DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00012-30 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

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

B.O. Fowler, Research Chemist, BRB, NIDR

COOPERATING UNITS (if any)

ADAHF, NIST, Gaithersburg, MD; NIST, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.00	1.00	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 objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy as well as chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (hydroxide, fluoride, chloride, carbonate, water and acid phosphate). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish compositional and structural details of the apatites in question, which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00074-20 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Bone and Tooth Matrix Biochemistry and Metabolism

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

L.W. Fisher, Research Chemist, BRB, NIDR
D.I. Deutsch, Visiting Scientist, BRB, NIDR
H. Green, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Sackler School of Medicine, Tel Aviv, Israel; University of New Mexico, Albuquerque, NM; Universita "La Sapieza", Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.29	1.08	.21

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 extracellular matrices of bones and teeth that give these unique tissues their shape and strength are synthesized and maintained throughout life by highly specialized cells. The cell's ability to assemble, mineralize and maintain the matrices is generally thought to be modulated through a variety of noncollagenous proteins (NCP). The goal of this project is to study the structure and function of several of these noncollagenous proteins. We have completed the cloning and sequencing of human and certain useful animal model noncollagenous proteins including bone sialoprotein (BSP), osteopontin (OPN), decorin (DCN), and biglycan (BGN). The human biglycan gene has been sequenced and localized to the end of the long arm of the X chromosome. The human decorin gene has recently been cloned, mapped and partially sequenced. The intron-exon structure of decorin and biglycan are identical, thus supporting our original hypothesis that the two are a direct consequence of gene duplication and divergent evolution. The DCN gene is located at 12q21.3. We have continued to be successful producing monospecific antisera to all of the major NCP in human and several animal model systems. In collaboration with Dr. Paolo Bianco, we have used both the antisera and cDNA probes to begin studies on the developmental pattern of expression of these proteins in human and rodent models. Production of the mg quantities of nondenatured protein necessary for structure-function studies has been successful for rat BSP. We have preliminary evidence for a cryptic cell attachment domain other than the ArgGlyAsp (RGD) tripeptide typical of the integrin-binding class of receptors, and have recently proposed that this second site may be homologous to the second attachment site of fibrinogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00088-19 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Chemical, Structural and Morphological Studies on Calcium Phosphates

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

E.D. Eanes, Chief, MCSS, BRB, NIDR
A.W. Hailer, Chemist, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	.5	1.0

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to study the physical, chemical, and ultrastructural properties of calcium phosphate salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable calcium phosphate salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued involves artificial lipid vesicles (liposomes) as in vitro models to investigate physico-chemical aspects of matrix vesicle (MV)-mediated calcification in vivo. Present findings show that enzymatic breakdown of extraliposomal proteoglycans (PG) does not necessarily destroy the retarding effect PGs have on calcium phosphate precipitation in liposomal suspensions. Core protein as well as glycosaminoglycan components, but not hyaluronic acid, are equally as effective as intact PG in delaying precipitate development. On the other hand, the breakdown products of chondroitinase digestion of the glycosaminoglycan components and of proteinase digestion of the core protein do not have a strong inhibitory effect on the precipitation. These data suggest that the core protein and glycosaminoglycan chains may have to be destroyed before PG loses its inhibitory influence on biomineral development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00157-17 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

NMR Studies of the Structure and Dynamics of Staphylococcal Nuclease

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

D.A. Torchia, Chief, PBS, BRB, NIDR
H.B.R. Cole, Senior Staff Fellow, BRB, NIDR
T. Yamazaki, Staff Fellow, BRB, NIDR
L.K. Nicholson, IRTA Fellow, BRB, NIDR

COOPERATING UNITS (if any)

University of Maryland; LCP, NIDDK; CUNY

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.9	2.9	0

CHECK APPROPRIATE BOX(ES)

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

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

NMR studies of the structure and dynamics of staphylococcal nuclease (SNase) were carried in order to elucidate the catalytic function of this model enzyme. We studied (1) the molecular dynamics of wild type SNase and (2) the three dimensional (3D) structure of an SNase mutant.

(1) SNase dynamics. Novel pulse sequences were developed that permitted the accurate measurement of relaxation parameters of heteronuclei (^{15}N and ^{13}C) in proteins. The relaxation parameters of the 22 leucine methyl carbons in SNase were measured. An analysis of the relaxation data showed significant internal motion of nearly one half of the leucine sidechains in SNase liganded to Ca^{2+} and pdTp. In the absence of the ligands, internal motions increased substantially. These results were interesting because all leucine sidechains are buried, suggesting a dynamic environment in portions of the protein interior.

(2) Mutant structure. The SNase mutant, G50F/V51N ΔSNase, is considerably more stable than the wild type enzyme, but is 100-500 fold less active. We have shown that the conformations of the mutant and wild type proteins are essentially identical except for a few residues near the active site. The difference in stability is due to the replacement of a disordered loop of the wild type enzyme with a well structured tight turn in the mutant. Several thousand NOE's have been measured for the mutant protein, and work is currently underway to determine its full 3D structure. A comparison of the wild type and mutant structures will be made in order to elucidate the reason for low level of catalytic activity of the mutant protein.

The significance of the project lies in the information about (1) the range and time scales of protein structural fluctuations provided by dynamics studies and (2) the relationship between structure and function that comes from comparing the structures of wild type and mutant proteins. In addition, many novel NMR experiments have been developed using SNase as a model system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00379-09 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Structure and Bone Matrix Gene Expression

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

M.F. Young, Research Biologist, BRB, NIDR
J.M. Kerr, IRTA, BRB, NIDR
K. Ibaraki, Visiting Associate, BRB, NIDR
A.M. Heegaard, Visiting Fellow, BRB, NIDR
D. Jondle, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.2	4.0	.2

CHECK APPROPRIATE BOX(ES)

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

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

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective is to study the structure and function of these macromolecules and to understand the regulation of their expression. The structures of bone and tooth matrix proteins have been studied by constructing recombinant cDNA libraries from bone or ameloblast cell mRNA. cDNAs encoding several bone and tooth matrix proteins were isolated using expression vectors and mono-specific antisera directed against individual bone and ameloblast proteins were produced. The clones and antibodies were used to determine the primary structure and mode of expression of the genes in cultured cells and intact tissue. The corresponding genomic DNAs have also been isolated and used to determine the intron-exon organization of these genes and the elements that potentially regulate their expression during development. Studies are underway using transgenic mice to identify the function of the matrix proteins and the elements that regulate their expression during development and aging in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00380-09 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Metabolism of Bone Cells

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

P. Gehron Robey, Biologist, BRB, NIDR
N.S. Fedarko, Staff Fellow, BRB, NIDR
T.E. Hefferan, Biol. Lab Technician, BRB, NIDR
U.K. Vetter, Visiting Associate, BRB, NIDR
W.J. Grzesik, Visiting Associate, BRB, NIDR
A.J. Friedenstein, Visiting Scientist, BRB, NIDR
G. van der Pluijm, Visiting Fellow, BRB, NIDR
M. Atkinson, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Department of Biopathology, Universita "La Sapienza", Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.0	1.50

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 primary goals are to determine the composition and functional features of the Bone Matrix Unit, the supramolecular complex that calcifies, and how cells in the osteoblastic lineage regulate this process. Towards these aims, bone cell cultures were established for biochemical analysis, and for studies at the genomic level in collaboration with Drs. Marian F. Young and Larry W. Fisher. Intrinsic factors were found to influence the biosynthetic output of these cells such as the animal species, position within the cell cycle, and importantly, the developmental age of the donor. Each protein has a different pattern of expression with increasing age. Extrinsic factors (nutrient and factor levels, time in culture, cell density, attachment to different substrata) also affected cell metabolism. Bone cells and their products were compared to those from other tissues, and from patients with different diseases. By histochemistry, bone sialoprotein was found only in osteoblasts, certain hypertrophic chondrocytes, osteoclasts and trophoblasts of the placenta. Osteonectin, a major Ca^{++}-binding bone protein, was found in renal epithelium, suggesting a role in ion transport. In cells from osteogenesis imperfecta patients, there were changes in post-translational modifications of not only collagen, but also proteoglycans, and in the absolute and relative amounts of the various components. These changes may cause the altered crystal structure found in OI bone, which ultimately results in fragility. In Turner's syndrome (karyotype 45, X0 and characterized by short stature and early onset osteoporosis), biglycan (whose gene is on the X chromosome) was found to be reduced by 50%. In various forms of osteosarcoma in rats and humans, both qualitative and quantitative differences in bone matrix proteins were detected, which may contribute to derangements in growth and structure observed in these tumors. Continued characterization of the interrelationship between bone cells and their extracellular environment will provide a clearer understanding of bone metabolism in health and in disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00507-03 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

NMR Structural Studies of TGF-β1 and HIV-1 Protease

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

D.A. Torchia, Chief, PBS, BRB, NIDR
S. Archer, Guest Researcher, BRB, NIDR
L.K. Nicholson, IRTA Fellow, BRB, NIDR

COOPERATING UNITS (if any)

LCP, NCI; Celtrix Laboratories; R&D Systems

LAB/BRANCH

Bone Research Branch

SECTION

Protein Biophysics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.65	.65	0

CHECK APPROPRIATE BOX(ES)

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

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

Two HIV related proteins, TGF-β1 and the HIV-1 protease, were studied by NMR spectroscopy. Studies were carried out to (1) elucidate the solution structure of TGF-β1 and (2) determine the feasibility of an NMR structural determination of the HIV-1 protease in solution.

(1) TGF-β1 structure. TGF-β1 up- and down-regulates proliferation of HIV infected cells. In order to understand the activity of this multifunctional cytokine, we have undertaken to determine the structure of TGF-β1 in solution. We have sequentially assigned essentially all proton signals of the protein, and have measured several hundred NOEs. Using the NOE data we have determined the secondary structure of the protein in solution. The structure determined in solution is in agreement with an independently determined X-ray structure of TGF-β2, except in a domain that distinguishes the activities of the β1 and β2 isoforms.

(2) HIV-1 protease. Several months ago we obtained 2-dimensional NMR spectra of the HIV-1 protease complexed with an inhibitor. These spectra showed that the complex was stable for several weeks in solution, and had the hydrodynamic properties of a non-aggregating homodimer. Recently, our colleagues at DuPont-Merck have worked out conditions for increasing the solubility of the protease to a level of 1mM. This development opens the way to obtain 3- and 4-dimensional NMR spectra, from which detailed structural information can be obtained.

The significance of this project arises from the unique, detailed structural information that is being obtained about HIV related proteins in solution. This information will form the basis for a rational drug design based upon the understanding of the function of these proteins in terms of interactions at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00510-03 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Regulation of Cartilage Matrix Metabolism

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

T.I. Morales, Expert, BRB, NIDR
K.D. Smith, Biologist, BRB, NIDR
P.E. Long, Biologist, BRB, NIDR
M. Montgomery, Biological Aide, BRB, NIDR

COOPERATING UNITS (if any)

Laboratory of Chemoprevention, NCI, NIH
Orthopedic Hospital, Univ. of Southern CA

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	.8

CHECK APPROPRIATE BOX(ES)

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

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

The long term objective of this program is to elucidate the intrinsic regulatory mechanisms that control the structure and function of the resilient layer of articular cartilage that covers and protects the ends of bone. We showed that TGF-β has the ability to prevent the spontaneous proteoglycan loss that occurs in basal cartilage organ cultures by increasing biosynthetic rates and decreasing degradation. We have now explored the interactions between the response elements for TGF-β and vitamin A (retinoic acid) in articular cartilage organ cultures. At physiological concentrations, vitamin A acts as a potent degradative signal for articular cartilage. However, this signal is dampened by the simultaneous addition of TGF-β to the cultures. Furthermore, if the tissue is first treated with vitamin A alone so that 80% of the matrix proteoglycans are released and then treatment is discontinued, the intrinsic ability of the tissue for repair is low, but is powerfully enhanced by addition of TGF-β. Thus, in the presence of TGF-β, the rates of proteoglycan synthesis rise more than 20 fold and there is a slow re-accumulation of matrix proteoglycans. The ability of other effectors to interact with TGF-β and amplify the repair response is presently being explored. The present findings extend our knowledge of the regulatory mechanisms that maintain cartilage matrix structure and function. Further, the repair model in vitro that we have set up and will continue to study should yield important insights concerning the regulation of repair processes in cartilage, an area that is critical for the understanding and eventual management of joint diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00546-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Hyaluronic Acid Synthesis by Ovarian Cumulus and Granulosa Cells

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

V.C. Hascall, Chief, PCS, BRB, NIDR
M. Yanagishita, Visiting Scientist, BRB, NIDR
A. Camaioni, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

2nd University of Rome, Rome, Italy

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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 purpose of this project is to study the biological and biochemical processes involved in synthesizing and organizing the hyaluronic acid-rich extracellular matrix which surrounds most mammalian oocytes at the time of ovulation. This matrix is produced primarily by the ~1,000 cumulus cells which are initially closely adherent to the oocyte. In response to a gonadotropin surge these cells initiate hyaluronic acid synthesis and deposit it in the extracellular matrix. This process enlarges the cumulus cell-oocyte complex, and the fully expanded complex is ovulated ~10 hours later. We are studying this process with mouse cumulus cell-oocyte complexes in vitro. Three factors have been identified that are necessary for expansion: (1) a soluble factor produced by the oocyte which induces hyaluronic acid synthesis, (2) FSH (or cAMP) which amplifies the synthetic response, and (3) a factor in serum required to retain the newly synthesized hyaluronic acid in the matrix. Topics of present interest include: (1) identifying the factor produced by the oocyte that is required to induce hyaluronic acid synthesis by the cumulus cells, (2) determining how the cumulus cells respond to this factor via second messenger systems, (3) identifying the ~40 kDa protein which is synthesized by the cells during the expansion process, (4) determining the role of this protein, which binds to the newly synthesized hyaluronic acid, in matrix formation, and (5) determining the role of the serum factor required to retain the newly synthesized hyaluronic acid in the matrix.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00547-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Novel Methods for Analyzing Glycosaminoglycan Substructure

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

V.C. Hascall, Chief, PCS, BRB
R.J. Midura, Staff Fellow, PCS, BRB
D. Hiscock, Visiting Fellow, PCS, BRB
A. Calabro, Staff Fellow, PCS, BRB
M. Yanagishita, Visiting Scientist, PCS, BRB
C.K. Ng, Visiting Fellow, PCS, BRB

COOPERATING UNITS (if any)

University of Iowa, Iowa City, IA
University of Lund, Lund, Sweden

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to develop new methods for analyzing the substructure of glycosaminoglycans with high resolution and high sensitivity. High performance anion exchangers with exceptional stability and resolving power have been developed by Dionex (CarboPac PA1) for separation of sugars and oligosaccharides. When used with appropriate monitors (UV or pulsed amperometric detectors), detection limits in the ng range can be achieved. Glycosaminoglycans can be selectively degraded with enzymes: chondroitinases digests chondroitin sulfates and hyaluronic acid, heparinases digest heparin and heparan sulfate, and keratanases digest keratan sulfate. The major product is often a mixture of monosaccharides and disaccharides, with various positions carrying a sulfate residue. These disaccharides are unstable to the alkali conditions used to elute the anion exchange column. We have developed a borohydride reduction method to reduce the disaccharides which both stabilizes them to alkali and eliminates the α and β anomers. The method has been optimized with disaccharides generated from chondroitin sulfate and hyaluronic acid by the bacterial eliminases, chondroitinase ABC and chondroitinase AC. Topics of present interest include: (1) the use of mercuric acetate to remove the unsaturated uronic acid from the eliminase digestion reaction which alters all the digestion products except the non-reducing ends and allows the latter to be identified, (2) adaptation of the method to resolve and purify oligosaccharides with different lengths from partial digests of hyaluronic acid with lyase and eliminase enzymes specific for this glycosaminoglycan, (3) resolution and identification of the disaccharides generated from heparan sulfate with various enzymes specific for this glycosaminoglycan and by nitrous acid treatments, and (4) the application of the procedure to analyze the contents and compositions of the chondroitin sulfate and hyaluronic acid in synovial fluid samples from patients with osteoarthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00548-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Study of Proteoglycan Biosynthesis in the Golgi Apparatus Using Brefeldin A

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

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Lars Uhlin-Hansen, Visiting Fellow, BRB, NIDR
Anthony Calabro, Staff Fellow, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR

COOPERATING UNITS (If any)

University of Tromso, Norway

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	0

CHECK APPROPRIATE BOX(ES)

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

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

The glycosaminoglycan components of proteoglycans are biosynthesized and modified in the golgi apparatus by highly organized carbohydrate transfer enzymes and sulfotransferases. The purpose of this project is to investigate the functional organization and subcellular localization of these enzyme complexes. Brefeldin A is a chemical which specifically blocks anterograde protein transport within the golgi apparatus. It was used to disrupt the normal biosynthetic processes for adding glycosaminoglycan chains onto proteoglycans. When ovarian granulosa cells were treated with Brefeldin A, dermatan sulfate proteoglycan synthesis was abolished whereas heparan sulfate proteoglycan synthesis was only partially inhibited, suggesting that dermatan sulfate and heparan sulfate assembly on proteoglycans occurs in different subcellular compartments. Topics of present interest include: (1) the use of xylosides as glycosaminoglycan initiators to determine if the galactosyl transferases which synthesize the linkage region can produce $(gal)_2$ xyloside in the presence of a brefeldin A block; (2) determine which core proteins are substituted with heparan sulfate in the presence of a brefeldin A block; and (3) determine the effects of this compound on hyaluronic acid synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00549-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Metabolism of Cell Surface Heparan Sulfate Proteoglycans

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

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Duncan Hiscock, Visiting Fellow, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR

COOPERATING UNITS (if any)

Department of Microbiology and Immunology, University of Michigan; Division of Cytokine Biology, CBER, FDA

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 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.)

Cell surface heparan sulfate proteoglycans are widely distributed throughout animal tissues, and are involved in critical cell functions such as cell-cell and cell-extracellular matrix interactions. Their interaction with a variety of molecules including growth factors, viruses, and extracellular matrix proteins, have important biological functions. The purpose of this project is to study the metabolism of cell surface heparan sulfate proteoglycans with focus on mechanisms involved in their endocytosis and subsequent intracellular processing. Topics of present interest include: (1) characterization of the intracellular trafficking and processing pathway for the dermatan sulfate proteoglycan which may enter the nuclear compartment; (2) further development of the procedure to isolate quantitatively and purify nuclei from UMR 106 osteoblastic cells and granulosa cells; and (3) define the intracellular subcompartments where the intercalated heparan sulfate proteoglycans are selectively degraded.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00550-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Biosynthesis and Extracellular Matrix Organization of Hyaluronic Acid

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

Masaki Yanagishita, Visiting Scientist, BRB, NIDR
Yumi Imai, Visiting Associate, BRB, NIDR
Juan Carlos Calvo, Visiting Scientist, BRB, NIDR
Vincent Hascall, Chief, PCS, BRB, NIDR
Aruna Das, Summer IRTA, BRB, NIDR

COOPERATING UNITS (if any)

Orthopaedic Research, Montefiore Hospital, New York

LAB/BRANCH

Bone Research Branch

SECTION

Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.56	2.4	.16

CHECK APPROPRIATE BOX(ES)

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

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

Hyaluronic acid (HA) is a unique glycosaminoglycan. Unlike others, which are synthesized on core proteins in the golgi to form proteoglycans, HA is not assembled on a core protein. Rather, it is synthesized at sites associated with the plasma membrane with the elongating chain being extended into the extracellular matrix. The biological functions of HA in the extracellular matrix are based on the ability of the HA molecules to occupy large hydrodynamic domains and to interact with various specific proteins which constitute structural components in the matrix or are associated with the cell surface. There are conflicting data concerning the nature of the HA synthetase, whether it is a single enzyme or a multi-enzyme complex. Additionally, studies on the regulation of HA synthesis have been technically difficult. The purpose of this project is to study the properties of the HA synthesizing enzyme(s) and the organization and biological roles of HA in the extracellular matrix in model cell culture systems. Topics of current interest include: (1) develop a rapid, sensitive assay for HA using HA immobilized to Sepharose beads and a biotinylated, highly specific HA-binding protein derived from the HA-binding region (G1 domain) of aggrecan; (2) characterization of the HA biosynthetic parameters and identification of the HA-synthetase complex in a human fetal skin fibroblast cell line; and (3) determine the mechanism by which newly synthesized HA is organized into a highly viscoelastic network during differentiation of 3T3 L1 cells into adipocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00552-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Role of Differentiation Factors In Cartilage and Bone Formation and Regeneration

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

F.P. Luyten, Visiting Associate, BRB, NIDR
S. Vukicevic, Visiting Scientist, BRB, NIDR
P. Chen, Visiting Fellow, BRB, NIDR
V. Paralkar, Visiting Associate, BRB, NIDR
M. Krosin, Biological Aide, BRB, NIDR
S. Chang, Summer IRTA, BRB, NIDR

COOPERATING UNITS (If any)

School of Medicine, Zagreb, Croatia; Pediatric Otolaryngology, Children's National Medical Center, George Washington University, Washington, D.C.; CBER, FDA; Johns Hopkins, Baltimore, MD

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Unit, Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.065	1.565	.50

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 objectives of this project are to study the cartilage and bone inducing factors and to define their role in embryogenesis and in postnatal life, both in tissue formation and in disease. As tissue regeneration recapitulates the developmental sequence of embryonic tissue formation, it is conceivable that understanding the mechanisms of action of the soluble differentiation factors is a key step towards biologically controlled regeneration of skeletal tissues. This will have a significant impact on the treatment of congenital and/or acquired skeletal diseases such as large bone defects, impaired fracture healing, osteoarthritis, osteoporosis and periodontitis. This project focuses on the further characterization of cartilage and bone inducing molecules and their binding proteins. Protein fractions with cartilage inducing activity in vivo, have been isolated from articular cartilage and purified to homogeneity; protein sequencing data from tryptic peptides have been obtained; databank searches did not reveal homology with any known sequences. The tissue specific localization of the new protein preparation was shown by immunolocalization using a polyclonal antibody against the N-terminal sequence. Further characterization by cDNA cloning is in progress. Using the molecular probes for the recently characterized bone morphogenetic proteins, we are studying their respective contribution to cartilage, endochondral and membranous bone formation. Immunohistochemical localization and in situ hybridization of cartilage and bone inducing proteins, as well as studies in vitro, indicate the selective contribution of these molecules to initiation, enhancement, maintenance and maturation of the chondrocytic and osteoblastic phenotype. This work contributes to the basic understanding of de novo cartilage and bone formation, and sets the stage for the proper indication and use of these soluble differentiation factors in a clinical setting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00553-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Role of Extracellular Matrix and Differentiation Factors in Skeletogenesis

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

S. Vukicevic, Visiting Scientist, BRB, NIDR
F.P. Luyten, Visiting Associate, BRB, NIDR
P. Chen, Visiting Fellow, BRB, NIDR
V. Paralkar, Visiting Associate, BRB, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; Laboratory of Pathology, NCI; Johns Hopkins University, Baltimore, MD

LAB/BRANCH

Bone Research Branch

SECTION

Bone Cell Biology Unit, Proteoglycan Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.015	1.015	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 objective of this project is to define the role of extracellular matrix in conjunction with soluble inductive molecules in cartilage and bone formation, both in embryogenesis and in postnatal life. The understanding of this functional entity is a key step towards biologically controlled regeneration of skeletal tissues, and could have an important impact on the therapeutic approaches in skeletal diseases. Our discovery of the role of basement membrane components in bone differentiation, indicates the importance of extracellular matrix in bone formation. We have also identified various active growth and differentiation factors bound to basement membrane. Some of them, like bone morphogenetic protein-3 and transforming growth factor-β bound to type IV collagen of the basement membrane and influenced the chondrogenic and osteoblastic phenotype in vitro. We showed that TGF-β remained bound to purified collagen type IV, suggesting caution in the interpretation of cellular activity related to extracellular matrix components. The colocalization of type IV collagen, laminin and bone differentiation factors in development and disease, supports the concept of a biologically active extracellular matrix-growth factor functional complex (EMGFC). Therefore, apart from expanding the basic scientific insight of the biological activity and importance of EMGFC, additional experiments in vivo will define the best carrier and conditions for the application of cartilage and bone inducing factors in skeletal diseases such as Paget's disease, periodontitis, osteoporosis and osteoarthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00554-01 BRB

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Physicochemical Studies on Calcium Phosphate Cements

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

E.D. Eanes, Chief, MCSS, BRB, NIDR
K. Ishikawa, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST, Gaithersburg, MD;
ADAHF Paffenbarger Research Center, NIST, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.36	1.36	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.)

