rigid body assumptions are used to provide resultant forces and moments at the ankle (early protocol) and at the knee and hip (amended protocol).

Early methodological studies found that joint power curves during stance in walking typically showed high frequency components. This was curious since the joint moment and angular velocity data used to calculate powers showed little or no high frequencies. We found that digital low-pass filtering of GRF, still preserving 99% of the signal content, attenuated the high frequency peaks in joint powers. As more patients are studied at the ankle, knee, and hip, joint powers may be used to show functional changes among the lower extremity joints. However, since only a few patients have pre- and post-operative data available for all three joints of the lower extremity, continued joint power analyses are not feasible at this time. We are shifting our emphasis away from joint powers and toward segmental powers at the proximal end of the foot to determine energy flow patterns.

U.S. GOVERNMENT PRINTING OFFICE 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41098-01 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

A study of falling in patients with muscle weakness due to myositis

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

PI:	F.L. Buczek	Staff Fellow	ARB/NIAMS
	R.L. Leff	Staff Fellow	ARB/NIAMS
Others:	L.H. Gerber	Chairman, DRM	DRM/CC
	J.E. Hicks	Dep. Chairman, DRM	DRM/CC

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH Biomechanics Laboratory

SECTION

INSTITUTE AND LOCATION NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 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.)

Falling can be a serious problem, especially for the elderly and those with musculoskeletal problems. During gait, falling may result from "slips," a condition that occurs during stance phase when friction is inadequate to meet slip-resistance needs, or "trips," when there is inadequate ground clearance during swing phase. To determine possible mechanisms for falls in patients with decreased strength and normal joints, we have begun studies in patients with muscle disease. Measurements of required slip-resistance and ground clearance were made in a patient with Inclusion Body Myositis (IBM) and a high incidence of falls, using two strain gauge force plates and a passive infra-red motion analysis system. Manual muscle testing was performed to measure relative muscle strength, and a questionnaire was used to gather facts pertinent to falls.

Marked differences were seen between the right and left legs. Ground clearances for the right toe and heel did not seem to put this patient at risk for tripping. Required slip-resistance at heel-strike was abnormally high at 0.66 +/- 0.03 (n=6), consistently above normal values (<0.25) and architectural guidelines (0.50). This put the patient at risk for slipping on the right foot. Although questionnaire responses did not identify slipping as a precursor to falls, knowledge of the increased risk was considered useful for patient safety. For the left leg, abduction was pronounced during swing, which terminated with a prolonged medial sweep of the foot over the final 40% of swing. Ground clearance of the left heel was below 1 cm during this sweep, increasing the likelihood of tripping. In this situation, the patient could fall because weak hip and knee musculature may not be strong enough to stabilize the body. Indeed, the patient did identify tripping on the left side as a precursor to falls. We conclude that detailed biomechanical analyses identify gait characteristics that put patients at risk for falling, and may facilitate directed clinical interventions to prevent falls. We are expanding the scope of this study to other IBM patients.

U.S. GOVERNMENT PRINTING OFFICE: 1991 0-864-939

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41099-01 ARB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

The genetic basis for metabolic myopathies.

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

PI:	Paul H. Plotz, M.D.	Chief, Connective Tissue Diseases Section	ARB/NIAMS
Others:	Nina Raben	Visiting Scientist	ARB/NIAMS
	Jeffrey Sherman	Senior Staff Fellow	ARB/NIAMS
	Cornelius Boerkoel,III		ARB/NIAMS
	Frederick Miller	Special Volunteer	ARB/NIAMS
	Mary Beth Anania	Summer Irta	ARB/NIAMS

COOPERATING UNITS (if any)

LAB/BRANCH: Arthritis and Rheumatism Branch

SECTION: Connective Tissue Diseases Section

INSTITUTE AND LOCATION: NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

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

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

A family with two patients with muscle weakness was referred for studies of familial myositis. The correct diagnosis was discovered to be phophofructokinase deficiency. A single base mutation in an intron leading to a splicing defect and the excision of an exon was discovered in the DNA of the patients and has been traced in the family. The influence of the deletion on the structure and function of the enzyme is being studied.

A family with two patients with muscle weakness and respiratory insufficiency was referred for studies of familial myositis. The correct diagnosis was discovered to be acid maltase deficiency. The genetic basis of the defect has been studied. The patient seen at NIH and her father were shown to have a deletion encompassing an entire exon and parts of both surrounding introns. An identical deletion was found in two patients (one Canadian and one Dutch) with the fatal infantile form of the disease. Hence, the identity of the mutations on the other alleles in these patients should reveal the reason that the disease is sometimes expressed in infancy and sometimes in midlife.

Two patients with mitochondrial myopathies and one with myoadenylate deaminase deficiency were uncovered in the past year and will be studied in further detail.

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