Self-setting calcium phosphate cements (CPC) are promising materials which have a variety of possible medical and dental applications. In situ setting and biocompatibility properties make CPCs potentially useful as endodontic filling materials, as implants for bony defects, and as a binder for other implant materials. CPCs are formed by moistening biphasic mixtures of calcium phosphate salts, usually anhydrous dicalcium phosphate (DCPA) and tetracalcium phosphate (TTCP), with limited amounts of water. Although relatively simple materials in composition, other chemical as well as physical properties, e.g. setting times, porosity and strength, are dependent in a complex manner upon a number of poorly understood parameters associated with the chemistry of the setting process. The purpose of this project is to study factors which influence the conversion of the DCPA/TTCP mixture to apatite, the principal end product in the setting reaction. The principal endeavor currently being pursued is an examination of the hydrolysis of the DCPA component to apatite under controlled experimental conditions in order to obtain mechanistic information on the role of DCPA in the CPC setting reaction. Findings to date suggest that DCPA can initiate apatite formation by surface nucleation but that solution parameters such as pH and supersaturation control the time required to complete the conversion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00555-01 BRB

PERIOD COVERED
October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
NMR Studies of the Structure and Function of PTS Proteins

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

D.A. Torchia, Chief, PBS, BRB, NIDR
J.G. Pelton, Senior Staff Fellow, BRB, NIDR

COOPERATING UNITS (if any)
Johns Hopkins University

LAB/BRANCH
Bone Research Branch

SECTION
Protein Biophysics Section

INSTITUTE AND LOCATION
NIDR, NIH, 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.)

The Phosphoenolpyruvate:glycose phosphotransferase system (PTS) protein III^{Glc} was investigated by nuclear magnetic resonance (NMR) techniques in order to better understand the function of the protein. Studies were carried out to characterize (1) the three-dimensional (3D) structure of phosphorylated III^{Glc} and (2) the chemistry of the active site histidines.

(1) 3D protein structure. Although the 3D protein structure of III^{Glc} had been characterized in solution, by NMR, and in the crystalline state, by X-ray diffraction, the effect of phosphorylation on the structure of III^{Glc} was unknown. Phosphorylation of III^{Glc} affects binding of the protein to sugar permeases, and thereby regulates the uptake of specific sugars by the cell. A regeneration system was developed that maintained III^{Glc} in the phosphorylated state for many days. This made it possible to perform multidimensional NMR experiments on P-III^{Glc} which yielded information about its 3D structure. It was found that the structure of P-III^{Glc} was essentially identical to that of III^{Glc}. This result indicates that the placement of a negatively charged phosphate in the hydrophobic active site of the protein, rather than a conformation change, causes the change of the binding of III^{Glc} to sugar permeases.

(2) Histidine chemistry. III^{Glc} contains two active site His residues. Using NMR we have shown that (a) both His residues are uncharged and have anomalously low pK_a values, < 5, and (b) the two His residues are in the opposite tautomeric forms. The NMR results are the first information about the chemical state of the active site His residues and provide a basis for understanding the mechanism of phosphoryl transfer in the PTS.

The significance of this project lies in its potential for providing a rational quantitative understanding of the function of the PTS. The PTS has essential and diverse physiological roles in many bacterial cells, including those responsible for dental caries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00001-40 LCDO

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Transglutaminases: Specificity and Control

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

PI: Folk, J.E. Chemist LCDO NIDR

OTHER: Goo, Y.M. Guest Researcher LCDO NIDR

COOPERATING UNITS (if any)

Dr. M. Fink, Baylor University, Waco, Texas.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, 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.)

Transgluaminases are enzymes that occur ubiquitously in eukaryotic cells, as well as in many extracellular regions. Although they vary significantly in molecular form, they catalyze a single covalent modification reaction, the outcome of which is the permanent attachment of certain protein molecules to one another subsequent to the assembly of their polypeptide chains. The importance of this post-translational event, which occur through so called ε(γ-glutamyl)lysine or bis-(γ-glutamyl)polyamine crosslinks is evident in fibrin clot stabilization in hemastasis, vaginal plug formation as a result of postejaculatory clotting of seminal plasma, and production of the cell envelope of the stratum corneum during terminal differentiation of keratinocytes in the epidermis. Each of these reactions is catalyzed by a different transglutaminase and the characteristics of each reflects the individual specificity of the enzyme involved. The purposes of this project are to gain understanding of the molecular basis for specificity differences among the transglutaminases, to construct specific inhibitors for the various enzymes based on this knowledge of specificity differences, and to apply these inhibitors as a means of determining further biological roles for the transglutaminases. Methods have been developed for detecting specificity differences for lysine residues and preliminary tests are encouraging. A number of inhibitors for transglutaminases are under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00049-21LCDO

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.)

Physiological Function of Transglutaminases

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

PI: Chung, S.I. Research Chemist LCDO NIDR

OTHERS:

Kwon, S.W. Visiting Fellow LCDO NIDR
Folk, J.E. Chief, ECS LCDO NIDR

COOPERATING UNITS (if any)

Peter Steinert, Laboratory of Skin Biology, NIAMSD
Soo Yeol Kim, Laboratory of Skin Biology, NIAMSD

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD 20892-0030

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.75	

CHECK APPROPRIATE BOX(ES)

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

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

The physiological function and mode of regulation of transglutaminases are being studied as to their role in the formation of the cornified cell envelope in terminally differentiated mucosal epithelial cells and in mucosal pellicle formation in the human oral cavity.

In the stratum distendum of human buccal epithelium, terminally differentiated cells form a stable cornified cell envelope as a result of crosslinking of specific cellular proteins. Salivary proteins are crosslinked to these cells to form the salivary pellicle. This pellicle that is found to be present in several cellular layers of the stratum distendum may constitute a multi-cell layered protective barrier of the oral mucosa.

A cDNA encoding the cytosolic zymogen, for epidermal transglutaminase E was isolated. The primary structure of the zymogen was deduced from the nucleotide sequences and by partial amino acid sequence determination. This zymogen shares 45-49% sequence identidy with other transglutaminases. There are four highly conserved sequence located in regions carboxyl-terminal to the active site.

Expression of membrane-associated transglutaminase K and its variant mutant clones in E. Coli provided a means to determine that there is a highly conserved sequence of amino acids, residues 105-572 essential for enzyme activity. The observation of a several-fold increase in catalytic activity with the mutant enzyme that lacks an amino-terminal peptide, residues 1-57, suggests that this peptide domain regulates activation of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00311-12-LCDO

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.)

Hypusine in eIF-4D: Biosynthesis and Function

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

PI:	Park, M.H.	Research Chemist	LCDO NIDR
OTHERS:			
	Folk, J.E.	Chemist	LCDO NIDR
	Wolff, E.C.	Expert	LCDO NIDR
	Jakus, J.	Visiting Fellow	LCDO NIDR
	Rinaudo, M.	Visiting Associate	LCDO NIDR

COOPERATING UNITS (if any)

Dr. H. Hanauske-Abel, Cornell University, Medical College, New York, NY; Dr. Marc Lalande, Harvard Medical School, Boston, MA; Dr. W.C. Merrick, Case Western Reserve University, Cleveland, OH.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.25	4.25	

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.)

Eukaryotic protein translation iniation factor 5A (eIF-5A) contains one residue of hypusine and appears to be the only cellular protein with this unique amino acid. Hypusine is produced post-translationally by transfer of the butylamine portion of the polyamine spermidine to a lysine residue in the eIF-5A precursor to form deoxyhypusine followed by hydroxylation to form hypusine. These findings reveal a novel cellular metabolic pathway. Hypusine is essential for the biological activity of eIF-5A in an *in vitro* translation initiation assay and hypusine and eIF-5A appear to be vital elements for growth of eukaryotic cells. Thus, the hypusine biosynthetic steps, deoxyhypusine synthesis and deoxyhypusine hydroxylation present special potential targets for intervention in cellular proliferation. Several inhibitors of the enzymes deoxyhypusine synthase, and deoxyhypusine hydroxylase were developed and their cellular effects have been examined. Studies are underway to relate the structure of hypusine to the physiological function of eIF-5A in mammalian cells..

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00433-06 LCDO

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.)

Functional aspects of C-reactive protein.

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

PI:	Robey, F.A.	Chief, PIU	LCDO NIDR
OTHERS:			
	Batinic, D.	Visiting Fellow	LCDO NIDR
	Heegaard, N.	Visiting Fellow	LCDO NIDR
	Nguyen, A.	IRTA Fellow	LCDO NIDR

COOPERATING UNITS (if any)

Henry Gewurz, Rush Medical School, Chicago, IL; Robert Kisilevski, Queens University, Kingston, Ontario, Canada

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.41	2.41	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

C-Reactive protein (CRP) and serum amyloid P component (SAP) are two closely related proteins with respect to their primary structure and their pentameric appearance under the electron microscope. The two proteins have unknown functions. A common property shared by CRP and SAP is their ability to bind to sulfated polysaccharides and to fibronectin in a calcium-dependent manner.

Using techniques including cell attachment assays, tissue culture, peptide synthesis and immunoassay, a peptide modeled after the primary sequence of SAP was found to bind strongly and specifically to heparin and certain other sulfated polysaccharides. This binding was independent of calcium. The homologous peptide from CRP also bound heparin.

When native SAP was denatured or digested with trypsin the heparin binding property remained, but was no longer calcium-dependent. Thus calcium may influence the conformation of SAP to expose the active peptide region. Consistent with this suggestion is the finding that the synthetic peptide competes with the native protein for heparin.

Because SAP is associated with all forms of amyloid including those found in Down's Syndrome and Alzheimer's Disease, an active peptide from SAP may lead to diagnostic or therapeutic materials for these conditions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00434-06 LCDO

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 HIV-1 Targeted Drug Delivery Systems

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

PI: Robey, F.A. Chief, PIU LCDO NIDR

OTHERS:

Batinic, D.	Visiting Fellow	LCDO NIDR
Harris-Kelson, T.	Staff Fellow	LCDO NIDR
Ivanov, B.	Visiting Fellow	LCDO

COOPERATING UNITS (if any)

Marjorie Roberts-Guroff, National Cancer Institute; Marian Neutra, Harvard University, Washington, DC; John Inman, NIAID.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	

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.)

HIV-1 is the causative agent of AIDS. CD4 is the cellular receptor for HIV-1 and its amino acid sequence is known. The region of HIV-1 that binds to CD-4 is termed gp160 and this is the envelope glycoprotein that is composed of gp120 and gp41. The gp120 region specifically binds to CD4 and the sequences of amino acids in both CD4 and gp120 that are responsible for the high affinity binding of the virus are now known.

The interaction of HIV-1 with CD4-bearing cells extends beyond the binding process and there are other events necessary for infection. We have found that the principle neutralizing epitope of HIV-1's gp120 binds sulfated polysaccharides and quite possibly, a highly anionic region of CD4. This region of gp120 is necessary for the infection process and those agents that have been found to block the HIV-1 infection process are probably doing so by a common mechanism. This mechanism involves binding to the highly cationic region of gp120 which extends from amino acids 310 to approximately 330.

The significance lies in the fact that complete understanding the molecular processes by which HIV-1 and its target cells interact should lead to a treatment for AIDS that is based on rational drug and vaccine design.

New methods in peptide chemistry have been developed as part of this project for the purpose of approaching problems encountered with current synthetic therapeutic and vaccine design strategies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00479-04-LCDO

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.)

Molecular Mechanisms Responsible for Oncogenesis

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

PI: Robbins, K.C. Chief, MCBS LCDO NIDR

OTHERS:

Matoskova, B. Visiting Fellow LCDO NIDR

COOPERATING UNITS (if any)

Joseph B. Bolen, Bristol-Myers Squibb, William J. LaRochelle, LCMB, NCI; Timothey J. Ley, Washington University, St. Louis, MIssouri, Stuart A. Aaronson, LCBM, NCI

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.96	1.30	.66

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 approaches were taken to address the mechanism of cellular transformation induced by nonreceptor protein-tyrosine kinases. One involves overexpression of fgr genes specifying normal or aberrant kinases in NIH/3T3 cells. Our findings document a rare malignant transformation by high levels of p55-c-fgr. Furthermore, it was shown that by mutation of sequences encoding the carboxyl terminus of p55-c-fgr the c-fgr gene is converted into a potent, dominant acting oncogene. A search for substrates for these activated tyrosine kinases has identified molecules of 135 kd and 70 kd that preferentially interact with and are tyrosine phosphorylated by transforming as compared to normal versions of src, fyn, and fgr kinases. The third approach involves searching for evidence of activated tyrosine kinases in naturally occurring human neoplasia, especially squamous cell carcinomas of the head and neck. We have found that the receptor for epidermal growth factor is activated in a majority of oral squamous cell carcinomas and have identified a novel mechanism for activation of this receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00480-04-LCDO

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.)

Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

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

PI: Robbins, K.C. Chief, MCBS LCDO NIDR

OTHERS:

Gutkind, J.S. Visiting Associate LCDO NIDR
Agarwal, A. Visiting Fellow LCDO NIDR
Salem, P. Visiting Fellow LCDO NIDR

COOPERATING UNITS (if any)

R.F. Sirgaganian, LI, NIDR

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.01	2.35	.66

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 working hypothesis states that nonreceptor protein-tyrosine kinases transduce environmental signals in fully mature cells. During the current reporting period important evidence has been obtained supporting this hypothesis. In an effort to define possible subcellular locations where the tyrosine kinase activity of p55-c-fgr might be exerted, human PMN was fractionated and assayed for the enzyme. The findings demonstrated that p55-c-fgr is associated with plasma membrane as well as functional secretory granules and is redistributed within normal neutrophils in response to their activation. It was also sought to determine whether platelets, a rich source for p59 association with PI-3 kinase, might serve as a measure of nonreceptor protein-tyrosine kinase activation under physiologic conditions. p60, as well as p59, the product of another member of the src family of proto-oncogenes, was found to physically associate with PI-3 kinase within 5 s after exposure to thrombin. The possible involvement of tyrosine phosphorylation in signalling degranulation through the high-affinity IgE receptor (FcϵRI) was examined. Tyrosine phosphorylation was shown to be an early signal following FcϵRI aggregation, independent of the exocytotic process itself. These findings functionally link protein phosphorylation on tyrosine residues to FcϵRI-mediated signal transduction leading to degranulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE0551-01-LCDO

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.)

Role of G Proteins in Growth Control and Carcinogenesis

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

PI: Gutkind, J.S. Visiting Associate LCDO NIDR

OTHERS:

Robbins, K.C. Chief, LCDO LCDO NIDR

Xu, N. Visiting Fellow LCDO NIDR

COOPERATING UNITS (if any)

Sylvie Hermouet, MPB, NIDDK, Allan Spiegel, MPB, NIDDK, Jurgen Wess, MBL, NINDS.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular Signalling Group, Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.75	2.0	.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.)

The objective of the project is to study the role of heterotrimeric G proteins and their coupled receptors in normal cell growth and oncogenesis. We have genetically engineered NIH 3T3 mouse fibroblasts to express the family of human acetylcholine muscarinic receptors (mAChRs). Using this model, we have shown that genes for mAChRs subtypes coupled to the activation of phosphatidylinositol (PI) hydrolysis can act as ligand-dependent oncogenes, whereas those coupled to the inhibition of the adenylyl cyclase (AC) are not. Furthermore, expression of GTPase deficient α subunit of G_q, an activator of PI-phospholipase C, induces focus-formation in NIH 3T3 cells. In contrast, expression of activated G_{i2}, an inhibitor of AC, fails to transform the same cells. The region of the mAChR that confers transforming potential has been mapped by a receptor chimera approach. We have found that the region responsible for coupling to other signal transduction pathways, such as the activation of phospholipases A_2 or D, is also responsible for transforming activity. Furthermore, available evidence suggests that activation of the phospholipase A_2-eicosanoid pathway is strictly necessary for mitogenesis as well as transformation induced by receptors coupled to G proteins, but not by activated tyrosine-kinase receptors. Mitogenic signalling through G protein-coupled receptors does not require Ca^{2+}-dependent protein kinase C, but involves rapid tyrosine phosphorylation of cellular proteins, affects the functions of p21^{ras}, the c-raf serine-threonine kinase, and induces expression of certain early-responsive proto-oncogene. Work is in progress to determine the molecular basis for these interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00558-01-LCDO

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.)

Oral Carcinogenesis

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

PI: Robbins, K.C. Chief, MCBS LCDO NIDR

OTHERS:

Cardinali, M. Visiting Associate LCDO NIDR

COOPERATING UNITS (if any)

John Ensley, Wayne State University

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.03	1.35	.68

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 approached oral carcinogenesis by initially searching for evidence of activated tyrosine kinases in naturally occurring human neoplasia, especially squamous cell carcinomas of the head and neck. Initial results have shown that the receptor for epidermal growth factor (EGF) is activated in a number of oral cavity tumors. As a probe for the mechanism of this activation, we have investigated the effect of suramin treatment on EGF receptor activity. It was expected that suramin would greatly reduce the level of intracellular protein-tyrosine phosphorylation by the EGF receptor. Instead, the drug dramatically enhanced protein-tyrosine phosphorylation in epithelial cell tumors. The mechanism at play was shown to involve activation of the growth factor, namely transforming growth factor alpha, that in turn stimulates the tyrosine kinase activity of the EGF receptor. These results suggested that suramin may stimulate the growth of certain tumors, and indeed suramin enhances the growth of oral carcinoma cells in culture. Cancer patients receiving suramin chemotherapy currently are being examined for expression of TGFα.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00212 16 CI

PERIOD COVERED: October 1, 1991 - September 30, 1992

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

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

Weiffenbach, James	Research Psychologist	CIPC	NIDR
Baum, Bruce J.	Clin. Dir/Chief	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Schwartz, Lisa K.	Special Volunteer	CIPC	NIDR

COOPERATING UNITS (if any)
LSB, NIA;

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.925	.925	0

CHECK APPROPRIATE BOX(ES)

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

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

This project seeks to elucidate the mechanisms by which oral perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. Olfactory function is routinely assessed by a standardized test of odor identification. Assessments of sensitivity to local pressure on the tongue and to variation in the temperature or the viscosity, of an oral bolus are also available. These methods, applied to the study of age associated changes, have provided insights into basic mechanisms of normal chemosensory perception. Oral perceptual changes may occur with oral or systemic disease and its treatment, salivary gland dysfunction, or as an isolated complaint. Documentation that sensory function is impaired (eg olfaction in Sjogren's Syndrome) or that it is unaffected (eg taste intensity following therapeutic irradiation) can each advance the understanding of the mechanisms by which the complex oral stimuli encountered in everyday life are perceived.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00332-11 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Clinical Investigations and Case Reports

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

Guckes, Albert D., Deputy Clinical Director, CIPC NIDR
Atkinson, Jane C., Senior Staff Fellow, CIPC NIDR
Baum, Bruce J., Clinical Director/Chief, CIPC NIDR
Brahim, Jaime S., Senior Staff Dentist, CIPC NIDR
Cooper. Lyndon C., Staff Fellow, CIPC NIDR
McCarthy, George M., Dental Officer, CIPC NIDR
Ship, Jonathan, Dental Officer, CIPC NIDR

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Pediatric Branch, NCI; Inter-Institute Genetics Program, CC; Nursing, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.15	,85	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.)

Clinical case studies of unusual interest and clinically related research are being conducted on a variety of dentally related subjects. Research techniques being utilized include chart and literature reviews, and evaluation of various therapeutic regimens. A recent case documented the use of oral endosseous titanium implants to replace the mandibular teeth of a patient with an edentulous patient with Papillon-Lefevre syndrome, which is also referred to as generalized juvenile periodontosis. This condition can cause the early loss of the permanent teeth due to rapid bone destruction of unknown etiology. The use of endosseous implants in patients with this syndrome has been questioned because of the possibility of rapid bone loss around implants similar to that which occurs around the natural teeth. Four mandibular endosseous implants have successfully integrated in this patient. The maxillae is being treated with a combination of bone grafting and endosseous implants.

Professional Personnel, continued

Vermillion, Cheryl	Dental Hygienist	CIPC	NIDR
Royce, Leah	Staff Fellow	CIPC	NIDR
Valdez, Ingrid	Senior Staff Fellow	CIPC	NIDR
Davis, Vincent	Clinical Associate	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00336-11 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Salivary Gland Secretion Mechanisms During Normal and Altered Functional States

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

Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Dai, Y.	Visiting Fellow	CIPC	NIDR
Hiramatsu, Yukiharu	Visiting Fellow	CIPC	NIDR
Li, Jun	Special Volunteer	CIPC	NIDR
Royce, Leah	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

DNM, CC, NIH; Dept. of Nuclear Medicine, Univ. of Chicago, Dept. of Neurology, Cornell University

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.65	.35

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We utilize in vitro dispersed cells of salivary glands to understand mechanisms controlling saliva formation. We have focused our studies on neurotransmitter regulation of secretory events and associated signalling mechanisms. During this reporting period the primary focus of study continues to be muscarinic receptors (mAChRs) in rat parotid gland acinar cells and their coupling to functional responses via specific G proteins. We have also initiated studies of α-adrenergic receptors (α_1-ARs) in these cells. In parotid cells, stimulation of mAChRs results in the generation of inositol phosphates via the activation of a phosphatidylinositol 4,5-bisphosphate specific phospholipase C. Subsequently this response leads to the elevation of cytosolic Ca^{2+} levels and fluid secretion. Additionally, mAChRs can mediate the inhibition of agonist induced cAMP formation. We have characterized the binding of a subtype non-selective antagonist (quinuclidinyl benzilate, QNB) to mAChRs in intact rat parotid cells. Specific binding is saturable, time- and temperature-dependent (Bmax ~80 fmol/mg protein; Kd ~ 100 pM). Also, we have assessed the relationship between mAChR occupancy and second messenger formation, determining that a moderate population (~20-40%) of spare receptors exist for inositol trisphosphate (IP_3) formation. We previously reported that rat parotid M_3-mAChRs couple to two different signal transducing G proteins. This year we determined the nucleotide sequence encoding the region of this receptor gene involved in G-protein coupling and we observed that it is virtually identical to other reported M_3 sequences. Thus, the mAChR itself is unlikely to be responsible for the divergent coupling. Additionally, we have shown that the α_1-adrenergic receptor subtype involved in the fluid secretory response is α_{1A}.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00337-11 CI

PERIOD COVERED
October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Oral Physiological Processes: Normal Function and Disease Perturbation

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

Fox, Philip C.	Dental Officer	CIPC	NIDR
Atkinson, Jane C.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Kohn, William	Dental Officer	DIR	NIDR
Kousvelari, Eleni E.	Senior Staff Fellow	CIPC	NIDR
Kurrasch, Regina	Medical Staff Fellow	CIPC	NIDR
Katz, Joseph	Special Volunteer	CIPC	NIDR

(see the attached continuing sheet)

COOPERATING UNITS (if any) RM, CC; Dr, CC; DD, NIDDK; LNS, NIA; Nursing, CC
Columbia University; University of Utah; University of California, San Francisco; University of Colorado; University of Ioannina, Ioannina, Greece; University of Liverpool, Liverpool, England

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Clinical Investigations Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	3.8	1.0

CHECK APPROPRIATE BOX(ES)

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

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

This project examines the function of the salivary glands and other oral tissues in individuals with alterations of normal oral function due to disease or therapeutic procedures. Major efforts have been directed at the evaluation of patients complaining of xerostomia (oral dryness). Entry into all studies is through the Dry Mouth Clinic. Utilizing outpatient and inpatient services, specific evaluative and diagnostic approaches have been developed to aid in establishing the extent and causes of salivary gland dysfunction in the "dry mouth" patient. Major patient groups studied include individuals with Sjögren's syndrome, an autoimmune exocrinopathy, and those with salivary hypofunction secondary to therapeutic irradiation to the head and neck region. Oral and secretory effects of a number of other systemic diseases also are evaluated. Recent treatment protocols have utilized the parasympathomimetic drug pilocarpine for salivary stimulation in the post-radiation group and steroid and non-steroidal anti-inflammatory drugs for Sjögren's syndrome patients. Clinical and laboratory studies focusing on the immunological basis of the salivary component of Sjögren's syndrome have advanced and represent the main focus of our work. An anti-salivary duct antibody present in serum and saliva of Sjögren's syndrome patients has been characterized. Markers of salivary gland disease activity in serum of patients with Sjögren's syndrome have been identified. The effects of cytokines and other immune mediators on a cultured human salivary cell line have been investigated. In addition, our detailed studies of associated oral complaints in the salivary hypofunction group (taste, oro-pharyngeal swallow, and mucosal status) have progressed.

Professional Personnel, continued

Macynski, Alice A.	Research Nurse	CIPC	NIDR
Nagler, Rafi	Visiting Fellow	CIPC	NIDR
Valdez, Ingrid H.	Dental Officer	CIPC	NIDR
Weiffenbach, James M.	Research Psychologist	CIPC	NIDR
Wu, Ava J.	Dental Staff Fellow	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00412-07 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Oral Endosseous Titanium Implants in Edentulous and Ectodermal Dysplasia Subjects

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

Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Guckes, Albert D.	Dep Clinical Director	CIPC	NIDR
McCarthy, George M.	Dental Officer	CIPC	NIDR
Cooper, Lyndon F.	Staff Fellow	CIPC	NIDR
Gracely, Richard H.	Research Psychologist	NAB	NIDR
Morgan, Victor L.	Dental Lab. Technician	CIPC	NIDR
Li, Shou Hua	Statistician (Health)	EB	NIDR

(see the attached continuing sheet)

COOPERATING UNITS (if any)

RM, CC; NUTR, CC; DR, CC; HGB, NICHD; DD, NKDDK; LNS, NIA; LSB, NIA; Nursing, CC; Columbia University; University of Utah; University of California, San Francisco

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.85	2.0	2.85

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 examines the use of endosseous dental implants in completely edentulous patients, or patients with ectodermal dysplasia and several congenitally missing permanent teeth. Removable dentures are considered a significant handicap related to mastication, speech, esthetics, reduction of the residual ridges of the mandible and maxillae, and body self image. Individuals with ectodermal dysplasia often have several congenitally missing teeth resulting in a lack of development of the alveolar bone which normally is present to support the permanent teeth. Lack of alveolar bone not only makes it difficult for a patient to wear a removable denture but also makes the placement of dental implants more difficult and possibly less successful. This study is attempting to determine if dental implants can be used successfully to replace missing teeth in conventional adult patients and adult and pre-adolescent patients with ectodermal dysplasia. Further, we are trying to assess if such treatment with an implant supported fixed denture significantly affects loss of vertical dimension of occlusion, satisfaction with treatment, food choice and nutrition, perception of difficulty of chewing selected food, and body self image, when compared to treatment with a conventional removable denture. Also, the project is seeking to determine the effects of mandibular dental implants on the growth and development of the craniofacial complex of pre-adolescent patients with Ectodermal Dysplasia and hypodontia. In addition the project asks if patients identified as being difficult to satisfy with conventional dentures are more satisfied when the prosthesis is fixed. Data from this project should provide information concerning the relationship of personality to body image and the ability to adapt to oral prostheses of various types. During this reporting period we have continued laboratory investigations to study the biology of the bone:implant interface. The goals of this project are to develop a cell culture model to evaluate the responses of osteoblast-like cells to three stresses; heat, mechanochemical, and alloplastic interaction, associated with the placement of titanium implants in intramembranous bone.

Professional Personnel, continued

Folio, John	Consultant	CIPC	NIDR
Ruttimann, Urs E.	Biomedical Engineer	DSB	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00415-07 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Ion Transport and Fluid Secretion in Salivary Glands

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

Turner, Roy James	Visting Scientist	CIPC	NIDR
Paulais, Marc	Visiting Fellow	CIPC	NIDR
Moran, Arie	Visiting Associate	CIPC	NIDR
Valdez, Ingrid H.	Dental Staff Fellow	CIPC	NIDR
Reshkin, Stephen	Staff Fellow	CIPC	NIDR
Davis, Vincent	Dental Staff Fellow	CIPC	NIDR
Eidelman, Ofer	Special Expert	CIPC	NIDR
Ferri, Concetta	Special Volunteer	CIPC	NIDR
Casavola, Valeria	Guest Worker	CIPC	NIDR

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIDR, NIH Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.1	4.1	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.)

Saliva is the principle protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations of salivary secretory mechanisms can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of salivary fluid secretion and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following.

(1) Studies of the regulation of the rat parotid acinar Na-K-2Cl cotransporter and Na/H exchanger by secretagogues and other stimuli were continued.

(2) Investigations of the functional properties of salivary ducts, in particular their ion transport properties and their responses to various secretagogues, were continued using microfluorometric methods.

(3) The ion transport systems and secretory responses of human labial salivary glands were studied.

(4) The responses of the human salivary ductal cell line HSY to hypotonic shock were characterized.

(5) The rabbit parotid Na-K-2Cl cotransport protein was purified in preparation for molecular cloning attempts.

(6) Fluorescence video imaging studies of intracellular calcium oscillations were begun in parotid and pancreatic acinar cells and in HSY cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00438-06 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Molecular Mechanisms Regulating Calcium Flux in Salivary Glands

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

Ambudkar, Indu S.	Senior Staff Fellow	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Hiramatsu, Yukiharu	Visiting Fellow	CIPC	NIDR
Lockwich, Timothy	Staff Fellow	CIPC	NIDR
Sawaki, Kohei	Special Volunteer	CIPC	NIDR
Kaplan, Mitch	Dental Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Department of Biological Chemistry, University of Maryland School of Medicine

LAB/BRANCH

Clinical INvestigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	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.)

Fluid secretion in salivary glands is modulated by changes in the cytosolic [Ca], which involves internal Ca release and Ca entry from the external medium. This project is directed towards understanding the processes which regulate cytosolic [Ca]. Three main areas were investigated during this reporting period: (i) regulation of phosphatidylinositolbisphosphate-specific phospholipase C (PLC), (ii) regulation of Ca entry in salivary cells, and (iii) regulation of Ca flux in rat parotid gland basolateral membrane vesicles. In isolated rat parotid membranes we have characterized an enzyme with high specificity for phosphatidylinositol-4,5,bisphosphate, which is immunologically distinct from the PLCβ_1 enzyme found in rat brain membranes. Agonist stimulation of this enzyme is dependent on the presence of lipids and detergent. Agonists such as carbachol, stimulate Ca entry into parotid acini which results is sustained elevation of Ca in the cytosol. However, during refill of the internal Ca pools Ca entry is not accompanied by substantial rise in cytosolic Ca. Our results indicate that low cytosolic Ca is maintained due to a highly active, thapsigargin sensitive, internal Ca accumulating mechanism. In a human salivary gland cell line, HSG, we have identified a Ca entry mechanism which is directly activated by muscarinic receptor stimulation. Consistent with our previous data with intact acini we have observed that Ca influx into basolateral membrane vesicles is sensitive to pH and is inhibited by both hydrophobic and hydrophilic carbodiimides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00458-05 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Beta-Adrenoreceptors and Gene Regulation

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

Kousvelari, Eleni	Expert	CIPC NIDR
Ambudkar, Indu S.	Senior Staff Fellow	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR
Lazowski, Krzysztof W.	Visiting Fellow	CIPC NIDR
Mertz, Prema M.	Staff Fellow	CIPC NIDR
Yeh, Chih-Ko	Staff Fellow	CIPC NIDR

COOPERATING UNITS (if any)

Department of Pharmacology, University of Minnesota; Squibb Institute of Medical Research; VA Medical Center

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretory Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.1	3.1	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The studies presented here are designed to; (i) identify the molecular mechanisms involved in gene regulation and hyperplasia caused in rats by ß-adrenergic receptor (ß-AR) stimulation; (ii) examine the effect(s) of irradiation on ß-AR induced gene expression and salivary protein composition; (iii) understand the role of the extracellular matrix components and their integrin receptors in rat parotid gland proliferation and differentiation during gland development; (iv) determine the different signal transduction pathways involved in the expression of c-fos, c-jun and jun B genes, in a salivary cell line (A5). During this reporting period we have; (1) constructed cDNA libraries from parotid glands of isoproterenol treated rats and used sub-tractive hybridization for the identification of cDNA clones corresponding to specific mRNAs in the parotid glands of isoproterenol treated rats; (2) isolated the acidic PRP (PRP33) gene;(3) demonstrated that a single dose (15Gy) of ionizing radiation has no effect on ß-AR stimulation induced changes in PRP transcription rates and salivary composition; (3) shown that laminin B1, B2, collagen IV genes and α6 and ß1 integrin genes and ß1 protein, are highly expressed during the early stages (1, 7 and 14) of parotid gland development and are present at basal levels at older ages (21, 42 and 90 days); and (4) identified the intracellular signals involved in the differential expression of c-fos, c-jun and jun B genes in A5 cells, after different stimuli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00499-03 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Oral health and salivary function in HIV-1 infected patients

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

Atkinson, Jane C.	Dental Officer	CIPC	NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC	NIDR
Fox, Philip C.	Dental Officer	CIPC	NIDR
Riley, Chiara	Staff Fellow	LME	NIDR
Valdez, Ingrid	Dental Officer	CIPC	NIDR
Yeh, Chih-Ko	Staff Fellow	CIPC	NIDR
Rooney, James	Special Expert	LOM	NIDR

COOPERATING UNITS (if any)

POB, NCI; University of California, San Francisco, Virginia Polytechnical Institute, Medical College of Virginia

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.525	.425	.1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project focuses on oral changes associated with HIV-1 infection. We have examined saliva and salivary glands, critical components of the oral defense system, for changes following infection. Forty-four percent of patients with HIV-associated salivary gland disease (HIV-SGD) had salivary antibodies that recognized the cytoplasm of a human salivary epithelial cell line, but the autoantibodies were not anti-SS-A, anti-SS-B, or anti-DNA. In contrast, 73% of patients with primary Sjogren's syndrome had anti-SS-A or anti-SS-B in their saliva. Periodontal status in approximately 200 HIV-1 seropositive patients was assessed (see Z01-DE-000498-03). HIV-associated periodontitis was not found, and HIV-associated gingivitis was more common. The subgingival flora of 39 of these subjects with gingivitis or adult periodontitis was cultured quantitatively. In general, the same types of bacteria were isolated as from the subgingiva of non-HIV subjects. Mycoplasma salivarium was significantly elevated in the HIV+ subjects examined. Yeasts were isolated from only 10% of the samples. Pediatric patients with HIV-1 infection appear to have normal dental development, and one-third of the patients referred for dental care have nursing bottle caries. Data suggest that saliva likely has several means by which to inhibit HIV-1 infectivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DE-00500-03 CI

PERIOD COVERED
October 1, 1991 - September 30, 1992

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

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

Ship, Jonathan A.	Dental Officer	CIPC NIDR
Atkinson, Jane	Senior Staff Fellow	CIPC NIDR
Baum, Bruce J.	Clin Dir/Chf	CIPC NIDR
Cherry-Peppers, Gail	NRSA Staff Fellow	CIPC NIDR
Ebbs, William L.	Dental Staff Fellow	CIPC NIDR
Fox, Philip C.	Dental Officer	CIPC NIDR
Streckfus, Charles	Senior Staff Fellow	EODPP NIDR
Weiffenbach, James M.	Research Psychologist	CIPC NIDR
Wu, Ava J.	Dental Staff Fellow	CIPC NIDR

COOPERATING UNITS (if any)
LNS, NIA; LSB, NIA; Howard University; University of North Carolina

LAB/BRANCH
Clinical Investigations and Patient Care Branch

SECTION
Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.01	2.01	0

CHECK APPROPRIATE BOX(ES)

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

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

This project examines the status of various oral tissues during physiologic aging. The current emphasis is to study cross-sectional and longitudinal effects of aging on oral physiology in a variety of populations: healthy whites and blacks of different ages, and subjects with well controlled systemic diseases. The influence of systemic disease and its treatment on aging and the oral cavity has become an important focus in our investigations. Clinical evaluation of participants involves an oral health questionnaire, collection of unstimulated and stimulated parotid and submandibular gland salivas, a comprehensive examination of dental, periodontal, and mucosal tissues, an oral motor exam, and the determination of pressure, gustatory, and olfactory sensitivities. During this reporting period, results from cross-sectional studies suggest that subjects with impaired glucose tolerance and diabetes generally have similar dental, gingival, periodontal, and oral mucosal health compared to healthy controls. Cross-sectional studies reveal that smell identification diminishes with increased age, females have superior smell function compared to males across the life-span, and that individuals with medical problems and taking prescription medications are more likely to be anosmic. Generally healthy, community-dwelling and urban older blacks have more tooth loss, greater attachment levels, and greater number of teeth with coronal restorations compared to younger, healthy blacks. However, the general oral condition of older blacks is good, perhaps due to regular general and oral health care. Results from longitudinal studies in healthy individuals of different ages reveal that 5 constituents commonly found in stimulated parotid saliva are not diminished over a 10 year period. A ten year longitudinal periodontal study indicates that attachment level increases over time which is independent of age, accounted primarily by recession and not pocket depth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00502-03 CI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Maxillofacial Surgery-Rigid Versus Nonrigid Fixation Following Orthognathic Surgery

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

Brahim, Jaime S.	Senior Staff Fellow	CIPC	NIDR
Folio, John	Consultant	CIPC	NIDR
Gracely, Richard	Research Psychologist	NAB	NIDR

COOPERATING UNITS (if any)

Rehabilitation Medicine Department CC; Nutrition Department, CC; Surgical Services Department, CC; Commissioned Officers Dental Clinic, CC; Nursing, CC

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.15	.05	.10

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose of this study is to determine the preferred method of fixation to avoid relapse following maxillary and mandibular osteotomy to correct facial developmental deformities. Correlations are being established between semirigid and nonrigid fixation techniques, and the degree of relapse as measured by cephalometric techniques utilizing metallic markers implanted in the maxillae and mandible 6 months prior to orthognathic surgery. Changes in the height or the width of the attached gingiva are being recorded. Pre and post-operative changes in facial contours and occlusion are being measured. In addition, movements of the tongue, mandible and associated soft tissues during speech and swallowing are being assessed with ultrasound imaging procedures. Initial results observed at 3, 6 and 12 months did not demonstrate a significant difference in relapse between the two groups. However, patients treated with semirigid fixation experienced better diet, speech, and oral hygiene compared to patients with rigid internal fixation. Initial results of the swallowing tests demonstrate that maximal physical adjustment in the mandibular-hyoid relationship occurs in the first three months following the surgery. Functional changes continue to occur throughout the year. There appears to be a positive relationship between the surgical procedure and swallowing function. Both procedures serve to normalize the rate of swallowing, i.e. to decrease the duration of swallowing after surgery. Mandibular reduction patients were more abnormal in their pre-surgery duration (i.e. they took longer and required more effort to swallow before surgery).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-16 LDB

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.)*

Proteins in Tissue Architecture and Cell Function

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

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:			
	Kibbey MC	Biologist	LDB, NIDR
	Grant DS	Visiting Associate	LDB, NIDR
	Weeks BS	Biologist	LDB, NIDR
	Holloway E	Bio Lab Tech	LDB, NIDR

COOPERATING UNITS *(if any)*

NINCDS (Wujek J); NIA (Jucker M); NIAID (Hoffman G); UC San Diego (Williamson M); Johns Hopkins Med Sch, Balt MD (Walker L); Georgetown U Med Sch Wash DC (Dym M); Yale U Med Sch (Rosen E); U Wisconsin Med Sch (Auerbach R).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	1.65	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 extracellular matrix has been found to be important in embryogenesis and in repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate, and differentiate in response to tissue and cell-specific matrix molecules has been established. We have found that the basement membrane, the extracellular matrix which underlies all epithelial cells and endothelial cells and surrounds nerve cells, promotes cell differentiation in vitro. Endothelial cells form capillary-like structures with a lumen, bone cells form canaliculi, salivary cells form glands, etc. Our goal is to define the molecular and cellular events involved in this process. Our approach has been to identify the (1) biologically active matrix components, (2) localize active sites on the matrix component with site specific antibodies and synthetic peptides, (3) identify and characterize cellular receptors, (4) gain an understanding of the intracellular events involved in the biological response, and (5) identify genes induced by the extracellular matrix. Specifically, we have defined YIGSR as an antiangiogenic site on laminin and SIKVAV as an angiogenic and neurite promoting site. Two new angiogenic factors, scatter factor and haptoglobin, were defined using in vitro and in vivo assays developed by us. In addition, estrogens have been found to promote angiogenesis on basement membrane and to increase leukocyte-endothelial cell adhesion. A brain derived cellular receptor for SIKVAV shares homology with the amyloid precursor protein and may define the role of this protein in development and in Alzheimer's disease. Subtractive cDNA cloning of endothelial cells on plastic vs basement membrane has identified several novel genes as well as thymosin and calmodulin as induced during differentiation into vessels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00481-04 LDB

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.)*

Connective Tissue Gene Expression in Development and Disease

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

PI:	Klotman PE	Special Expert	LDB, NIDR
Others:			
	Bruggeman LA	Staff Fellow	LDB, NIDR
	Ray PE	Visiting Scientist	LDB, NIDR
	Kopp JB	Staff Fellow	LDB, NIDR
	Weeks BS	Biologist	LDB, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Chemoprevention, NCI (Sporn M); University of Maryland, Baltimore MD (Hansen B); Duke University, Durham NC (Coffman T).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Disease Pathogenesis Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.05	2.05	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.)*

During nephrogenesis, basement membrane genes are expressed in a coordinate fashion in different portions of the nephron. After nephrogenesis is complete and during adult life, matrix proteins are synthesized at low levels, presumably representing on-going replacement of basement membrane and mesangial matrix. A common feature of response to injury or inflammation by many tissues is the increased deposition of matrix proteins. The kidney is exquisitely sensitive to malfunction as a consequence of scarring stemming from the repair process. Whereas many mesenchymal tissues (skin, bone, muscle, liver) and certain epithelial tissues (spleen intestine) can maintain function despite a degree of structural disorganization, normal renal function is predicated on the precise tissue structure. Thus, scarring anywhere along the nephron threatens the filtration process. Progressive renal disease of many etiologies is characterized by cellular proliferation and increased accumulation of a cellular material within the glomerular mesangium and the renal interstitium. The purpose of these studies is to determine the molecular mechanisms responsible for the regulation of extracellular matrix proteins, to explore inducible factors that modulate mitogenesis and cellular matrix protein production such as hormones, autacoids, cytokines, and growth factors, and to develop novel therapeutic strategies to prevent progressive fibrosis and sclerosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00482-04 LDB

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.)*

Tumor Growth and Metastases

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

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:			
	Yamamura K	Visiting Fellow	LDB, NIDR
	Kibbey MC	Biologist	LDB, NIDR
	Grant DS	Visiting Associate	LDB, NIDR
	Mosley GL	Bio Lab Tech	LDB, NIDR
	Bui T	Biologist	LDB, NIDR

COOPERATING UNITS *(if any)*

Molecular Oncology Inc, Gaithersburg, MD (Fridman R); UCSF, San Francisco, CA (Kim K); NIA, (Passaniti A); Developmental Biology Center, University Wisconsin, Madison, WI (Auerbach R); NCI (Yamelli Y).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.55	2.25	0.30

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 are conducted to define the mechanisms involved in tumor growth and metastasis and to develop new animal models of human cancers. We have found that a basement membrane extract (Matrigel) when premixed with human tumor cells (which do not grow well in mice) promotes their incidence and growth. Very low cell numbers can be used. We have been able to culture new highly differentiated human tumor cells lines from the tumors grown in mice. Laminin, a major basement membrane component, has been found to promote the malignant phenotype. Various biologically active laminin-derived synthetic peptides have been identified. YIGSR from the B1 chain blocks lung colonization, reduces tumor growth, and inhibits angiogenesis. It inhibits tumor growth in the subcutaneous Matrigel model when daily injections are begun 10 days after tumor cell inoculation when the tumors are palpable. Another laminin-derived peptide containing SIKVAV from the A chain has been found to increase tumor growth, lung colonization, and angiogenesis as well as collagenase IV activity and plasminogen activation. Adhesion of cells to these peptides has been used to select for melanoma sub-populations of different malignant potential. The YIGSR adherent cells form more tumors in lung colony assays and larger tumors in the subcutaneous model than the parent cells. The YIGSR non-adherent cells formed the least number of tumors. Our data demonstrate the roles of laminin receptors on tumor cells and of angiogenic factors in regulating tumor growth and spread. Using this information and the newly developed models of human tumors, the development of new therapeutic strategies for cancer should be facilitated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00483-04 LDB

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.)*

Gene Regulation and Function of Cartilage

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

PI:	Yamada Y	Section Chief	LDB, NIDR
Others:	Hatano O	Visiting Fellow	LDB, NIDR
	Matsuki U	Visiting Fellow	LDB, NIDR
	Sanchez Y	Visiting Fellow	LDB, NIDR
	Line S	Latin American Fellow	LDB, NIDR
	Rhodes C	Biologist	LDB, NIDR
	Bui T	Biologist	LDB, NIDR
	Mosley G	Bio Lab Tech	LDB, NIDR

COOPERATING UNITS *(if any)*

Shriner's Hospital (Doege); Johns Hopkins University (Francomano C); Aichi Medical University (Kimata K); Wistar Institute (Caton CA); University of Tennessee (Yoo TJ).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.45	3.02	0.43

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Cartilage is a unique tissue that consists of extensive extracellular matrix whose function is to absorb shock in the joint. Genetic defects of cartilage proteins are responsible for certain types of osteoarthritis. Abnormal expression and degradation of cartilage components also lead to impaired function of joints. The purpose of this project is to understand the molecular mechanisms underlying cartilage formation in normal development and to identify factors involved in the differentiation of chondrocytes. Several major cartilage components have been cloned and their primary structures have been determined. The structure and function of these proteins have been studied using both synthetic peptides and recombinant proteins produced in bacteria and mammalian cells. Sequences regulating transcription of the collagen II gene have been identified in the promoter and the enhancer region. These sequences have been shown to interact with multiple nuclear factors. Several protein factors which bind to the enhancer have been cloned and characterized. The function of these factors is being tested by DNA transfection. Glucocorticoid responsive sequences have been identified in the promoter and the first intron of the link protein gene. Without these sequences, the promoter activity of the link protein gene is significantly reduced in chondrocytes. Retinoic acid decreases the expression of the link protein gene. The region with which retinoic acid interacts to produce this effect has been localized within the first intron of the link protein gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00484-04 LDB

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.)*

Animal Models of Connective Tissue Disease in Transgenic Mice

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

PI:	Yamada Y	Section Chief	LDB, NIDR
Others:			
	Yamada K	Chief	LDB, NIDR
	Hatano O	Visiting Fellow	LDB, NIDR
	Bui T	Biologist	LDB, NIDR
	Gabriel V	Bio Lab Tech	LDB, NIDR
	Mosley G	Bio Lab Tech	LDB, NIDR
	Strong D	Bio Lab Tech	LDB, NIDR

COOPERATING UNITS *(if any)*

Shriner's Hospital (Doege K); Osaka University (Kimura T); Wistar Institute (Caton GA); University of Tennessee (Yoo TT).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.3	0.7	2.6

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.)*

Creation of transgenic mice by introduction of new or mutated genes into the germ line of a mouse has been proven to be useful for understanding function of proteins and developmental regulation of genes. The purpose of this project is to create transgenic mice as animal models for studying the molecular basis of genetic and acquired connective tissue diseases. These transgenic mice will also be used to elucidate the mechanisms by which the genes for extracellular matrix proteins and their receptors are regulated in a developmental-specific manner.

Creation of transgenic animals which carry mutated exogenous genes for basement membrane and cartilage components have been exploited. Constructs containing a reporter gene under the direction of the promoter and the enhancer of these genes have been injected into mouse oocytes to identify sequences necessary for tissue specific regulation of these genes. These regulatory sequences are being used to express a foreign gene in specific tissues of transgenic mice, and these transgenic animals may be used as models for human diseases such as arthritis and diabetes. The creation of specific alterations in basement membrane and cartilage genes at their native chromosomal loci in mice by homologous recombination has also been attempted. By using gene targeting in embryonic stem cells, mutations of specific sites within genes can be introduced in the mouse germ line to assess the role of these genes in the whole animal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00485-04 LDB

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.)*

Gene Regulation and Function of Basement Membrane

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

PI:	Burbelo PD	Senior Staff Fellow	LDB, NIDR
Others:			
	Yamada Y	Chief	LDB. NIDR
	Utani A	Visiting Fellow	LDB, NIDR
	Nomizu M	Visiting Associate	LDB, NIDR
	Shibuya M	NCI-JFCR	LDB, NIDR
	Gabriel G	Q Student	LDB, NIDR
	Kubota S	Visiting Associate	LDB, NIDR

COOPERATING UNITS *(if any)*

NINCDS (Wujek J, Kedar V); Max-Plank-Institute (Timpl R); Univ. of Pittsburgh (Hassell J); Univ of Genova (Noonan D); MD Anderson Cancer Center (Carson D); INSERM U49 (Clement B).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.66	4.46	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.)*

Basement membranes are the principal extracellular matrices for most tissues, providing physical support, molecular filtering, and cell regulatory functions. Basement membranes consist of a unique set of proteins, and their composition and function are different in various tissues. Recombinant DNA techniques have been used to determine the structure and functions of basement membranes proteins. New components of basement membranes have been identified and studies of their tissue specificity and function have been in progress. Approaches of synthetic peptides and expression of recombinant proteins have been applied to understand the mechanisms of the molecular assembly of basement membrane molecules. DNA elements which regulate genes for basement membrane proteins have been localized in the promoter and enhancer regions and examined for their gene specificity. Nuclear factors which bind to some of these DNA elements have been cloned and sequenced. The protein factors have been expressed in bacteria and mammalian cells to study their cell type specificity and their function.

"Professional Personnel, continued"

Mosley GM	Bio Lab Tech	LDB, NIDR
Fukuda K	Visiting Fellow	LDB, NIDR
Bui T	Biologist	LDB, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00508-03 LDB

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 Human Immunodeficiency Virus I (HIV-1)

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

PI:	Klotman PE	Special Expert	LDB, NIDR
Others:			
	Kopp JB	Staff Fellow	LDB, NIDR
	Weeks BS	Biologist	LDB, NIDR
	Ray PE	Staff Fellow	LDB, NIDR
	Bruggeman LA	Staff Fellow	LDB, NIDR
	Kleinman HK	Section Chief	LDB, NIDR
	Yamada KM	Chief	LDB, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Tumor Cell Biology, NCI (Klotman M, Browning P, Gallo R); Laboratory of Chemoprevention, NCI (Roberts A, Sporn M).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Disease Pathogenesis Group

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Patients infected with human immunodeficiency virus type 1 (HIV-1) develop a syndrome of cachexia and wasting associated with recurrent infections with opportunistic organisms, thought to be the result of CD4+ T-cell depletion. In addition, patients develop tissue-specific syndromes that may be more directly related to interactions of the host tissues with replicating virus or viral gene products such as Kaposi's Sarcoma, AIDS dementia complex, cutaneous disorders associated with AIDS, and HIV-associated nephropathy. The objective of these studies is to explore the cellular and molecular mechanisms responsible for the the generation of these HIV-associated diseases. To explore the role of viral gene products in the absence of replicating virus, we have created transgenic mice using a non-infectious HIV-1 proviral construct lacking the gag and pol genes but encoding env, and the accessory genes tat, rev, vif, vpr, and vpu. In the F2 generation, homozygous affected animals develop growth retardation, skin lesions, lymphoid hyperplasia, thymic involution, splenomegaly, profound wasting, and early death by day 25. Heterozygous animals appear phenotypically normal at birth but approximately 60% develop renal disease within 200 days of life. The majority of heterozygous animals also develop epidermal papillomatous lesions (60%) and a rare myopathy associated with weight loss and wasting (2%). These studies substantiate an important role for viral proteins in AIDS pathogenesis. As a result, we are investigating mechanisms for the targeting of injected T-cells to specific host tissues. We have shown that T-cells infected with HIV-1 attach to fibronectin and that attachment is mediated by increased expression of $\alpha 5\beta 1$ integrin on the cell surface. Once attached to the basement membrane, HIV-1 infected T-cells produce collagenase and invade artificial basement membranes. Current studies in the laboratory are directed to a better understanding of the specific viral proteins responsible for inducing these syndromes as well as the development of strategies for therapeutic intervention. New development of strategies for therapeutic intervention. New transgenic mice are being generated that target HIV genes to specific sites. These studies are intended to explore HIV pathogenesis and to to develop novel therapeutic strategies directed at the prevention of HIV associated syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00524-02 LDB

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.)*

Functions and Developmental Regulation of Matrix Receptors

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

PI:	Yamada KM	Chief	LDB, NIDR
Others:			
	Brown KE	IRTA Fellow	LDB, NIDR
	Yamada S	Visiting Fellow	LDB, NIDR
	Kennedy DW	Biol Lab Tech	LDB, NIDR
	Tran MN	Biologist	LDB, NIDR

COOPERATING UNITS *(if any)*

University of Manchester, England (Humphries M); CNRS, Paris (Thiery JP); Kyoto University, Japan (Takeichi M); University of Turku, Finland (Larjava H); Scripps Research Institute, La Jolla, CA (Ginsberg M).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.25	2.3	0.95

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Integrins and other receptors for extracellular matrix proteins such as fibronectin, vitronectin, and collagen are thought to play important roles in embryonic development, wound healing, and tumor cell metastasis. Recent studies suggest important roles for integrins in early development including neural crest migration, as well as keratinocyte migration during wound healing. Defects in integrin-related functions might contribute to a variety of human congenital defects involving mistakes in morphogenesis. Tissue-specific receptors are likely to provide a means of providing specificity of interactions with individual molecules such as fibronectin and collagen. Collaborative studies have identified unique and common features of fibronectin- and collagen-binding receptors from fibroblasts and platelets; these data may facilitate the development of tissue-specific inhibitors. Monoclonal antibody inhibition experiments with gingival keratinocytes established that specific integrin receptors are used for adhesion to fibronectin and collagen. The binding of antibodies to β1 integrins stimulated the secretion of type IV collagenase by gingival keratinocytes, whereas binding of a variety of known integrin ligands did not. Integrin signalling may therefore help regulate secretion of an enzyme implicated in gingival wound healing. We are exploring various approaches to develop novel recombinant DNA and immunological probes against various integrin subunits to establish their roles in early development using animal models, including in the formation of craniofacial structures. Other studies will characterize the roles of the integrins and of cell-to-cell adhesion systems in mutual cross-regulation of function during morphogenesis and other developmental events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00525-02 LDB

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.)*
Mechanisms and Regulation of Cell Adhesion, Migration, and Morphogenesis

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

PI:	Yamada KM	Chief	LDB, NIDR
Others:			
	Aota S	Visiting Associate	LDB, NIDR
	Thomas LA	Biologist	LDB, NIDR
	Lee C-C	Visiting Fellow	LDB, NIDR
	Yamakawa N	Visiting Fellow	LDB, NIDR

COOPERATING UNITS *(if any)*
CBER, FDA (Komoriya A, Shinagawa S); Univ. of Manchester, England (Humphries M); Dept. Anatomy, Univ. of PA (Lash J); Dept. Anatomy and Cell Biology, Georgetown University (Chen W-T); Aichi Medical University, Japan (Kimata K).

LAB/BRANCH
Laboratory of Developmental Biology

SECTION
Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.48	3.48	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.)*

Cell adhesion, cell migration, and specific morphogenetic events are crucial processes in normal embryonic and fetal development, and errors in them can produce anomalies. Specific structural molecules that mediate these processes and the mechanisms that regulate them are being identified and characterized. Fibronectin and related molecules are thought to be crucial for normal morphogenesis, e.g. for neural crest cell migration. Regions of fibronectin required for cell adhesion are being characterized in detail by site-directed deletion and biological analysis of synthetic peptide inhibitors. Our recent studies demonstrate requirements for helper or synergy regions for the functions of both the central cell-binding domain and the alternatively spliced IIICS regions of fibronectin. Such synergy regions cooperate with the short tripeptide sequences Arg-Gly-Asp and Leu-Asp-Val, increasing their activities up to 20-100 fold. The mechanisms of synergy site function in cell adhesion and migration are being analyzed by fibronectin-fibronectin and fibronectin-vitronectin chimeras, as well as by studies of variant synthetic peptides. The functions of another matrix molecule that we found to contain a biologically active Arg-Gly-Asp sequence, type XII collagen, are being analyzed and compared to fibronectin by monoclonal antibodies. Cell migratory interactions with extracellular molecules can be regulated by a novel process we have discovered and termed "contact stimulation of migration." Neural crest cells or derivatives can show up to 200-fold stimulation of migration after such contact. In addition, cytokine receptors such as the c-met proto-oncogene product can also regulate migration. Molecular requirements for function of these regulatory mechanisms are under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00559-01 LDB

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.)*
Biological Activities of HIV Proteins

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

PI:	Kleinman HK	Section Chief	LDB, NIDR
Others:			
	Weeks BS	Biologist	LDB, NIDR
	Holloway E	Biol. Lab Tech.	LDB, NIDR
	Johnson BA	IRTA Fellow	LDB, NIDR
	Yamada KM	Chief	LDB, NIDR

COOPERATING UNITS *(if any)*
St. Louis University, (Green M, Desai K); NCI, NINCDS (Lieberman D).

LAB/BRANCH
Laboratory of Developmental Biology

SECTION
Cell Biology Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.52	1.12	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 patients with HIV infection (AIDS), there is an unexplained dementia. Active virus is not observed in brain cells so it is proposed that a soluble factor released from the virus may be affecting the patients. We find that the HIV viral protein TAT (which is a transactivator of the HIV-LTR promoter) promotes neural cell adhesion in vitro and blocks laminin-mediated process outgrowth. Using recombinant TAT, synthetic TAT (it is 86 amino acids long) and smaller synthetic peptides duplicating sequences in TAT, we find that residues 49-57 are responsible for the biological activity. Using peptide affinity chromatography and coimmuno-precipitation of labeled cell membranes, a 90 kd TAT receptor on neuronal cells was identified. Direct injection of TAT into the brains of rats caused impaired motor function and destruction of large amounts of brain tissue. These data demonstrate that TAT has a strong effect on neural cells and suggest a possible mechanism to explain the neurologic changes and dementia observed in AIDS patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00560-01 LDB

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.)*

Molecular and Functional Analysis of Integrin Cytoplasmic Domains

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

PI:	LaFlamme SE	Senior Staff Fellow	LDB, NIDR
Others:			
	Yamada KM	Chief	LDB, NIDR
	Tran MN	Biologist	LDB, NIDR
	Akiyama SK	Senior Staff Fellow	LDB, NIDR

COOPERATING UNITS *(if any)*

Oncology, John Hopkins Hospital, Baltimore, MD, (Tucker R, Wilhide C).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

Integrins are the major class of receptors by which cells interact with the extracellular matrix, promoting cell adhesion and cell migration. Signalling from extracellular matrix molecules to the inside of cells can occur via integrin receptors. These signalling events are thought to play roles in processes important for embryonic development such as induction of cell-cell adhesion molecules, stimulation of enzyme secretion, and tissue organization. Integrins generally contain two relatively short cytoplasmic domains. The roles of these domains in controlling the location of receptors, signalling, and regulating cell behavior is being explored using molecular biology and biochemical methods. Functions of isolated domains are being tested using chimeric receptors containing a reporter domain consisting of a subunit of the interleukin-2 receptor and various integrin cytoplasmic tails. The β1 cytoplasmic domain of the fibronectin receptor contains sufficient information to target receptors to adhesion sites of cells, whereas the α5 domain of this same integrin does not. Preliminary studies indicate that β3 tails can also provide such targeting information, and that this activity can be regulated by alternative splicing of precursor RNA. Since the chimeric β1 receptor concentrated at sites where endogenous ligand-occupied receptors normally localize, the possibility was tested that localization of endogenous receptors is regulated by occupancy with its ligand molecule. Fibronectin fragments or synthetic peptide containing the binding site for the fibronectin receptor were able to drive redistribution of the integrin fibronectin receptor directly by occupancy; similar results were obtained with β3 integrin receptors. Further studies are characterizing the roles of other integrin domains in localization as well as in regulation of phosphorylation and other messenger systems. These studies should help provide an in-depth understanding of how cells communicate with their extracellular environment in normal and abnormal embryonic development, where such signalling is essential for coordinating the complex rearrangements and final organization of oral, facial, and other developing tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE 00563-01 LDB

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.)*

Molecular Mechanisms of Cell-Substrate Interactions

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

PI:	Akiyama SK	Senior Staff Fellow	LDB, NIDR
Others:			
	Yamada KM	Chief	LDB, NIDR
	Tran MN	Biologist	LDB, NIDR
	Aota S	Visiting Associate	LDB, NIDR
	Lee C-C	Visiting Fellow	LDB, NIDR
	LaFlamme SE	Senior Staff Fellow	LDB, NIDR

COOPERATING UNITS *(if any)*

Dept Cell Biol & Neuroscience, U of Texas Southwestern Med Ctr, Dallas TX (Grinnell F); Lab de Biol Experimentale, U P. et M Curie, Paris (Darribere T, Boucaut JC); Dept Anatomy, U of Okla Health Sci Ctr (Tomasek JJ); INSERM (Lesot H).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.4	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.)*

Interactions of fibronectin with its integrin receptors play important roles in embryonic development, wound healing and the progression of diseases such as cancer. Approaches involving monoclonal antibodies, molecular and cell biology, and physical chemistry are being employed to elucidate molecular mechanisms underlying the interactions of fibronectin with integrins with the goal of understanding the roles of these glycoproteins in complex biological processes in order to develop bioadhesive substrates and to provide the bases for rational medical intervention in diseases involving abnormal cellular adhesion and migration. Besides the RGD and the synergistic regions on fibronectin, a third site to the amino-terminal side of the synergistic region has been identified that is required for assembly of fibronectin matrices in vivo, though not for binding of fibronectin to integrins. Fibronectin-integrin interactions are not required for fibroblast-mediated collagen gel contraction nor for odontoblast differentiation, although such interactions play a role in the adhesion of these cells. Up-regulation of keratinocyte migration during wound healing depends on the redistribution of integrins, expression of the $\alpha 5\beta 1$ fibronectin receptor integrin, and the acquisition of a migratory phenotype. Studies currently underway will examine the three-dimensional structure of the fibronectin cell-adhesive region in solution, and the role of integrins in transmembrane signal transduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00034-24 LI

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.)

Mechanisms of Histamine Release

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

Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI NIDR
Hook, William A.; Research Microbiologist, LI NIDR
Berenstein, Elsa H.; Microbiologist, LI NIDR
Swieter, Mark; Senior Staff Fellow, LI NIDR
Benhamou, Marc; Visiting Fellow, LI NIDR
Bader, Greta; Biologist, LI NIDR
Kihara, Hidetoshi; Visiting Fellow, LI NIDR
Minoguchi, Kenji; Visiting Fellow, LI NIDR

COOPERATING UNITS (if any)

NICHD ODCPR, NIH (M. Karten)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.22	6.22	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.)

Histamine release from mast cells and blood basophils is being studied as one of the immunological mechanisms involved in inflammation. It is also a model for cell secretion. Among the histamine releasing agents employed are IgE antibody, other secretagogues, LHRH peptides, and the calcium ionophore A23187. Cultured rat basophilic leukemia cells are used as a model for the studies of the IgE receptor and of biochemical changes during cell activation. Large numbers of cells can be obtained for biochemical studies and biochemical variants have been selected which are defective at different sites in the pathway of cell activation and secretion.

Professional Personnel, continued

Tomlinson, Nicola	Visiting Fellow	LI	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00046-21 LI

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.)

Normal and Pathologic Mechanisms of Inflammation and Repair

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

PI: Sharon M. Wahl LI, NIDR
Henry Wong LI, NIDR
Janice Allen LI, NIDR
Sue Dougherty LI, NIDR
Gina Costa LI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.48	1.31	1.17

CHECK APPROPRIATE BOX(ES)

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

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

Evolution of inflammatory and immune reactions is dependent upon the recruitment, migration and activation of circulating leukocytes at a site of injury or antigen deposition. Long range goals of this laboratory foucs on characterizing and modulating these events. By suppressing monocyte functions and promoting the expression of an IL-1 receptor antagonist (IL-1ra) that blocks the action f IL-1 by speicifically binding to the IL-1 receptor without initiating signal transduction, IL-4 may contribute to the down-regulation and resolution of an inflammatory response. In vitro analysis of IL-4 regulation of monocyte phenotype and function revealed a dose-dependent induction of IL-1ra mRNA within 2 to 4 hours without a concomitant effect on the expression of IL-1 mRNA. Increased IL-1ra mRNA was not due to RNA stabilization, but occurred at the level of transcription. In the presence of LPS, IL-4 not only augmented IL-1ra levels, but markedly inhibited LPS-induced IL-1 mRNA expression. Peripheral blood monocytes from cancer patients, obtained prior to and immediately after a regimen of IL-4 immunotherapy, were also examined for IL-1ra gene expression. After IL-4 treatment, monocytes from the patients showed a marked increase in the expression of IL-1ra mRNA. This induction of IL-1ra in circulating monocytes was reflected by significantly enhanced serum levels of IL-1ra ($P<0.05$) during IL-4 therapy which subsequently declined. The selective upregulation of IL-1ra by resting or activated monocytes, coupled with inhibition of IL-1 production by activated monocytes, as we have demonstrated both in vitro and in vivo, suggest that IL-4 may prove clinically useful as an anti-inflammatory agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00199-16 LI

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.)

In Vitro Studies of Secretory Cell Structure and Function

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

Oliver, Constance; Guest Worker, LI NIDR
Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI NIDR
Waters, Judith F.; Biologist, LI NIDR
Weedon, Lynda L.; Biologist, LI NIDR
Swaim, William D.; Senior Staff Fellow, LI NIDR

COOPERATING UNITS (if any)

Dr. A. Robbins, LBM NIDDK

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	1.2	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.)

Secretory and endocytic processes in several cell types are currently under investigation. The rat basophilic leukemia cell line (RBL-2H3), and other cultured cells are being used to study various aspects of endocytic and secretory processes. Emphasis is placed on morphological, cytochemical and biochemical characterization of these processes in the cultured cells. Events involved in receptor activation, signal transduction and endocytic mechanisms are under investigation. The lysosomal system and its role in endocytic and secretory pathways is also under study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00290-13 LI

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.)

Production of Hybridomas

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

Siraganian, Reuben P.; Chief, Receptors and Signal Transduction Section, LI NIDR
Hook, William A.; Research Microbiologist, LI NIDR
Berenstein, Elsa H.; Microbiologist, LI NIDR
Fischler, Cynthia; Biological Lab Technician, LI NIDR
Hamawy, Majed; Staff Fellow, LI NIDR
Nishikata, Hikaru; Visiting Fellow, LI NIDR
Mergenhagen, Stephan E.; Chief, Laboratory of Immunology, LI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.22	3.22	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.)

Hybridomas are being produced which secrete monoclonal antibodies of defined antigen specificity. Hybridomas have been produced against the Fcε receptor of mast cells and to human IgE. These monoclonal antibodies are being used for biochemical and biological studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00392-09 LI

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.)

Role of Mucosal Macrophages in Host Defenses

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

PI: Phillip D. Smith	Senior Investigator	LI, NIDR
Nandita Chopra	Microbiologist	LI, NIDR
Michael Handy	Microbiologist	LI, NIDR
Larry M. Wahl	Research Biologist	LI, NIDR
Sharon M. Wahl	Chief, CIS	LI, NIDR

COOPERATING UNITS (if any)

Edward N. Janoff, M.D., University of Minnesota Medical School; Thomas Kossmann, M.D., University of Zurich and Cristina Morganit-Kossmann, University of Zurich

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	1.2	1.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The focus of this laboratory is characterization of the phenotypic and functional features of mucosal macrophages. The major accomplishments of the past year include the following. 1.) We succeeded in routinely purifying lamina propria cells with morphologic and surface antigen features of macrophages. 2.) In addition to expressing accessory cell function, lamina propria macrophages were shown to secrete cytokines, including IL-1, which is necessary for successful antigen presentation, as well as TNF-α and TGF-β. Gene expression studies suggested transcriptional regulation of the TNF-α peptide. 3.) We identified TNF-α mRNA in mucosal lesions associated with H. pylori gastritis and cytomegalovirus (CMV) colitis, suggesting a potential role for TNF-α in mediating mucosal inflammation. 4.) Relevant to CMV mucosal disease, we showed that CMV, but not HIV, primed monocytes for enhanced LPS-induced TNF-α secretion. In related studies, CMV infection of astrocytes was shown to induce TGF-β, which then upregulated astrocyte expression of the virus. Whether a similar regulatory mechanism applies to CMV expression by CMV-infected lamina propria macrophages will be pursued in future studies. 5.) We also demonstrated that lamina propria macrophages are capable of infection with HIV and that IgA may enhance this infection. 6.) Arachidonic acid metabolites were shown to be secreted by lamina propria macrophages, but not mucosal epithelial cells, in response to H. pylori. These studies implicate potentially important roles for lamina propria macrophages in mediating mucosal responses to mucosal pathogens and foreign antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00424-07 LI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Molecular Analysis of Monocyte Phenotype and Function

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

PI: Nancy McCartney-Francis; Special Expert, LI NIDR
Sharon M. Wahl; Chief, CIS, LI NIDR
Larry M. Wahl; Research Biologist, LI NIDR
Diane Mizel; Chemist, LI NIDR
Suanne Dougherty; Chemist, LI NIDR
Janice B. Allen; Biologist, LI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.27	1.1	1.17

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 objective of this research program is to define the molecular mechanisms that regulate the functional and phenotypic properties of monocytes. Previous studies have elucidated several proinflammatory activities of TGF-β including chemotaxis and cytokine induction in monocytes. Prolonged exposure to TGF-β enhances the responsiveness of monocytes to other inflammatory stimuli including interferon (IFN γ), interleukin-1 (IL-1), and lipopolysaccharide (LPS). Pretreatment with TGF-β for 6-48 hr and IL-6, within 30 min after exposure to LPS as compared to untreated cells. In contrast, simultaneous exposure to TGF-β and another activation signal suppresses the cytokine response. These activation process in monocytes, with TGF-β providing the initial cytokine. The interaction between these primed cells and inflammatory mediators may contribute to chronic inflammation. Although a variety of cytokines have been identified in inflamed tissue, another compound, nitric oxide, may also play a role in the inflammatory process, providing another type of signal to mediate monocyte function, in this case, microbicidal activity. Preliminary studies have identified increased levels of nitric oxide as measured by nitrite (7 to 30 μM) in synovial cell and tissue culture fluids as well as synovial fluids from rheumatoid arthritis patients. These studies provide evidence for the existence of inducible nitric oxide biosynthesis in human inflammatory disease. Ongoing studies are addressing the possible therapeutic intervention of nitric oxide synthase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00441-06 LI

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Immunoregulation of Experimentally Induced Immune Responses

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

PI: Janice B. Allen; Chemist, LI NIDR
Sharon M. Wahl; Chief, CIS, LI NIDR
Tomozumi Imamichi; Fogarty Fellow, LI NIDR
Nancy Francis; Special Expert, LI NIDR
Stephan E. Mergenhagen; Chief,LI NIDR

COOPERATING UNITS (if any)

M. Bienkowski, A. Berger, Hoffman-LaRoche; T. Kossmann, Switzerland; C. Manthey, USUHS; J. Dasch, Celtrix Laboratories; B. Sartor, UNC-Chapel Hill.

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

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

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

Group A streptococcal cell wall (SCW) fragments induce biphasic of chronic inflammation in the LEW, but not the genetically similar F344, rat. In continuing studies to identify the cellular and molecular mechanisms of differential genetic susceptibility, individual cell populations and tissues from the two strains were compared for their responsiveness to SCW in vitro and in vivo. Two inflammatory mediators, TGF-β and IL-1 have been identified in inflamed synovium and gingiva. A single intraarticular injection of a monoclonal antibody to TGF-β reduces both the acute and chronic SCW-induced arthrits. Histological analysis revealed a decrease in mononuclear infiltration, and bone and cartilage destruction. In parallel studies, systemic administration of IL-4, which inhibits chronic arthritis, due in part to the induction of IL-1ra, which neutralizes IL-1 activity. Histopathological evaluation confirmed the clinical observations. These studies show that by neutralizing certain pro-inflammatory cytokines, chronic lesions can be suppressed. Additional studies revealed that Kupffer cells are an important source of inflammatory cytokines during SCW-induced hepatic inflammation by expressing and secreting the cytokines TNFα, IL-1β and TGF-β. Granuloma formation id dependent, therefore, upon a mechanism of attraction and migration of mononuclear cells. Utilizing another experimental model, studies were performed to investigate the mechanisms involved in the pathogeneisis of chronic intestinal inflammation after a single subserosal injection of SCW/into LEW rats. Collectively, these studies try to elucidate the mechanisms of pathogenesis of chronic, inflammatory diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00456-05 LI

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.)*

Signal transduction in the monocyte/macrophage.

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

PI:	Larry M. Wahl	Research Biologist	LI,NIDR
	Marta L. Corcoran	Chemist	LI, NIDR
	Mary R. Stofega	Biologist	LI, NIDR
	Susan Hopkinson	Chemist	LI, NIDR

COOPERATING UNITS *(if any)*

D.S. Finbloom, FDA; I. Katona, USUHS; A. Spiegel, NIDDK; J. Weinstein and J. Szebeni, NCI, W. Stetler-Stevenson, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.80	.80	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project focusses on the biochemical pathways involved in signal transduction in the monocyte that lead to the production of metalloproteinases. Our previous studies have demonstrated that the production of these enzymes occurs through a a PGE2-cAMP dependent pathway involving G proteins. These studies demonstrated a potential coupling between prostaglandin synthetase (PGS) and a 46-kDa Gsα G protein that is ribosylated only in activated monocytes. Our recent findings have shown that the Con A induced ADP-ribosylation of the 46-kDa Gsα is suppressed by PGS inhibitors such as indomethacin and aspirin. This finding, along with the ability of cholera toxin (CT) to enhance prostaglandin synthesis only in Con A stimulated monocytes, indicates that the stimulant induced coupling between PGS and the 46-kDA GSα is required for the activation of the 46-kDa Gsα. Studies on PGS have revealed that a constitutive level of PGS-1 is expressed in the membranes of control monocytes. However, stimulation with Con A or LPS results in the induction of an inducible form of PGS (PGS-2). Moreover, the level of PGS-2 in stimulated monocytes is significantly enhanced by CT or TGFβ. Thus, the ability of CT or TGF-β to enhance prostaglandin production and, as a result, metalloproteinase production is due, in part, to the elevation of PGS-2 by these agents. Additionally, the suppression of PGS-2 by PT is most likely related to the inhibition of prostagladins and metalloproteinases by this toxin. Similarly, the ability of IL-4 to significantly inhibit the production of PGS-2 may explain the potent ability of this cytokine to suppress PGE2 production that results in the inhibition of metalloproteinases production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00513-03 LI

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.)*

Role of Monocytes in AIDS and as Targets for Antiviral Therapy

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

PI: Sharon M. Wahl Chief, CIS LI, NIDR
Michael Handy Biologist LI, NIDR

COOPERATING UNITS *(if any)*

Jan Orenstein, GWU; Thomas Kossmann, Cristina Kossmann, Zurich, Switzerland

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.15	.35	.80

CHECK APPROPRIATE BOX(ES)

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

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

Inflammatory processes in the brain require the cooperation of immunocompetent cells and glial cells, which communicate by secreting bidirectional mediators. Resident cells within the nervous system can synthesize and secrete inflammatory cytokines, as well as neuropeptides, contributing to the response within the CNS to injury or immunological challenge. Although the mechanisms of cell activation and immune interaction are poorly understood, accumulating evidence implicates these pathways in neuropathogenesis. For example, in the acquired immunodeficiency syndrome (AIDS), HIV-1-induced nervous system dysfunction and dementia are associated with the presence of infiltrating leukocytes and the release of inflammatory cytokines including transforming growth factor beta. Defining the pathways of cytokine dysregulation and neurotoxicity invoked by the infiltrating leukocytes, as well as the contribution of the neural cells themselves, may help to identify mechanisms of intervention in this and other debilitating CNS diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00533-02 LI

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.)

Regulation of leukocyte recruitment, activation and survival by cytokines

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

PI: Mary E. Brandes Intramural Branch Training Awardee LI, NIDR
Dennis F. Mangan NRSA Senior Postdoctoral Fellow LI, NIDR

COOPERATING UNITS (if any)

Lalage Wakefield, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.38	1.38	

CHECK APPROPRIATE BOX(ES)

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

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

Cytokines elaborated by and affecting peripheral blood monocytes are pivotal mediators in controlling inflammation. The focus of this laboratory is on understanding the regulatory events and mechanisms which control the inflammatory response, specifically: 1) elucidating cytokine signal tranduction pathways whcih, if inhibitied, can alter cytokine effects, 2) examining programmed cell death (PCD) as a means by which the number of cells at an inflammatory site is controlled. With regard to the first focus, our studies have showed that human monocytes express only the type II receptor for tumor necrosis factor-α (TNF-α), (2000 receptors/cell, K_D=230 pM). Although this receptor has been shown not to contain any intrinsic kinase activity in its intracellular domain, our signal transduction studies showed the TNFα receptor/ligand interaction indirectly triggers tyrosine kinase activity/resulting in greatly enhanced tyrosine phosphorylation of a 43 kD protein. Inhibition of this tyrosine kinase activity eliminated the TNFα-stimulated functions of reactive oxygen intermediate generation and inflammatory cytokine mRNA production. Additional studies have identified a pivotal role for specific phosphatases as an off-signal for cytokine-triggered cytokine production. Inhibition of these enzymes greatly increases the magnitude and duration of cytokine synthesis triggered by inflammatory stimuli. Prolonged cytokine synthesis may further regulate inflammatory cell accumulation and activation by preventing activation of an endogenous endonuclease responsible for PCD.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00561-01 LI

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.)

Taste and Smell

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

Ryba, Nicholas; Visiting Fellow; LI NIDR
Hall, Matthew; Visiting Fellow: LI NIDR
Hirano, Fuki; Guest Worker, LI NIDR
Siraganian, Reuben P.; Chief, Receptors and Signal Transduction, LI NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.12	2.12	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 aim of this project is to investigate the molecular mechanisms involved in signal reception and transduction of the chemical senses, taste and smell. Major goals are characterization of the receptors involved, their distribution and specificity. Studies will also characterize the coupling of these receptors to production of secondary messengers, including the proteins with which the receptors interact and the mechanisms of desensitization and adaptation of the signal pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00254-15 LME

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.)*

Microbial Antigens Associated with Specific Adherence

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

Cisar, John O.	Research Microbiologist	LME, NIDR
Hsu, S. Dana	Microbiologist	LME, NIDR
Sandberg, Ann L.	Chief, Microbial Receptors & Pathogenesis Sec.	LME, NIDR

COOPERATING UNITS *(if any)*

University of Florida; University of Maryland; Royal Dental College Aarhus, Denmark

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.00	1.00	1.00

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Seventy-one strains of viridans streptococci, classified as *Streptococcus sanguis, S. gordonii, S. oralis, S. mitis* or *S. anginosus*, by a revised taxonomic scheme, were characterized and compared by their specific adherence related properties. Significant differences were noted between species. Strains of *S. sanguis, S. gordonii* and *S. oralis* commonly expressed neuraminidase sensitive hemagglutinating activities while aggregation of acidic proline rich protein coated latex beads was noted primarily with strains of the latter two species. More strains of *S. sanguis, S. gordonii* and *S. oralis* were specifically adherent to saliva-coated hydroxyapatite than were strains of *S. mitis* and *S. anginosus*. Lactose resistant coaggregations with actinomyces were noted most frequently with *S. gordonii* and *S. anginosus*, species not involved in the primary colonization of teeth. In contrast, lactose sensitive coaggregations with actinomyces were observed with all strains of *S. oralis* and less frequently with the other streptococcal species. Many of these streptococci also participated in GalNAc sensitive coaagregations with certain *S. sanguis* and *S. gordonii* strains. Actinomyces with Gal and GalNAc sensitive lectins and streptococci with GalNAc sensitive lectins both coaggregated with *S. oralis* 34 but these bacteria did not coaggregate with a spontaneous mutant of strain 34 that lacked a cell wall polysaccharide previously shown to contain GalNAcβ1→3Galα receptor regions. Other strains known to possess cell wall polysaccharides containing this disaccharide also coaggregated with streptococci expressing GalNAc sensitive lectins. Unlike actinomyces lectins, the streptococcal lectins failed to recognize Galβ1→3GalNAcα in polysaccharide receptors of other streptococci. The association of certain adherence properties with different taxonomic groups of viridans streptococci and definition of the specificities of some of these interactions provide a basis for further ecological characterization of these oral bacteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00273-14 LME

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Cell-Cell Interactions Between Oral Actinomyces and Other Bacteria

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

Kolenbrander, Paul	Research Microbiologist	LME, NIDR
Andersen, Roxanna	Microbiologist	LME, NIDR
Brenchley, Jean	Guest Researcher	LME, NIDR
Clemans, Daniel	Guest Researcher	LME, NIDR
Ganeshkumar, Nadarajah	Visiting Associate	LME, NIDR
Klier, Christiane	Visiting Fellow	LME, NIDR
London, Jack	Chief, Clinical Microbiology Section	LME, NIDR

COOPERATING UNITS *(if any)*

Dr. L.V.H. Moore, VPI and SU, Blacksburg, VA; Dr. B.C. McBride, Univ. of British Columbia, Vancouver, Canada; Dr. E. Weiss, Tel Aviv Univ., Tel Aviv, Israel; Dr. N. Stromberg, Univ. of Goteborg, Sweden; Dr. A. Callaway, Erlangen, Germany.

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	1.00

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The focus of our research program is to understand the role of coaggregation in bacterial accretion of early colonizing bacteria on a clean tooth surface. The primary colonizers include actinomyces, streptococci, and veillonellae. Antiserum against the 38-kDa surface adhesin from S. *gordonii* PK488 cross reacts with a similar size protein from all of those streptococci (so far tested) that coaggregate with *A. naeslundii* PK606. The gene encoding this protein in S. *gordonii* PK488 has been cloned in a 2.1 kb DNA fragment and expressed in *E. coli*. Southern blots using the radioactively labeled 2.1 kb fragment as a probe identified a single restriction enzyme fragment in the genomic DNA of all streptococcal strains that coaggregate with *A. naeslundii* PK606. Identical fragments reacted with a 30-mer probe prepared from a gene encoding a 34.7-kDa saliva-binding adhesin of *S. sanguis* 12. This protein was shown to be a lipoprotein. These results suggest that a 34 to 38-kDa adhesin/lipoprotein may be present on most, if not all, early colonizing streptococci, and it may be important in mediating colonization of the tooth surface.

The adhesins on several actinomyces including *A. naeslundii* PK606 are being investigated to determine the nature of functionally similar actinomyces adhesins. While intergeneric coaggregation among oral bacteria is commonplace, intrageneric coaggregation among oral bacteria is highly unusual, except among streptococci. Transposon mutagenesis has been used to identify a 110-kDa adhesin on *S. gordonii* DL1 that mediates intrageneric coaggregation with other streptococci. The gene encoding this adhesin is being cloned and sequenced. The long range goal of these studies, collectively, is to elucidate the molecular mechanisms responsible for bacterial colonization in the human oral ecosystem.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00341-11-LME

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Regulation of sugar transport and metabolism in lactic acid and oral bacteria

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

Thompson, John	Visiting Scientist	LME, NIDR
Donkersloot, J.A.	Research Microbiologist	LME, NIDR
Robrish, S.A.	Research Microbiologist	LME, NIDR
Gentry-Weeks, C.R.	Staff Fellow	LME, NIDR

COOPERATING UNITS *(if any)*

Miller, S.P.F.	Staff Fellow	DMNB, NINCDS

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project concentrates on a basic science research program emphasizing the physiology, biochemistry, and genetic basis of regulation of amino acid and sugar fermentation pathways of oral bacteria. This fundamental knowledge is essential for identification of the causes, as well as, formulation of treatments and prevention of oral diseases. The following accomplishments have been made during this period:

1. Two enzymes: (a) sucrose 6-phosphate hydrolase, and (b) ATP-dependent fructokinase, are required for sucrose fermentation by *Fusobacterium mortiferum*. These two enzymes have been purified to electrophoretic homogeneity. The physicochemical properties, catalytic functions and N-terminal amino acid sequences of the proteins have been established.

2. The roles(s) of sulfur-transducing enzymes in pathogenicity of *Fusobacteria* are being defined. One of the enzymes, cystathionase, has been partially purified from *F. mortiferum*.

3. The mechanism of toxicity of extracts of *Bordetella avium* toward mammalian osteoblast cells has been delineated, and attributed to the desulfuration of L-cystine by β-cystathionase. This enzyme has been purified to homogeneity, and the gene (met c) has been cloned and sequenced.

4. The cloning, expression and sequencing of the gene encoding N(5)-(carboxyethyl) ornithine synthase from *Lactococcus lactis* have been accomplished.

5. Two new N-(carboxyalkyl) amino acids have been synthesized via enzyme catalyzed reactions mediated by N(5)-(CE)ornithine synthase. N(5)-(Carboxymethyl) ornithine and N(6)-(carboxymethyl) lysine have been purified, and characterized by $1_H, 13_C$-NMR and GC-mass spectroscopy.

6. The results of the preceding investigations have been presented, and discussed in six peer-reviewed publications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00382-09 LME

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.)*

Growth and interaction of oral microorganisms

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

Robrish, Stanley A.	Research Microbiologist	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR
Gomez, Irma M.	Microbiologist	LME, NIDR
Gentry-Weeks, Claudia	Staff Fellow	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, 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.)*

The first definitive evidence for a phosphenolpyruvate-dependent sugar phosphotransferase sugar transport system in the family *Bacteroidaceae* was shown in our studies of sucrose utilization by *Fusobacterium mortiferum* ATCC 25557. Growth of *F. mortiferum* on sucrose resulted in cells which fermented sucrose to principally acetic and butyric acids and whose phosphorylation of sucrose was dependent on phosphoenolpyruvate. Anaerobic conditions were necessary for sucrose use by *F. mortiferum*. Two enzymes necessary for sucrose use, a sucrose-6-phosphate hydrolase and an ATP dependent fructokinase, were purified to electrophoretic homogeneity from extracts of sucrose grown *F. mortiferum*. The physicochemical and catalytic properties of these enzymes have been examined, and the N-terminal amino acid sequences of both proteins determined.

Disaccharide hydrolases have been demonstrated in sonic extracts of *F. mortiferum* grown on disaccharides and raffinose. A sucrose hydrolase has been demonstrated in extracts of melibiose grown *F. mortiferum* which is very similar to the sucrose-6-phosphate hydrolase obtained from sucrose grown cells. Similar hydrolases and alpha galactosidases have been shown in melibiose and raffinose grown cells of *F. mortiferum* suggesting that these are parts of a raffinose operon in this organism.

A cystathionase has been demonstrated in extracts of *F. mortiferum* which is cross reactive with antibody to a homogeneously purified cystathionase from *Bordetella avium* having toxicity to osteoblasts in tissue culture. Similar activities have been shown in cultures of a variety of other fusobacteria.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00454-06 LME

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.)

Role of Surface Molecules in Metabolism and Ecology of Oral Bacteria

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

London, Jack P.	Chief, Clinical Microbiology Section	LME, NIDR
Allen, Janet	Microbiologist	LME, NIDR
Cavedon, Katherine	IRTA Fellow	LME, NIDR
Citron, Jean	NRSA Fellow	LME, NIDR
Kolenbrander, Paul E.	Microbiologist	LME, NIDR
Lunsford, Dwayne R.	Staff Fellow	LME, NIDR
Linehan, Lisa	NRSA Fellow	LME, NIDR
Riley, Chiara	NRSA Fellow	LME, NIDR

COOPERATING UNITS (if any)

University of Connecticut and Tel Aviv University, Tel-Aviv, Israel

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.45	4.45	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 2.23 kb gene encoding the fimbrial-associated adhesin of *P.loescheii* PK1295 has been cloned and sequenced. The gene contains a ribosomal slip which results in a reading frame shift and a failure to translate the 28 bases found prior to the shift. Attempts to express the gene in a number of *E.coli* host strains have met with limited success. Clones containing vector-gene constructs appear to be near lethal; in the two instances where expression was observed both plasmid vector and gene were quickly lost. Presently, new strategies are being tested to stabilize constructs. The receptor for the *Actinomyces israelii*-specific fimbrial adhesin of *P. loescheii* has been extracted from the cells of *A. israelii* PK14 and purified by column chromatography. Preliminary results indicate that it contains neither protein nor carbohydrate reducing sugar, but the material does contain phosphorus. The purified material is capable of aggregating *P. loescheii* and certain *Streptococcus sanguis* cells.

The gene encoding the xylitol and ribitol pathway enzymes are currently being cloned. A 6.2 kb chromosomal DNA fragment containing the entire ribitol-5-P dehydrogenase (RDH) gene has been ligated into pBluescript II SK and transformed into an *E. coli* host. Expression of the gene product was detected with anti-RDH IgG on immunoblots and active dehydrogenase was detected in cell extracts containing the cloned DNA. DNA fragments containing the xylitol-specific Factor III and xylitol-5-P dehydrogenase are currently being ligated into pBluescript II also.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00498-03 LME

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.)

Identification and Enumeration of Oral Microflora in HIV-1 Infected Subjects

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

London, Jack P. Chief, Clinical Microbiology Section LME, NIDR

Riley, Chiara Staff Fellow LME, NIDR

COOPERATING UNITS (if any)

NCI, NIH

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.05	1.05	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A study to determine the etiology of oral soft tissue lesions in HIV infected patients is underway. A significant number of these ulcerative lesions have been examined and were found to be Candida or Herpes infection, however, many of them cannot be attributed to either of these infectious agents. Attempts to cultivate fungi or bacteria from these tissue samples have been unsuccessful in the former instance and the bacterial flora has been identified as microorganisms normally associated with soft tissues; namely gram positive streptococci. Therefore, the study has focused on identifying viral agents that might be causing these lesions. Lesions of HIV-infected subjects are currently being cultured for *Herpes simplex* 1 and 2 (HSV-1, 2), Herpes Human Virus 6 (HHV-6), *Cytomegalovirus* (CMV) and Epstein-Barr virus (EBV). In addition, serum antibody titers for all of these viruses are being quantitated to correlate presence of the lesion to viral antibody levels.

Roughly 80% of HIV-infected patients being treated with antiviral agent, dideoxyinosine (ddI), suffer from an unusual form of xerostomia. The parameters of this salivary disorder are currently being examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00512-03 LME

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 Analysis of *Bordetella avium* Pathogenicity

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

Gentry-Weeks, Claudia	Senior Staff Fellow	LME, NIDR
Thompson, John	Visiting Scienties	LME, NIDR
Robrish, Stanley	Research Microbiologist	LME, NIDR
White, Deborah	Microbiologist	LME, NIDR
Spokes, Jennifer	Biologist	LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.85	1.25	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.)

Studies of the virulence of *Bordetella avium* revealed a novel cytotoxic protein, designated osteotoxin, that is lethal for MC3T3-E1 osteogenic cells, fetal bovine trabecular cells, embryonic bovine tracheal cells, and UMR106-01(BSP) rat osteosarcoma cells. The metabolic activity of IC-21 and ANA-1 mouse macrophage, and J774A.1 monocyte-macrophage cell lines is partially inhibited by the osteotoxin, whereas RAW264.7 mouse monocyte-macrophage cell cultures and L6 myoblasts are relatively unaffected. The osteotoxin lacks both hemolytic and dermonecrotic toxin activities, and is non-proteolytic. Osteotoxin was purified to homogeneity from *B. avium* 197 by conventional chromatographic procedures. The native protein (M_r 80,000; pI 4.5) is a homodimer, and the two, non-covalently linked subunits contain a pyridoxal phosphate co-factor. The N-terminal amino acid sequence of the osteotoxin was obtained and two oligonucleotides were generated for use as probes in screening a *B. avium* gene library. A *B. avium* gene library was constructed in *E. coli* and recombinant clones were identified that contained a recombinant plasmid, p4-25, which encoded *B. avium* osteotoxin. DNA sequence analysis of the osteotoxin gene indicated that it is composed of 1191 basepairs of DNA and encodes a protein with a molecular mass of 42,600. The *B. avium* osteotoxin is highly homologous (38% identity and 60% similarity) to cystathioninase of *E. coli* and *Salmonella typhimurium*. Exposure of MC3T3-E1 osteogenic cells to *B. avium* osteotoxin (cystathionase) in the presence of ^{35}S-cystine resulted in specific labeling of one major cell protein. We propose that *B. avium* cystathionase causes cell death by hydrolysis of cystine and generation of reactive sulfane sulfurs which inactivate or overactivate essential cellular enzymes. Seven *Fusobacterium* species were examined for the presence of a protein(s) which reacts with antibody against *B. avium* cystathionase. In this test, immunologically related proteins were found in, *F. periodonticum*, *F. necrophorum*, *F. necrogenes*, *F. varium and F. mortiferum*, but not in *F. nucleatum* and *F. russii*. Cystathionase purified from *F. mortiferum* was lethal for MC3T3-E1 osteogenic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00514-03 LME

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.)*

Anthrax Toxin - A Model for Bacterial Pathogenesis

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

Leppla, Stephen H.	Supervisor, Research Chemistry	LME, NIDR
Klimpel, Kurt R.	Staff Fellow	LME, NIDR
Arora, Nauveen	Visiting Associate	LME, NIDR
Uchida, Ikuo	Visiting Fellow	LME, NIDR
Haley, Sheila	Microbiologist	LME, NIDR
Fields, Raymond	Chemist	LME, NIDR
Keith, Jerry M.	Chief, Laboratory of Microbial Ecology	LME, NIDR

COOPERATING UNITS *(if any)*

Laboratory of Cellular and Molecular Biology, NCI, NIH (S. Aaronson)
Department of Microbiology and Molecular Genetics, Harvard Med. School (RJ Collier)
Laboratory of X-ray Crystallography, Dana-Farber Cancer Inst. (RC Liddington)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.8	3.6	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.)*

Structural and functional analyses of bacterial protein toxins were performed to discover how toxins contribute to bacterial pathogenesis. Studies focussed on identifying the routes of internalization and mechanisms of intracellular action of anthrax toxin proteins.

A. Additional evidence was obtained to support the hypothesis that the obligatory cleavage of anthrax toxin protective antigen (PA) is caused by the eukaryotic protease furin. Thus, the relative potencies of a set of protease inhibitors in blocking cleavage of PA bound on cellular receptors matched their inhibitor action on purified furin. PA mutants altered at the cleavage site were digested by purified furin at rates proportional to their toxicity for cells.

B. A fusion protein method was used to show that residues 1-254 of the anthrax toxin lethal factor (LF) are sufficient to cause binding to PA and internalization.

C. Initial work toward using components of anthrax toxin as cell-type specific therapeutic agents was accomplished by placing an amino acid sequence recognized by HIV protease at the cleavage site of PA. It is expected that this mutant PA will target the cytotoxic LF fusion proteins specifically to HIV-infected cells.

D. Characterization of the mechanism of toxin binding and uptake was advanced by isolation of CHO cell mutants lacking PA receptor. To clone and identify the receptor, a cDNA library was transfected into these mutants, and screening was begun for colonies which have regained receptor. Replicate plating and fluorescent cell sorting methods were developed to detect PA binding to its receptor.

E. Collaborative work on the structure of PA was continued with the successful crystallization of several cysteine-substituted PA mutant proteins, and the collection of a complete set of X-ray diffraction data.

F. Analysis of the control of anthrax toxin synthesis was advanced substantially by cloning and sequencing a positive regulatory gene needed for expression of all three toxin proteins. This gene does not have apparent sequence homology to known regulatory genes and may be of a novel regulatory type.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00518-03 LME

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.)*

Development of Detoxified Pertussis Toxin for Acellular Whooping Cough Vaccines

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

Keith, Jerry M.	Chief, Laboratory of Microbial Ecology	LME, NIDR
Nicholls, Peter	Visiting Associate	LME, NIDR
Fields, Raymond	Chemist	LME, NIDR

COOPERATING UNITS *(if any)*

National Institute of Health, Tokyo, Japan (H. Sato)
Washington University, St. Louis, MO (R. Curtiss III)
University of Missouri, Columbia, MO (C. Parker)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.05	0.55	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.)*

Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is pertussis toxin. Clinical trials of acellular pertussis products strongly indicate that pertussis toxin will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" pertussis toxin vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Through our gene expression experiments we discovered a molecular approach for inactivation of pertussis toxin. Using site-specific DNA mutagenesis, the S1 subunit was modified by either a single or double amino acid substitution. These mutations virtually eliminated toxic activity, yet the immunogenic protective epitope was retained. We have devised several methods to transfer these genetic changes into the chromosome of *B. pertussis*, thus creating several new mutant strains. Using these new mutant strains, a genetically detoxified pertussis toxin molecule has been produced. This nontoxic holotoxin has strong immunoprotective properties and can be used as a vaccine antigen without chemical inactivation. Immunoprotein studies as well as characterization of the biological activities associated with these new strains are currently underway in our laboratory and at the National Institute of Health in Tokyo, Japan. In addition to this effort, new constructs have been produced to utilize a live Salmonella oral vaccine. These construct are being tested in an animal model as a collaboration with researchers at Washington University in St. Louis and University of Missouri in Columbia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00537-02 LME

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.)*

Protein Virulence Factors of Bacterial Periodontal Pathogens

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

Leppla, Stephen H.	Supervisory Research Chemist	LME, NIDR
Klimpel, Kurt R.	Staff Fellow	LME, NIDR
Arora, Nauveen	Visiting Associate	LME, NIDR
Uchida, Ikuo	Visiting Fellow	LME, NIDR
Haley, Sheila	Microbiologist	LME, NIDR
Keith, Jerry M.	Chief, Laboratory of Microbial Ecology	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.7	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.)*

Additional strains were recovered and grown from the large set of isolates of bacterial strains collected from patients with both HIV infection and periodontal disease. These isolates were previously isolated and speciated at Virginia Polytechnic Institute (VPI), Blacksburg, Virginia. Most of these bacteria are facultative or obligate anaerobes, and many are difficult to grow. In some cases, adjustments of the growth media and repeated trials were needed to obtain adequate growth.

Although the VPI collection of bacteria is large, certain species previously associated with periodontal disease were not represented. Therefore, additional species were obtained from private individuals and from the ATCC. It is considered important to have a large and representative collection of strains so that the enzymatic and cytotoxicity assays will have a reasonable probability of detecting new virulence factors.

Assays for adenylate cyclase and ADP-ribosylation were completed on representative strains from approximately twelve species. No strains producing these activities were found. For several species, variations in growth medium were explored to mimic the in vivo growth environment and stimulate production of virulence factors. Media were varied by including addition of carbon dioxide and limiting the availability of iron.

Assays for additional virulence factors have been developed. Agar plate media were optimized for detection of protease, hemolysin, iron chelators, and lysozyme resistance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00557-01 LME

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.)*

Specific Interactions of Bacteria with Mammalian Cells

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

Sandberg, Ann L.	Chief, Microbial Receptors & Pathogenesis Sec.	LME, NIDR
Lee, Si Young	Visiting Fellow	LME, NIDR
Ruhl, Stefan	Visiting Associate	LME, NIDR
Cisar, John O	Research Microbiologist	LME, NIDR
Bryant, Joe	Chief, ACU	ACU, NIDR

COOPERATING UNITS *(if any)*

Dr. Mike Eckhaus, ARP, NIH

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.00	3.00	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.)*

Sialo-and asialoglycoconjugates on mammalian cells were found to serve as receptors for sialic acid and Gal/GalNAc reactive lectins on certain strains of oral viridans streptococci and actinomyces, respectively. Investigations initiated to examine the regulation of the expression of these receptors employed cytofluorometry to assess the binding of directly fluoresceinated *Streptococcus gordonii* DL1 and *Actinomyces naeslundii* WVU45 or plant lectins with specificities similar to that of the actinomyces lectin to HL60 cells. Incubation of HL60 cells with DMSO, an agent known to induce differentiation towards polymorphonuclear leukocytes (PMNs), resulted in a significant enhancement of bacterial binding. Similar but less dramatic results were obtained with fluoresceinated plant lectins. The specificities of the interactions were demonstrated by saccharide inhibition as well as the finding of markedly decreased or enhanced binding of the streptococci and actinomyces, respectively, to neuraminidase treated cells. In addition, a streptococcal strain and an actinomyces mutant strain lacking lectin activity failed to bind to the HL60 cells. The interactions between these bacterial adhesins and their complimentary receptors on mature PMNs stimulated the production of superoxide anions and the release of secondary granule contents. Both species of bacteria were ingested and the actinomyces were subsequently destroyed. However, five of seven strains of *S. gordonii* remained viable. These five strains induced endocarditis in a rat model system in which the aortic valve was damaged by catheterization. In contrast, the two *S. gordonii* strains that were susceptible to lectin-mediated killing produced minimal aortic valve colonization. Although other investigators have implicated binding of bacteria to fibronectin, laminin or fibrinogen and aggregation of platelets in the etiology of endocarditis, these parameters failed to correlate with the induction of the disease process. Thus, for *S. gordonii*, the resistance to lectin-mediated killing by PMNs appears to be a major determinant of virulence for the initiation of endocarditis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00564-01 LME

PERIOD COVERED

December 15, 1991 to September 30, 1992

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

Genetics and Toxic Mechanism of Leukotoxins from Pathogenic Oral Bacteria

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

Bouma, Carolyn L.	Staff Fellow	LME, NIDR
Haley, Sheila	Microbiologist	LME, NIDR
Holmes, Elisabeth	Biological Aide	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.15	0.8	0.35

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Pathogenic oral bacteria are often associated with the progression of oral diseases, such as periodontitis. The Gram-negative bacterium *Actinobacillus actinomycetemcomitans* (*Aa*) colonizes periodontal sites and produces a leukotoxin that is likely to play an important role in periodontitis. Understanding the role of the *Aa* leukotoxin and other virulence factors in oral disease requires knowledge of the conditions under which such factors are produced, and how they act.

Because of its probable role in periodontal disease, we have initiated studies of the genetics and mechanism of cytotoxicity of the *Aa* leukotoxin, LktA. *LktCA* has been isolated from *Aa* ATCC 29524 by PCR amplification, and we have developed fluorescent and isotopic assays for cytotoxicity. We have shown that cytoplasmic extracts of recombinant *E. coli* carrying *Aa lktCA* contain a protein that is cytotoxic to a human pre-monocyte cell line. The nucleotide sequence of the cloned *lktCA* genes was determined. While the sequence of these clones differs at several positions from *lktCA* of *Aa* JP2, the activity of LktA is unaffected. The protein has been partially purified by S-sepharose chromatography, and is stabilized in the presence of denaturing agents (4M urea, 1% CHAPS). We are using recombinant DNA techniques to generate a MalE-LktA fusion protein, from which the LktA domain will be purified and used to prepare an anti-LktA antibody. We propose to identify the sites of transcription initiation and termination for the *Aa lkt* operon, and to use Northern hybridizations to investigate the environmental factors that might affect LktA synthesis (temperature, Ca^{2+}, CO_2, and others).

We plan to use DNA hybridization as a tool to identify other oral bacteria that might produce leukotoxins. Genomic DNA from normal oral flora as well as organisms frequently isolated form diseased individuals will be included in this survey.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00421-07 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Herpes Simplex Virus and Persistent Infections

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

PI: J.F. Rooney, Special Expert, LOM, NIDR
Others: A.L. Notkins, Medical Director, LOM, NIDR
J. Dumois, Staff Fellow, LOM, NIDR
C. Wohlenberg, Microbiologist, LOM, NIDR
N. Marinos, Biological Lab Technician
S. Jagannath, Biologist, LOM, NIDR

COOPERATING UNITS (if any)

Laboratory of Clinical Investigation, NIAID

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.94	1.21	.73

CHECK APPROPRIATE BOX(ES)

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

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

Standard treatment for herpes labialis is conservative, as antiviral medications have not yet been approved by the FDA for treatment of labial herpes. Recently we completed a randomized double-blind placebo-controlled crossover trial to determine whether daily oral acyclovir (an antiviral agent) could be useful in preventing outbreaks of herpes labialis in patients with frequently recurrent disease (6 or more episodes yearly). Twenty patients with proven frequently recurrent labial herpes were randomized to receive acyclovir 400 mg PO BID or matched placebo for 4 months, and then were switched to the opposite therapy for 4 months. Recurrent disease was documented by examination in clinic and by viral culture. Treatment with acyclovir resulted in a 53% reduction in the mean number of clinically determined recurrences mean ($\pm$ SEM) of 1.80 $\pm$ 0.28 episodes in the placebo courses vs. 0.85$\pm$0.25 in the acyclovir courses ($P<.02$) and a 71% reduction in the mean number of virologically documented occurrences (1.40$\pm$0.22 in the placebo courses vs. 0.40$\pm$0.15 in the acyclovir courses) ($P<.005$) as compared to placebo therapy. We conclude that daily oral acyclovir can significantly reduce the incidence of herpes labialis in immunocompetent adults with proven frequently recurrent disease. This study will be used to hopefully extend FDA approval of acyclovir to include an indication for use in the treatment of herpes labialis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00423-07 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Cloning, Expression and Characterization of Human Autoantigens

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

PI: M.S. Lan, Senior Staff Fellow, LOM, NIDR
Others: M.G. DeSilva, Visiting Associate, LOM, NIDR
J. Lu, Visiting Fellow, LOM, NIDR
Q. Li, Visiting Fellow, LOM, NIDR
A.L. Notkins, Medical Director, LOM, NIDR

COOPERATING UNITS (if any)

Navy Medical Oncology Branch, NCI; National Naval Medical Center

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.49	3.49	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 novel cDNAs, IA-1 and IA-2, were isolated and characterized from a human insulinoma subtraction library (ISL-153). IA-1 cDNA clone has a 2838 bp sequence consisting of an open reading frame of 1530 nucleotides which translates into a protein of 510 amino acids. The IA-1 protein can be divided into two domains based upon the features of its amino acid sequence. The N-terminal domain of the deduced protein sequence (1-250 a.a.) has four classical pro-hormone dibasic conversion sites and an amidation signal sequence, Pro-Gly-Lys-Arg. The C-terminal domain (251-510 a.a.) contains five putative zinc-finger DNA-binding motifs of the Cys2-His2 DNA-binding protein class. Northern blot analysis revealed that IA-1 mRNA is expressed primarily in neuroendocrine tumors. The restricted tissue distribution and unique sequence motifs suggest that this novel cDNA clone may encode a protein associated with the transformation of neuroendocrine cells. IA-2 cDNA clone has a 2937 bp open reading frame. The predicted amino acid sequence of IA-2 revealed a 979 amino acid protein which is consistent with a signal peptide, an extracellular domain, a transmembrane region and an intracellular domain. The extracellular domain contains an unusual cysteins-rich region following the signal peptide. The intracellular cytoplasmic domain of IA-2 possesses highly conserved regions similar to the phosphatase (PTP) family. IA-2 may represent a new member of the receptor-type PTP family with a distinct extracellular domain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00471-05 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Transgenic Mice as Models for the Study of HIV-1 Pathogenesis

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

PI: R.R. Franks, Senior Staff Fellow, LOM, NIDR
Others: A.L. Notkins, Medical Director, LOM, NIDR
J. Rappaport, Senior Staff Fellow, LOM, NIDR
J. Rooney, Special Expert, LOM, NIDR
C. Wohlenberg, Microbiologist, LOM, NIDR
N. Marinos, Biological Lab Technician

COOPERATING UNITS (if any)

Laboratory of Tumor Cell Biology, NCI; Dermatology Branch, NCI

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.03	1.23	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.)

Transgenic mice bearing a noninfectious, (i.e., replication-deficient) HIV-1 proviral genome, which lacks only the viral *gag* and *pol* genes, were generated as models for HIV pathogenesis. Heterozygous transgenics develop progressive glomerulosclerosis, as well as hyperkeratotic skin lesions. These pathologies are not unlike the HIV-associated nephropathy and hyperkeratotic skin disorders, respectively, identified in patients with AIDS. Homozygous litter-mates, in addition to displaying cutaneous disorders, suffer a syndrome of cachexia and wasting that is evident in the neonate. A similar syndrome has been described in humans that have been infected with HIV-1 *in utero*. The pathological and molecular biological manesfestations of these disorders were examined in transgenics by a variety of methods, including RNA analysis, immunohistochemistry, and *in vitro* studies that identify the cellular and viral proteins that activate the HIV transgene. HIV mRNAs are differentially expressed in various tissues, with the highest levels of expression occurring in skin, skeletal muscle, and intestine. Lesser amounts are present in kidney, as well as thymus and lymph node. At least one cellular factor, NF-KB, has been identified as a component of the viral LTR promoter transcription complex, and may play a role in tissue-specific activation. Kidneys harvested from transgenic animals show a spectrum of pathologic changes that include microcystic tubular dilation and atrophy, and an interstitial infiltrate composed mainly of macrophages and some T lymphocytes. In skin, viral gene expression is limited to differentiated keratinocytes of the epidemal stratum granulosum. The viral proteins, gp41 and gp120, have been identified in these cells. Viral gene expression in skin is enhanced by a variety of physical and chemical insults, and is correlated with an increase in the incidence of skin lesions. Additional transgenic mouse lines are being generated to help determine the particular HIV-1 gene products responsible for these cytopathic effects that occur in the absence of replicating virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00534-02 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Human Immunoglobulin Genes and their Properties

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

PI: R.F. Rando, Senior Staff Fellow, LOM, NIDR
Others: S. Cheung, Junior Staff Fellow, LOM, NIDR
G. Donadel, Visiting Fellow, LOM, NIDR
N. Dorfman, Expert, LOM, NIDR
N. Harindranath, Visiting Associate, LOM, NIDR
W. Kajiyama, Visiting Associate, LOM, NIDR
S. Takeda, Visiting Associate, LOM, NIDR

COOPERATING UNITS (if any)

NIAID (C. Lane)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.66	6.66	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.)

Objective: To design methods for improving human hybridoma development; characterize human anti rabies virus mAbs capable of neutralizing virus, cloning of the Fab domains of these mAbs in heterologous systems; elucidate the mechanism(s) by which polyreactive antibodies recognize a variety of different antigens.
Major Findings: We have successfully cloned several different human monoclonal antibody Fab domains in E. Coli. One of these recombinants, derived from a mAb cell line (mAb57) capable of neuralizing rabies virus in vitro and in vivo, maintained virus neutralizing activity in vitro. In vivo activity has yet to be tested. We have developed 10 new anti-rabies virus mAbs which recognize the surface glycoprotein "G" of rabies. These antibodies will be screened for virus neutralization activity. We have also cloned and characterized two human anti-HIV-1 Fab domains derived from mAbs. One Fab (M7B5) binds to the HIV-1 gp120 protein and the second Fab (T15G1) binds to HIV-1 gp41.

In efforts aimed at defining the mechanism by which polyreactive antibodies recognize multiple antigens we have determined that the constant domain DNA and amino acidsequences of polyreactive antibodies do not differ from monoreactive antibodies and that the degree of glycosylation in these antibodies does not change their antigen recognition profile.

Professional Personnel, continued

A.L. Notkins, Medical Director, LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00536-02 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Polyreactive and Monoreactive Autoantibodies

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

PI: G. Sigounas, Visiting Associate, LOM, NIDR
Others: M. Kawamura, Visiting Fellow, LOM, NIDR
M. Kearns, Staff Fellow, LOM, NIDR
R. Kurrasch, Expert, LOM, NIDR
Bich-Thuy Le thi, Visiting Scientist, LOM, NIDR
A.L. Notkins, Medical Director, LOM, NIDR

COOPERATING UNITS (if any)

Washington University School of Medicine, St. Louis, Missouri (Dr. M. Holers)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.46	4.26	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.)

Polyreactive Autoantibodies To explore the mechanism(s) that operate polyreactivity, the intravascular survival and biodistribution of polyreactive antibodies, we performed in vitro alnd in vivo studies with a number of randomly-selected human monoclonal antibodies produced in our Laboratory. We found that the carbohydrate moieties of immunoglobulins are not associated with the multiple binding characteristic of the antibodies. We have shown for the first time that the half-life of polyreactive antibodies is significantly shorter compared to monoreactive counterparts. The short circulatory survival of polyreactive antibodies is an intrinsic property of the molecules associated with the multiple binding. Liver seems to be the major site for the removal of these antibodies from the circulation, perhaps in the form of immune complexes. We also have shown that purified neutrophils bind to polyreactive antibody in a dose-dependent manner. These antibodies, via binding to phagocytic cells could, therefore, facilitate the clearance of a variety of molecules released during cellular injury.
Previous work in LOM has shown that CD5 antigen-bearing B cells segregated with B cells producing polyreactive antibodies. To understand the relationship between the expression of the CD5 gene and the production of polyreactive antibodies, we tried to clone the genomic form of the human and mouse gene. We isolated a 17 Kb human clone containing the 3′ region of the gene. Attempts are currently made to isolate the 5′ region and the regulatory elements of the gene. Furthermore, we isolated a 10 Kb clone from a mouse spleen genomic DNA library. This clone contains most of the CD5 gene including the whole first exon and the intramembranous sequences. Mutated constructs of this clone will be used for gene knock-out studies.

CR2 Transgenic Mice. Several lines of transgenic mice which express the human CR2 (hCR2) gene at RNA and protein level have been developed. Low but significant binding of anti-CR2 antibodies and biotinylated EBV has been observed. Studies in progress will determine the ability of EBV to infect mouse cells expressing hCR2.

Professional Personnel, continued

J. Wheeler, Biologist, LOM, NIDR
E. Monell-Torrens, Biological Lab Technician, LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00562-01 LOM

PERIOD COVERED

October 1, 1991 - September 30, 1992

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

Molecular Biology of HIV and Gene Therapy

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

PI: J. Rappaport, Senior Staff Fellow, LOM, NIDR
Others: A.L Notkins, Medical Director, LOM, NIDR
R.R. Franks, Senior Staff Fellow, LOM, NIDR
M. Richardson, Biologist, LOM, NIDR
M. Zener, Biological Aid, LOM, NIDR
J.F. Rooney, M.D., Special Expert, LOM, NIDR

COOPERATING UNITS (If any)

Arn Hampel University of Northern Illinois; Staal University of California, San Diego (Flossie Wong)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.72	0.93	0.79

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 HIV transgenic mouse model has been established which exhibits some of the clinical manifestations seen in AIDS. Homozygous animals are born runted and usually die of wasting within 30 days of birth. Heterozygous animals are born with normal weight, but often develop nephropathy as adults. Histologically, the microcystic changes seen in the mouse kidney sections are similar to those seen in samples with HIV patients with renal disease. HIV transgenic mice exhibit additional abnormalities, including myopathy/myositis, lymphadenopathy, and papillomatous lesions of the skin. Given the similarities between the HIV transgenic phenotype and human AIDS, the HIV transgenic mouse system may provide a useful model to study HIV gene therapy. We have focuses our efforts on the development of therapeutic strategies based on the molecular regulation and life cycle of HIV. We have initiated experiments with antisense oligonucleotides, multimers of the TAR sequence, and ribozymes as pilot studies to evaluate gene therapy in this system. Transgenic animals have been developed containing multimers of HIV-1 TAR under the control of the HIV-1 LTR and an HIV specific ribozyme expressed from the human β-actin promoter. Results of genetic crosses with HIV transgenic animals may demonstrate the efficacy of these novel gene therapy strategies.

Professional Personnel, continued

C. Wohlenberg, Microbiologist, LOM, NIDR
N. Marinos, Biological Lab Technician, LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-24 NA

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.)*

Design and Computer Interfacing of Neurophysiological Instrumentation

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

Brown, Frederick J. Electronic Engineer (Instru) NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	.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.)*

These projects involve the design and construction of electronic and electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory and central computer installations. Electronic circuit design, microcomputers, and assembly of machine language programming may be used in these instruments or interfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-18 NA

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.)*

Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

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

Dionne, Raymond Research Pharmacologist NA NIDR

COOPERATING UNITS *(if any)*

Linda McCullough, CC Nursing
Oral Surgery, Georgetown University

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Inj

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials.

Previous work on the physiologic function of plasma beta-endorphin and regulation of its release has provided evidence of enhanced release by the nonsteroidal anti-inflammatory drug ibuprofen during surgical stress. A current study is attempting to replicate and extend these findings to postoperative pain. Subjects who have undergone oral surgery remain at the clinic until the onset of moderate to severe pain and are then administered either S(+)-ibuprofen, racemic ibuprofen, or placebo. Blood samples are collected for four hours following drug administration for the measurement of the isomers of ibuprofen and beta-endorphin. The S(+) isomer of ibuprofen is the active form of the drug and has been demonstrated to result in higher blood levels than administration of the racemic mixture which requires hepatic conversion of the R(-) isomer to the S(+) isomer and is associated with a delay in onset. Analysis of data from 175 completed subjects indicates that the S(+) isomer results in a faster onset of analgesic activity and greater peak analgesia than the racemic mixture. Laboratory analyses are continuing to determine if a relationship exists between the blood levels of the S(+) isomer, plasma levels of beta-endorphin, and pain relief.

A parallel clinical study is attempting to measure inflammatory mediators released following surgery in order to determine the relationship between local levels of inflammatory mediators, clinical reports of pain, and their modulation by ocal infusion of prototypic drugs into the site of inflammation. Subjects undergoing the removal of an impacted third molar have a microdialysis fiber placed under the mucoperiosteal flap which is then slowly perfused to allow diffusion of mediators from the extracellular fluid into the perfusion fluid and administration of drugs from the perfusion fluid into the extracellular space. Technical difficulties in the placement of the probes have resulted in an insufficient patient sample to date to permit conlcusions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-18 NA

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.)*

Assessment of Experimental and Clinical Pain

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

Gracely, Richard H.	Research Psychologist	NA NIDR
Dionne, Raymond	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

Oral Surgery, Georgetown University

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.7	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The interactive computer-based staircase scaling method and the continuous track-ball method were each used in two experiments. One track-ball study delivered 1.4 sec 49oC thermal stimuli, the other studies delivered stimuli, ranging from 37 to 53oC.

The first experiment is providing additional evidence that cardiac chest pain in patients with normal coronary arteries does not represent general hyperalgesia and is actually accompanied by reduced somatic pain sensitivity. The second experiment is examining the effects of imipramine and clonidine on the perception of painful thermal stimuli in this group of patients. The code will not be broken in either study until a total of 60 patients is completed for each.

The third study used the track-ball method to assess the effects of fentanyl or placebo on the magnitude and duration of pain sensations evoked by 3-sec thermal stimuli of varying intensity. A preliminary analysis of 40 subjects shows that fentanyl reduces both the magnitude and durations of thermally-evoked pain sensations.

The fourth study used track-ball assessment of trains of 49oC stimuli to assess the effects of fentanyl on first and second pain sensations. New computer programs are required for a preliminary analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00286-13 NA

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.)*

Experimental Therapeutics for Acute Pain

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

Dionne, Raymond	Research Pharmacologist	NA NIDR
Berthold, Charles	Guest Researcher	NA NIDR

COOPERATING UNITS *(if any)*

Allen, Chris	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model for minor surgical procedures with associated intraoperative and postoperative pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety. A recent study evaluated the analgesic efficacy of two anithistamine drugs in comparison to ibuprofen and placebo. Terfenadine, a H1 histamine receptor blocker, and ranitidine, a H2 histamine receptor blocker, were administered one hour prior to oral surgery and the onset and severity of postoperative pain monitored for four hours postoperatively. Final analysis demonstrated that dependent measures for analgeisa were sensitive to the effects of the positive control, 400 mg of ibuprofen, but that the two antihistamines could not be differentiated from the effects of placebo. These data indicate that pretreatment with a single dose of a histamine receptor antagonist does not produce analgesia in the oral surgery model, suggesting that antihistamines which act primarily at peripheral sites are devoid of analgesic activity.

A current study is evaluating the combination of ibuprofen and oxycodone to define an analgesic combination which results in additive analgesia for the management of pain not responsive to the use of a single agent such as ibuprofen and related drugs. Interim evaluation suggests that the highest dose of oxycodone is resulting in additive analgesia in comparison to ibuprofen alone or ibuprofen alone or ibuprofen plus lower doses of oxycodone. Demonstration of an additive effect for an opioid-nonsteroidal anti-inflmmatory drug combination may provide a basis for the management of severe acute pain with an oral drug combination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00288-13 NA

PERIOD COVERED

October 1, 1991 to September 31, 1992

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

Neuropharmacological Characterization of Synaptic Ciruitry in the Dorsal Horn

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

Ruda, Maryann	Chief, Cellular & Molecular Mechanisms Section	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Franklin, Emma L.	Biology Laboratory Tech. (Electron Microscope)	NA NIDR
De León, Marino A.	Staff Fellow	NA NIDR
Inagaki, Shinobu	Visiting Associate	NA NIDR
Besse, Dominique	Visiting Fellow	NA NIDR
Ren, Ke	Visiting Associate	NA NIDR

COOPERATING UNITS *(if any)*

D.M. Jacobowitz - NIMH, Laboratory of Clinical Science

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.45	2.32	1.13

CHECK APPROPRIATE BOX(ES)

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

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

Our studies are designed to delineate the neuronal response to noxious stimulation of the periphery and nerve injury. The dorsal root ganglion and spinal cord are the first levels of processing of neuronal information. Using cellular and molecular techniques, we are able to identify important elements in this neuronal network.

Excitotoxicity induced neuronal loss has been proposed as a possible cause of some types of chronic pain states. Calcium-binding proteins may function to buffer the effects of an excessive neuronal barrage. We have studied calretinin (CR) a recently identified calcium-binding protein using immunocytochemical techniques. CR immunoreactivity is present in a small subpopulation of dorsal root ganglia neurons and neurons in many spinal cord laminae. In the spinal cord the densest axonal and cell body staining occurs in laminae I and II, an important area for processing of noxious inputs. The spinal distribution of CR is unique from that of other calcium binding proteins. Experiments are underway to examine regulation of calcium-binding proteins in animal models of nerve injury and nociception.

Nitric oxide (NO), a free radial gas, has been proposed to be a novel neuronal messenger molecule. One potential role for this gas is in excitotoxicity. Using in situ hybridization histochemistry, RNA blot analysis, and NADPH histochemistry we have localized NO to subpopulations of dorsal root ganglion neurons and spinal cord neurons. Following nerve injury, nitric oxide synthase is induced in dorsal root ganglion neurons. The subpopulation of neurons containing NO likely play important roles in nerve injury and nociception.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00291-13 NA

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.)*

Microinjection of Analgesic Agents into the Medullary Dorsal Horn of Monkeys

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

Thomas, David A.	Postdoctoral Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Kenshalo Jr., Daniel R.	Research Biologist	NA NIDR
Williams, Gene M.	IRTA Fellow	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.66	1.35	.31

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 exist both noradrenergic and opioid based systems of pain control. We examined the effects of activating these systems by injecting antinociceptive agents into the medullary dorsal horn (MDH) and examining the ability of monkeys to detect small increases in noxious thermal stimuli. The effects of these agents on facial scratching behavior in separate experiments were also examined. In the detection paradigm, the monkeys were required to detect temperature changes of 0.4, 0.6 and 1.0°C (T2) superimposed on a 46.0°C (T1) stimuli. Consistent with previous research, 10 micrograms of the noradrenergic agonist, ST-91, and also 3 micrograms of the opioid agonist, morphine, each increased the time to detection of the heating stimuli. Both morphine and ST-91 also produced small increases in the monkeys' time to detection of innocuous cooling stimuli. Neither morphine nor ST-91 interfered with the detection of visual stimuli, indicating the effects of these agents are not the result of changes in motoric, attentional, or motivational aspects of the monkeys' behavior. In separate experiments, morphine administered into the MDH, but not ST-91, was found to produce a great amount of facial scratching behavior. In both the detection and the scratching paradigms, the noradrenergic receptor antagonist, idazoxan (1.0 mg/kg: I.M.) attenuated the effects of both ST-91 and morphine. In the detection paradigm, the opioid receptor antagonist, naloxone (1.0 mg/kg: I.M.) was only effective at attenuating the effects of morphine, not ST-91. These findings demonstrate that the opioid and noradrenergic systems are involved in both pain and scratch behavior. The present data has increased our understanding of how pain control systems interact, which may lead to precise co-activation of opioid and noradrenergic pain control systems in clinical settings. The findings related to scratching indicate that noradrenergic antagonists may be effective antipruritic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-11 NA

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.)*

Discrimination of Thermal Stimuli Applied to the Face in Monkey

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

Kenshalo, Jr. Daniel R.	Research Biologist	NA, NIDR
Dubner, Ronald	Chief, NAB	NA, NIDR
Thomas, David	Postdoctoral Fellow	NA, NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.41	1.10	0.31

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 investigates the neural mechanisms that subserve the monkey's ability to detect innocuous cutaneous stimuli (air puffs) delivered to the face. The magnitude of sensations produced by small increases in air puff stimuli was studied by the use of a reaction time paradigm. The monkey initiated a trial by pressing an illuminated button. Subsequently air puff stimuli (AP1) of identical intensity were delivered to the face at the rate of one per second. After a variable time period between 4 and 10 seconds, a larger air puff (AP2) was presented. The subject was required to release the button as soon as the larger air puff stimuli was detected. Detection latency was defined as the time interval between the onset of the large air puff and the release of the button. Humans subjects were trained in a similar paradigm except that they were not reinforced with cherry juice for correct responses.

Both the monkey's and human's detection latencies to stimuli presented on the face were dependent on the intensity of the AP2. The psychophysical functions obtained from the monkeys face were monotonically related to the intensity of AP2. As the intensity of AP2 increased, the monkey's detections latencies became shorter. The humans' detection latencies to AP2 stimuli were also monotonically related to the intensity of the air puff. The psychophysical functions obtained form the humans' face were equivelent to those obtained from the monkey face. The monkey's and human's reaction time to air puff stimuli appear to be an accurate measure of the perceived intensity of innocuous cutaneous stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DE 00366-10 NA

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.)*
Analgesic Mechanisms in Patients with Chronic and Acute Postoperative Pain

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

Max, Mitchell	Neurologist	NA NIDR
Byas-Smith, Michael	Medical Staff Fellow	NA NIDR
Gracely, Richard	Research Psychologist	NA NIDR
Bennett, Gary J.	Chief, NPPM Section	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR
Park, Karen	NRSA Fellow	NA NIDR

COOPERATING UNITS *(if any)*

Shoaf, Susan	Head, Clin Biochem Sect.	LCA, NIAAA
Muir, Joanne	Nurse	CC Nursing

LAB/BRANCH
Neurobiology and Anesthesiology Branch

SECTION
Neuropathic Pain Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.7	.2

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to elucidate the principles of treatment of acute and chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury.

In an interim analysis of a double-blind, crossover study in 22 patients with painful diabetic neuropathy, 7 patients obtained significantly better pain relief with clonidine than placebo and had that response confirmed by two subsequent double-blind crossover comparisons of clonidine and placebo. Clonidine is an a2-adrenergic agonist commonly used as an antihypertensive. This confirms the result of a previous trial showing that a subset of patients were clonidine-responsive, and suggests that clonidine may be a useful advance in treatment for a subset of patients with neuropathic pain.

Because of growing evidence from animal studies that neuropathic pain may be mediated by spinal cord neurons depolarized by NMDA channels, the NMDA antagonist ketamine is being studied in two conditions: chronic pain in patients with nerve damage or reflex sympathetic dystrophy, and normal volunteers with hyperalgesia of the skin briefly induced by intradermal injection of capsaicin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-07 NA

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.)*

Experimental Neuropathy of Peripheral Nerve in Rat

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

Bennett, Gary J.	Chief, Neuropathic Pain and Pain Measurement Section	NA NIDR
Tal, Michael	Visiting Associate	NA NIDR

COOPERATING UNITS *(if any)*

Seybold, Virginia	Associate Prof., Dept. of Anatomy, Univ. of Minnesota
Aanonsen, Lin	Assistant Prof., Dept. of Biology, MacAlaster College
Wakisaka, Satoshi	Associate Prof., Dept. of Oral Biology, Osaka, JAPAN

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.62	1.42	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.)*

An animal model of painful peripheral neuropathy is produced in the rat by a chronic constriction injury to the sciatic nerve. Animals with this nerve injury have behavioral symptoms that indicate disordered pain sensations like those seen in human syndromes. In particular, the rats have hyperalgesia to thermal and mechanical stimuli, allodynia (pain from normally innocuous stimuli) to touch and cold, and spontaneous pain (or dysesthesias). Immunocytochemical analyses show that the damaged sciatic nerve's primary afferent neurons begin to synthesize neuropeptide Y (NPY) after several kinds of nerve injury (complete transection, the chronic constriction injury, and crush) but not after a painful inflammation of the hind paw. Cell body size and an analysis of the laminar pattern of the nerve injury-evoked NPY increase indicate that the response is largely or entirely within a particular functional class of neurons, the low- threshold mechanoreceptors with large somata and A-beta axons. The nerve injury is known to cause transsynaptic degeneration in small, presumably inhibitory, interneurons in laminae I-III, and this is believed to be due to a NMDA receptor-mediated excitotoxic effect of spontaneous discharge from the damaged primary afferent axons and cell bodies. Such damage would create a state of spinal disinhibition, and electrophysiological evidence for such a state has been found. Dextrorphan, a noncompetitive antagonist of NMDA receptors, reduces the neuropathic hyperalgesia in a dose-dependent way. Further investigation of the transsynaptic degeneration effect has shown that similar, but probably temporary, neuronal damage is caused by a surgical exploration that does not damage the nerve. This indicates that not only abnormal neuropathic pain, but also the ordinary pain of tissue injury can produce excitotoxic damage in the spinal cord. In the case of surgery, this damage is likely to contribute to postoperative pain, and this suggests a role for NMDA antagonists in the control of typical pain states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-07 NA

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.)*

CNS Neurotransmitter Regulation during Peripheral Inflammatory States

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

Iadarola, Michael J.	Research Pharmacologist	NA NIDR
Gu, Jun	Visiting Fellow	NA NIDR
Messersmith, Donna J.	IRTA Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project focuses on the transcriptional control of the prodynorphin gene, which codes for the dynorphin family of opioid peptides. We have shown that during peripheral inflammation prodynorphin gene expression is greatly increased in spinal cord and, thus, may play a role in modulating pain. Fos, a transcription control protein, is also increased in our inflammation model. Fos binds to an AP-1-like DNA sequence located at -1546 from the transcription start site in the prodynorphin promoter. We call this sequence the DAP site for dynorphin AP-1-like site.

A transient expression assay has been established to determine the functional efficacy of the DAP site in cell lines in vitro. Starting with ~2,000 bp of upstream sequence, several dynorphin promoter-CAT reporter plasmids have been constructed and analyzed by restriction digests or nucleotide sequencing. One series of clones contains the region surrounding the DAP site. We have found that this fragment confers high level constitutive expression when transiently transfected into HeLa cells or PC-12 cells. A 250 bp fragment adjacent to the DAP site was much less effective as an enhancer element. The entire 2,000 bp promoter fragment was also inefficient as an enhancer, suggesting that more proximal sequences have a modulatory role on expression driven by the DAP site. We have also obtained preliminary data on another region of the dynorphin promoter that showed strong complex formation with CNS nuclear protein extracts. This element, at -208, appears to bind a set of proteins distinct from the -1546 site and has homology to the Inr consensus YAYTCYYY. We have isolated a cDNA clone by southwestern screening of a rat brain cDNA library that binds to this element. This clone was sequenced and codes for a unique protein. Binding studies suggest that it forms a specific complex with the dynorphin Inr-like element. We are currently verifying our initial observations by producing the protein via recombinant methods and testing the purified protein for specific binding to the dynorphin Inr-like sequence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00440-06 NA

PERIOD COVERED

October 1, 1991 to September 1992

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

Dorsal Horn Circuitry Related to Pain: Inflammation-induced Plasticity

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

Ren, Ke	Visiting Associate	NA, NIDR
Williams, Gene M.	IRTA Fellow	NA, NIDR
Ruda, Maryann	Chief, CMM Section	NA, NIDR
Dubner, Ronald	Chief, NAB	NA, NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.21	1.45	.76

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 combination of behavioral, physiological, and pharmacological approaches was used to study the neural mechanisms involved in pain and analgesia. To address the mechanisms of dorsal horn plasticity and hyperalgesia following inflammation, a neurotoxin, capsaicin, was used to selectively destroy a subpopulation of small diameter primary afferents. The effects of adjuvant-induced inflammation on dorsal horn neuronal activity and nociception were studied in the neonatal capsaicin-treated rats. Capsaicin treatment resulted in an over 85% loss of unmyelinated fibers in L5 dorsal roots. The thermal nociceptive threshold was increased in capsaicin-treated rats, but behavioral hyperalgesia still developed. There was a significant decrease in the percentage of dorsal horn nociceptive neurons that responded to C-fiber stimulation and noxious heating of the skin. The expansion of receptive fields, a hallmark of the effect of inflammation-induced plasticity, was less extensive for nociceptive specific (NS), but not for wide dynamic range (WDR), neurons. Compared to vehicle-treated rats, a smaller population of NS neurons had background activity. An NMDA receptor antagonist, MK-801, reduced the receptive field size of dorsal horn neurons and attenuated the behavioral hyperalgesia in capsaicin-treated rats. These results indicate a complex effect of neonatal capsaicin treatment in rat model of inflammation/hyperalgesia. The data suggest that unmyelinated and fine myelinated primary afferents play important roles in triggering dorsal horn plasticity. Additionally, NMDA receptors appear to function at least in part independent of small diameter primary afferent axons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00460-05 NA

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.)*

Molecular Responses to Nerve Injury

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

Nahin, Richard L.	Senior Staff Fellow	NA NIDR
Ren, Ke	Visiting Associate	NA NIDR
De Leon, Marino A.	Staff Fellow	NA NIDR
Ruda, Maryann	Chief, CMM Section	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.83	1.25	.58

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 Chronic Constriction Injury (CCI), a model of nerve injury in the rat, is produced by tying loose ligatures of 4-0 chromic gut suture around the sciatic nerve. The CCI elicits behavioral hyperalgesia and allodynia. As such, the CCI resembles human neuropathies associated with several clinical syndromes including, causalgia and reflex sympathetic dystrophy. Using a number of complementary anatomical and molecular techniques, we studied the effects of the CCI on the peripheral nervous system of the rat. Dorsal root ganglia taken from animals with the CCI were analyzed for alterations in mRNA levels encoding growth associated protein-43 (GAP-43), calcitonin gene related peptide (CGRP), galanin (GAL), neuropeptide Y (NPY), substance P (SP), and vasoactive intestinal polypeptide (VIP). We found that GAP-43 expression increased 3-fold, peaking between 7 and 14 days after development of the CCI. However, within this same 7 to 14 day time frame, both CGRP and SP mRNAs fell to half their normally abundant basal levels. The most dramatic change in expression occurred for GAL, NPY and VIP mRNAs, which all rose rapidly (1 day) from nearly non-expressed states. These data suggest that gene expression is modified to increase production of molecules responding to nerve injury and necessary for regrowth, and to decrease production of molecules involved in normal neurotransmission.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00509-03 NA

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.)*

Mechanisms of Capsaicin Pain in Human

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

Gracely, Richard	Research Psychologist	NA NIDR
Bennett, Gary	Section Chief	NA NIDR
Byas-Smith, Michael	Staff Fellow	NA NIDR
Max, Mitchell	Neurologist	NA NIDR
Park, Karen	Staff Fellow	NA NIDR
Smith, Wendy	Psychologist	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.95	1.75	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.)*

New findings confirm that many features of neuropathic pain syndromes, including spontaneous pain, mechanical allodynia (pain evoked from stimulation of Ab low threshold mechanoreceptor (LTM) afferents, cold hyperalgesia, and sensory and motor abnormalities, likely result from a central process maintained dynamically by ongoing input from a site of peripheral injury. Local anesthesia of these sites but not adjacent sites (both within an area of Ab-LTM mechanical allodynia) alleviates all symptoms for the duration of the local anesthetic. Thus the relief is not likely due to non-specific effects of the local anesthetic or to a placebo effect. In a subset of patients with peripheral injury, modification of circulation by limb elevation or occlusion by a tourniquet abolished all symptoms within 2-6 minutes, too soon to result from neural blockade. Experiments using limb elevations and cuff blocks have incorporated placebo controls and identified two patterns of relief; one apparently related to limb perfusion and one apparently related to suppression of circulating epinephrine. Capsaicin an active ingredient in chili pepper, was injected into the volar forearm or dorsum of the foot in normal volunteer subjects. Injection of 100 mg produced both spontaneous pain and in some cases an area of mechanical allodynia which also appeard to be Ab-LTM mediated. This effect was observed in only one-half of the experiments. Increasing the dose up to 500 mg also produced variable amounts of mechanical allodynia which does not appear to be dose-related.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00526-02 NA

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.)*

Cloning of Genes Regulated during Neuronal Injury and Nerve Regeneration

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

De León, Marino A.	Staff Fellow	NA NIDR
Nahin, Richard L.	Senior Staff Fellow	NA NIDR
Ruda, Maryann	Chief, CMMS	NA NIDR
Allen, Barbara V.	Biologist	NA NIDR
Besse, Dominique	Visiting Fellow	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.44	1.0	.44

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 work focuses on the study of genes associated with neuronal injury and nerve regeneration.

We studied the regulation of SR13, a novel protein cloned from a sciatic nerve library. Our primary findings were as follows: (a) SR13 mRNA was localized to Schwann cells using in situ hybridization histochemistry, where it's expression was repressed after sciatic nerve transection; (b) SR 13-like immunoreactivity was found in neurons in the spinal cord and in fibers in both the dorsal root ganglia (DRG) and the spinal cord; (c) RNA blot analysis showed that SR13 message was induced in a Pheochromocytoma cell line (PC12) after nerve growth factor (NGF) treatment, suggesting that SR13 expression may be regulated by NGF.

The significance of this project has recently become evident due to recent reports showing that the SR13 gene is mutated in trembler mice (a model for peripheral neuropathy). SR13 was found to be duplicated in patients with Charcot-marie-tooth disease (CMT), a common inherited neuropathy in humans that involves both motor and sensory nerves and has a prevalence rate of 1 in 2500.

We continued the characterization of two cDNAs named DI12 and DA11. DI12 is similar to the human FK506BP and may play an important role in T cell activation. DA11 is a novel cDNA that is induced in the DRG after sciatic nerve cut. Its function is still unclear.

Finally, we also examined the pattern of expression of the transcription factors jun-D, jun B, c-*fos* and c-*jun* in the DRG after nerve transection. We found that only the mRNA of c-*jun* was upregulated ipsilateral to the nerve transection. These data suggest that c-*jun* may play an important role in neuronal injury and nerve regeneration.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00532-02 NA

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.)*

Pathophysiology of Chronic Pain

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

Reid, Kevin	Postdoctoral Fellow	NA NIDR
Dionne, Raymond	Research Pharmacologist	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

Rosenbaum, Lola	Senior Staff Physical Ther.	CC DRM
Frank, Joseph	Director, MIR Research	CC DRD
Lord, Dorothy	Staff Therapist	CC DRM

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neural Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The project utilizes new diagnostic methods and prototypic treatments in three controlled clinical trials attempting to better characterize the pathophysiology of chronic facial pain. The first study is a double-blind clinical evaluation of the effect of iontophoretically applied dexamethasone, in comparison to placebo, for temporomandibular joint pain. Subjects determined to have internal derangement of the temporomandibular joint complete a battery of analgesic and psychologic questionnaires prior to drug administration. Mandibular range of motion (including vertical, lateral, and protrusive movements) are assessed. Subjects randomly receive either 0.4% dexamethasone in a vehicle of 4% lidocaine or saline placebo by iontophoretic administration. Approximately 95% of the projected sample of 60 subjects have completed the protocol. In the second study, subjects are diagnosed into one of four groups as determined by clinical signs and symptoms: localized masticatory myalgia, fibromyalgia, polymyalgia rheumatica and normal controls. Magnetic resonance spectroscopy (MRS) is also performed and interpreted by a radiologist blind to diagnoses. After collection of baseline palpation and MRS data, subjects are asked to chew at a rate of approximately 80 chewing strokes per minute in order to stress the masticatory musculature. MRS is performed following the exercise in the same manner as the baseline measurements. Differences between groups in exercise tolerance, tissue pH, or phosphate energy metabolism may provide insight into pathophysiologic processes contributing to chronic facial pain of muscular origin. A third study uses pressure algometry to assess reproducibility of pain pressure thresholds in masseter and temporalis muscles in patients with chronic myogenic facial pain and in normal controls. These data show that patients and controls display site-specific reproducibility over time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00556-01 NA

PERIOD COVERED

December 1, 1991 to September 30, 1992

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

Neuropeptide Interactions with Excitatory Synapses

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

Caudle, Robert M.	Staff Fellow	NA NIDR
Dubner, Ronald	Chief, NAB	NA NIDR

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.12	.92	.20

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Endogenous neuropeptides were studied to determine their effects on communication between neurons in the central nervous system. Studies were carried out to characterize the effects of the peptides on A) synaptic currents and B) extracellularly recorded potentials from large populations of neurons.

A) In the CA3 region of the guinea pig hippocampus neurons receive a number of synaptic inputs from other areas of the brain. One of these inputs contains the endogenous neuropeptide dynorphin. Using whole cell voltage clamp in living slices of brain tissue, we have discovered that dynorphin produces an increase in the synaptic current evoked by a specific class of receptor known as the N-methyl-d-aspartate receptor. The increase in this current indicates that communication between the cells has been enhanced. Dynorphin and N-methyl-d-aspartate receptors in the spinal cord are an important part of the processes involved with chronic pain. Thus, efforts are underway to develop a living spinal cord slice preparation in order to determine the effect dynorphin has on synaptic currents in that tissue.

B) Using standard extracellular recording techniques in the CA1 region of the rat hippocampal slice, we have discovered a potential that corresponds to the event related potential (p300) that is studied in humans. This in vitro potential has not been reported in the literature previously. The p300 is associated with the perception of external stimuli, including pain. Efforts are underway to further characterize this potential and to determine the effect neuropeptides have on this potential.

The significance of this project is to understand the role neuropeptides play in pain processes at the molecular, cellular and network levels. With this knowledge novel treatment strategies for chronic pain can be developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00043-22 LME

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.)*
Physiological and Genetic Studies of Oral and Other Microorganisms

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

Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR
Harr, Robert J.	Biolaboratory Technician	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.9	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.)*

N^5-(carboxyethyl)ornithine synthase (EC 1.5.1.24) from *Lactococcus lactis* subsp. *lactis* mediates the NADPH-dependent reductive condensation between L-ornithine (or L-lysine) and pyruvate to form N^5-(L-1-carboxyethyl)-L-ornithine (or N^6-(L-1-carboxyethyl)-L-lysine). To study the relationship between this enzyme and the three other N-(carboxyalkyl)amino acid dehydrogenases that have been sequenced, the gene ceo was cloned (from *L. lactis* K1), expressed in *Escherichia coli*, and sequenced. The gene encodes a 313-amino-acid protein (M_r = 35,323) which showed relatively little overall sequence similarity to proteins currently catalogued in the data banks. When compared with the other N-(carboxyalkyl)amino acid dehydrogenases, N^5-(carboxyethyl)ornithine synthase was most similar to yeast saccharopine dehydrogenase (EC 1.5.1.7), which catalyzes the NADH-dependent condensation of lysine and α-ketoglutarate. These two proteins constitute a family of N^ω-(carboxyalkyl)amino acid dehydrogenases, which in turn, can be considered part of an amino acid dehydrogenase superfamily, that would also include N^2-(carboxyalkyl)amino acid dehydrogenases such as octopine synthase and nopaline synthase. With respect to the binding of its three substrates, N^5-(carboxyethyl)ornithine synthase contains a sequence segment that was virtually identical (eight out of nine residues) to one present in the $\beta\alpha\beta$-fold of the dinucleotide-binding domain of several microbial glutamate dehydrogenases. In addition, sequence motifs associated with the binding of pyruvate and ornithine were identified in the N-terminus of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00043-22 LME

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.)*
Physiological and Genetic Studies of Oral and Other Microorganisms

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

Donkersloot, Jacob A.	Research Microbiologist	LME, NIDR
Harr, Robert J.	Biolaboratory Technician	LME, NIDR
Thompson, John	Visiting Scientist	LME, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Ecology

SECTION
Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION
National Institute of Dental Research, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.7	0.9	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.)*

N^5-(carboxyethyl)ornithine synthase (EC 1.5.1.24) from *Lactococcus lactis* subsp. *lactis* mediates the NADPH-dependent reductive condensation between L-ornithine (or L-lysine) and pyruvate to form N^5-(L-1-carboxyethyl)-L-ornithine (or N^6-(L-1-carboxyethyl)-L-lysine). To study the relationship between this enzyme and the three other N-(carboxyalkyl)amino acid dehydrogenases that have been sequenced, the gene *ceo* was cloned (from *L. lactis* K1), expressed in *Escherichia coli*, and sequenced. The gene encodes a 313-amino-acid protein (M_r = 35,323) which showed relatively little overall sequence similarity to proteins currently catalogued in the data banks. When compared with the other N-(carboxyalkyl)amino acid dehydrogenases, N^5-(carboxyethyl)ornithine synthase was most similar to yeast saccharopine dehydrogenase (EC 1.5.1.7), which catalyzes the NADH-dependent condensation of lysine and α-ketoglutarate. These two proteins constitute a family of N^ω-(carboxyalkyl)amino acid dehydrogenases, which in turn, can be considered part of an amino acid dehydrogenase superfamily, that would also include N^2-(carboxyalkyl)amino acid dehydrogenases such as octopine synthase and nopaline synthase. With respect to the binding of its three substrates, N^5-(carboxyethyl)ornithine synthase contains a sequence segment that was virtually identical (eight out of nine residues) to one present in the βαβ-fold of the dinucleotide-binding domain of several microbial glutamate dehydrogenases. In addition, sequence motifs associated with the binding of pyruvate and ornithine were identified in the N-terminus of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00410-08 OD

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 Natural History of Periodontal Disease in Man

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

Löe, Harald, Director, NIDR PDS, EODPP, NIDR
Kingman, Albert, Chief Statistician, EODPP, NIDR

COOPERATING UNITS *(If any)*

University of Texas Dental School, San Antonio, TX
Department of Oral Biology, University of Buffalo, NY

LAB/BRANCH

Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.05	.05	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 study of the natural history of periodontal disease in Sri Lankan tea laborers and Norwegian males is continuing. This past year analyses have been completed both on the reliability and measurement errors associated with the assessment of plaque, calculus, gingival bleeding, and loss of periodontal attachment, and on the longitudinal relationships between gingival restorations and periodontal health. Future studies include the following: (1) Completing and submitting for publication a study of gingival recession in a population who practiced mechanical oral hygiene daily and in a population where oral hygiene never was practiced. (2) Study of the stability of the gingival lesion and conversion of gingivitis to periodontitis which are the critical issues in the quantitative destruction of the periodontium and which can only be studies with some precision in longitudinal materials. (3) Bacteriological and immunological studies of the two populations to relate the presence and absence of selected periodontal pathogens and peripheral blood antibody titers to the rate of periodontal destruction in the two groups. (4) Studies of the patterns and rates of tooth loss over the 25-year period in the Sri Lankan tea laborers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00520-03 OD

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.)*

Surface-specific attack rates in Iceland and the U.S.

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

Kingman, A., Chief Statistician, EODPP, NIDR

COOPERATING UNITS *(if any)*

Bjarnason S., Associate Professor, Göteborg, SWEDEN
Plöger, w., Chemist, Henkel, Duesseldorf, GERMANY

LAB/BRANCH

Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.05	.05	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.)*

Clinical data from 12 and 13 year old Icelanders (n=1032) obtained in 1984 were compared with those obtained for 12 and 13 year olds in the 1980 and 1986 NIDR National Children's Surveys (n=3256 and n=3460, respectively) for the U.S. population.
The surface-specific caries attack rates were consistently higher for the Icelandic population, in many instances 4 to 10 times as high. The notable difference was the strong inverse relationship observed between fluoride exposure and the level of smooth surface caries between the two groups. also noticed was that the prevalence of dental caries is rapidly becoming a pit and fissure phenomenon in the U.S. population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00521-03 OD

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.)*

Predictive value of salivary assays in predicting dental caries

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

Kingman, A., Chief Statistician, NIDR

COOPERATING UNITS *(if any)*

Bjarnason, S., Associate Professor Göteborg, SWEDEN
Plöger, W., Chemist, Henkel, Duesseldorf, GERMANY

LAB/BRANCH

Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.05	.05	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.)*

Identifying subjects who are likely to develop high levels of dental caries based on results from simple salivary assays for microorganism counts was the focal point of this study. It is know that these microorganism assays are of limited value in areas with low caries prevalence. Therefore, we studied an adolescent Icelandic population whose mean DMFS scores were roughly 3 times that of their US counterparts.

The basic findings were that the microorganism assays were also of limited value in a high caries prevalence population, although their positive predictive values were slightly higher than those typically reported in a low caries prevalence population. They also proved not to be significantly better than using the initial DMFS scores for individual subjects, suggesting that they were of no cost-benefit value in targeting subjects at high risk for caries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01DE00567-01 OD

PERIOD COVERED
June 1, 1992 - September 30, 1992

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

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

Streckfus, Charles F. Senior Staff Fellow EODPP

COOPERATING UNITS *(if any)*
CIPC, NIA

LAB/BRANCH
Office of the Director, EODPP

SECTION

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	0.00

CHECK APPROPRIATE BOX(ES)

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

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

The oral research component of the Baltimore Longitudinal Study of Aging (BLSA), since its inception in 1978, has been designed to evaluate the physiological and pathological factors that influence the oral health and function of individuals of different ages. Currently, the EODPP, is developing plans to broaden the scope of research to include studies of alveolar bone loss in the oral cavity, the detection and application of oral molecular biological markers for systemic disease, and an expanded periodontal evaluation implementing protein markers and DNA microbial probes for early disease detection. The oral epidemiology component is also working with the BLSA to increase minority enrollment thereby increasing the diversity of the BLSA population base. The implementation of these additional areas of investigation within the BLSA, present an opportunity to enhance the overall understanding of age related changes in the oral cavity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00044-22 MSS

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.)*

Handling of Microbial Strain Information by Computers

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

McManus, Candace	Microbiologist	MSS, MEDIB, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, MEDIB, EODPP, NIDR

COOPERATING UNITS *(if any)*

See attached page

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.30	1.09	1.21

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 MSS is developing a unified computer coding system for microbial information which is becoming an international standard for communicating strain data. The original bacterial system now includes the algae, yeasts, fungi, protozoa, and hybridomas.

Strain data are being entered into computers to provide: data on specific organisms; identification of unknown isolates; definition of parameters of taxa; aids in quality control of tests, methods, and laboratories; and communication of data via common format. Files of primary data on microorganisms found in the oral cavity and related types provide a resource for ecological and epidemiological dental research. Thus, indicator organisms for potential and/or on-going disease states can be found for diagnostic purposes.

The MSS analyzes the phenotypic data submitted by cooperating reference laboratories to the International Working Group on Mycobacterial Taxonomy in order to elucidate the taxonomic relationships within this genus. The latest analysis demonstrated at least one new distinct group of clinically important mycobacteria.

With EPA and ATCC staff, the MSS is building databases to aid in risk assessment of release of genetically engineered organisms in the environment, including features of microorganisms used in genetic manipulation and biotechnological processes and redefinition of taxonomic boundaries of such organisms. An identification matrix for fluorescent pseudomonads, organisms commonly used in biotechnology and genetic engineering, was developed from analysis of data collected in this effort.

Z01-DE-00044-22

COOPERATING UNITS: E. Baron, Wadsworth VA Hospital
M. Grahn, Food and Drug Administration
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture Collections
R. Atlas, University of Louisville
S. Socransky, Forsyth Dental Center
V. Levy-Frebault, Pasteur Institute
D. Jacobs, University of Maryland
W.E.C. Moore, Virginia Polytechnic Institute and State University
L.V.H. Moore, Virginia Polytechnic Institute and State University

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE-00250-15 MSS

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.)*

Algorithms for Microbial Systematics

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

Walczak, Cynthia A.	Computer Scientist	MSS, MEDIB, EODPP, NIDR
Krichevsky, Micah I.	Research Chemist	MSS, MEDIB, EODPP, NIDR
Mercer, Paula	Computer Programmer	MSS, MEDIB, EODPP, NIDR
McManus, Candace	Microbiologist	MSS, MEDIB, EODPP, NIDR

COOPERATING UNITS *(if any)*

See attached page

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

Microbial Systematics Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.38	2.17	0.21

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 Microbial Information System (MICRO-IS) is an ongoing project to enter, retrieve, and analyze microbiological data for epidemiological, diagnostic, taxonomic, ecological, and regulatory uses. The long term goal is to establish a worldwide data network at a series of cooperating centers. A mainframe version of the MICRO-IS is currently used extensively for management and analysis of strain data by the MSS and also by the FDA and EPA their regulatory roles. The latest thrust of this effort is development of a portable version of the MICRO-IS for installation on a wide range of computers including personal computers, minicomputers, and mainframes. This version is now being distributed and accepted on a worldwide basis.

The specifications (table structures) have been developed for transforming the data of the Hybridoma Data Bank into a relational model for ease of global editing and making special kinds of reports. A program for conversion of controlled vocabulary information in text records of the HDB into the relational database has been implemented by the MSS in collaboration with the HDB staff. The conversion of the HDB from flat files to the relational model is ongoing.

A related project is the development of techniques for format analysis and standardization of text images obtained by direct input of microbiological laboratory notebook information. A pilot project has been initiated to capture the phenotypic strain descriptions from the archival records of the VPI Anaerobe Laboratory.

COOPERATING UNITS: M. Grahn, Food and Drug Administration
E. Baron, Wadsworth VA Hospital
L. Blaine, American Type Culture Collection
M. Segal, Environmental Protection Agency
L. Wayne, Long Beach VA Hospital
B. Kirsop, World Federation for Culture Collections
S. Socransky, Forsyth Dental Center
V. Levy-Frebault, Pasteur Institute
D. Jacobs, University of Maryland
W.E.C. Moore, Virginia Polytechnic Institute and State University
L.V.H. Moore, Virginia Polytechnic Institute and State University
H. Sugawara, Institute for Physical and Chemical Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00462-05 SSDM

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.)*

Methods for Estimating Age-Specific DMFS and DMFT Scores in the U.S.

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

PI:	Li, Shou-Hua	Statistician (Health)	SSDM, NIDR
Others:			
	Kingman, Albert	Chief Statistician	OD, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	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.)*

Typically the prevalence of dental caries is determined by a full mouth examination for each subject. However, limitations of manpower or time may often preclude such a approach. Knutson showed that a screening examination using the presence or absence of caries could be used to predict DMFT scores for children. In this study we investigate how well DMFS scores for subjects under 35 years of age can be estimated by specific models based on age-specific prevalences, using data from the NIDR prevalence surveys (1987 children's survey and the 1985 adult survey). Data for adults older than 35 were not used, since the M component in DMFS is not caries specific for these adults.

Knutson in his original model studied the relationship between the age-specific mean DMFT and the age-specific caries prevalence. Here, we considered two models. One is Knuston's model with DMFS replacing DMFT. The second one is the linear regression model. The assumption for the linear regression model is that the prediction of DMFS can be expressed as a linear function of the log of the proportion of caries-free individuals and age.

Both Knutson's model and the regression model can be used in both children and young adults separately to describe the relationship between caries severity (DMFS) and caries prevalence. The regression model is easier to interpret and estimate than the nonlinear Knutson model. The regression model also emphasized the need to adjust for age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00464-05 FSS

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.)*

Natural History of Oral Manifestations of HIV Infection

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

Philip A. Swango	STCDPS, EB, EODPP, NIDR
Dushanka V. Kleinman	STCDPS, EB, EODPP, NIDR
Philip Fox	CIPCB, IRP, NIDR
Ruth Nowjack-Raymer	DPS,DPHPB,EODPP,NIDR
Carla Bock	EODPP, NIDR

COOPERATING UNITS *(if any)*

Walter Reed Army Institute of Research, SUNY Buffalo

LAB/BRANCH

Health Assessment Branch, EODPP, NIDR

SECTION

Field Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

United States Army personnel and dependents who have tested seropositive for the Human Immunodeficiency Virus (HIV) are given medical examinations and treatment at Walter Reed Army Medical Center. Subjects are also invited to participate in a research protocol to study the natural history of HIV infection, conducted by the Walter Reed Army Institute of Research. An oral health research component conducted by NIDR is a part of this natural history study.

The oral component documents the prevalence and incidence of oral pathologic conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions are also characterized, and the role of oral manifestations as early predictors or markers of disease progression are studied. Areas of emphasis are mucosal pathologies, periodontal conditions, candidal infections, and salivary constituents. Results to date show an increase in mucosal pathology as T4 cell counts decrease with progression of HIV disease. The most commonly observed mucosal pathologies were oral candidiasis and hairy leukoplakia. Destructive periodontal disease has occurred in about 25 percent of subjects examined. Longitudinal studies showed that about 30% of persons free from mucosal pathology at entry presented with pathology after six months of follow-up.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DE00501-03 SSDM

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.)*
Surface-Specific Attack Rates In Primary Teeth From Two National Surveys

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

PI:	Li, Shou-Hua	Statistician (Health)	SSDM, NIDR
Others:			
	Kingman, Albert	Chief Statistician	OD, NIDR
	Forthofer, Ronald	Private Consultant	

COOPERATING UNITS *(if any)*

LAB/BRANCH
Health Assessment Branch

SECTION
Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION
NIDR, NIH, Bethesda, Maryland 20892

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

The purpose of this study was to investigate whether changes had occurred in attack rates in primary teeth during the 1980's. Data on decayed and filled surfaces (df) in 5-9-year old children from the 1979-80 and 1986-87 NIDR dental caries surveys were analyzed. The attack rate was defined as the number of decayed and filled surfaces per 1000 surfaces at risk.

The prevalence of df surfaces declined from an overall mean of 5.31 in 1979-80 to 3.91 in 1986-87. Surface-specific caries attack rates were found to be similar in both surveys. The rank order of the largest six attack rates were: occlusal surfaces of 2nd and 1st molars, distal surface of 1st molar, mesial of 2nd molar, lingual of upper 2nd molar and buccal of lower 2nd molars. The tooth-specific attack rates were, from high to low: 2nd molar, 1st molar, central incisor, lateral incisor and cuspid.

There was no appreciable difference reduction in caries attack rates between the occlusal and proximal surfaces of primary teeth from the 1980 to 1987 NIDR survey. This was in contrast to greater reductions in caries attack rates reported in proximal surfaces of permanent teeth between the 1971-74 HANES survey and the 1980 NIDR survey and also permanent teeth between 1980 to 1987 survey.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00503-03 SSDM

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.)*

Documentation of Public Use Files--1986-87 and 1979-80 Surveys of School Children

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

PI: Snowden, Cecelia B. Chief, Samp., Stat., & Data Mgmt SSDM, EODPP, NIDR

Others:

Miller-Chisholm, Ann Health Scientist Administrator MEDI, EODPP, NIDR

COOPERATING UNITS *(if any)*

Westat, Inc.
Rockville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF 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.)*

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a replication and extension of the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this continuing collaborative effort is to document the survey designs and produce public use tapes for both the 1986-87 and the 1979-80 surveys, and to provide clinical protocols and statistical methodologies for calculating national or regional estimates, weights and sampling errors. The documented tapes generated by this effort were released for public use by the National Archives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00504-03 SSDM

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.)*

Statistical Analysis of the 1986-87 and 1979-80 NIDR Surveys of School Children

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

Snowden, Cecelia B. Chief, Samp., Stat,, & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

Westat Inc.
Rockville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

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

During 1986-87 the National Survey of Oral Health in School Children was conducted by Westat Inc. in cooperation with NIDR to monitor the prevalence of oral diseases in school children, grades K-12, throughout the contiguous United States and Hawaii. The 1986-87 survey was a replication and extension of the NIDR National Dental Caries Prevalence Survey conducted in 1979-80, also by Westat, which established baseline estimates on the prevalence of dental caries, gingivitis and dental restorative treatment needs. Both surveys utilized multi-stage probability samples of over 39,000 school children enrolled in grades K-12 to represent over 43 million children enrolled in public or private schools in the seven geographic regions of the U.S. In the 1986-87 survey, additional assessments were made for dental fluorosis, soft tissue lesions, and the use of smokeless tobacco. Residential histories, health and demographic data were collected for each child participating in the clinical examination.

The objective of this ongoing collaborative effort is to develop generalized variance models for selected non-binary statistics common to both surveys, to calculate correlations between the two surveys and to measure design effects in order to establish optimal cluster sizes for future national surveys.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00527-02 SSDM

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.)*

Software for Analyzing Data From Complex Dental Surveys

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

Snowden, Cecelia B. Chief, Samp., Stat., & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Center for Disease Control
Hyattsville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	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 development of a data analysis software package (SUDAAN) that is appropriate for the analysis of complex sample surveys is an ongoing project of the National Center for Health Statistics through a contract with Research Triangle Institute (RTI). SUDAAN is a collection of several high level statistical procedures that employ state-of-the-art methodology, Taylor series or Delta method of estimation for analyzing data from complex sample survey designs. Data from two Children's, Adults and Seniors Dental Surveys are being used to analytically test and evaluate SUDAAN in five areas:

(1) appropriate methodology for complex survey samples
(2) portability
(3) reliability/numerical accuracy
(4) computational efficiency
(5) ease of modification/enhancement

Work is proceeding in these areas with intermediate mainframe and PC software test versions of SUDAAN.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00528-02 SSDM

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.)*

Statistical Methods and Software for Analysis of Geographic Referenced Data

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

Snowden, Cecelia B. Chief, Samp., Stat., & Data Mgmt SSDM, EODPP, NIDR

COOPERATING UNITS *(if any)*

National Center for Health Statistics, Centers for Disease Control
Hyattsville, Maryland

LAB/BRANCH

Health Assessment Branch

SECTION

Sampling, Statistics, and Data Management Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	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.)*

This is an ongoing research and development project that includes automated cartography, geographic information systems, and related statistical methods and software, that will generally and/or specifically be used to meet NIDR data analysis goals in studying minority dental health status and other studies such as tooth loss. Included in this project is an analysis and documentation of existing technology and application of new statistical methods and technology to NIDR data sources.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00420-07 ASDSB

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.)*

Analysis of National Survey of Oral Health in School Children

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

Brunelle, Janet A. Statistician (Health) EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.10	0.10	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

A national survey of the Oral Health of School Children was conducted during the 1986-87 school year. Approximately 41,000 children were examined by 13 trained and calibrated dental teams. When compared to a similar survey conducted in 1979-80, DMFS had declined 37%. Mean DMFS was lower in all regions of the country; however, there were still regional variations as before. Approximately 50% of the children aged 5-17 were caries free in their permanent dentition. A monograph on caries status, "Oral Health of U.S. Children, 1986-87," was published. Mean caries experience for primary teeth for children aged 5-9 years showed a 26% decline from the 1979-80 survey. The dfs was lower in every region except Region VII, with the greatest decline observed in Reg. I. Approximately 50% of the children had no dfs; 28% had more than 4 surfaces d or f. Only 7.6% of the children aged 5-17 had sealants present. The average number of sealants in children with sealants was 4.2 per child. An estimate of the prevalence of dental fluorosis was made using Dean's Index on 2nd through 12th graders. 22% of children showed definite signs of fluorosis, 17% very mild, 4% mild, 1% moderate and 0.3% severe. Microbiological samples of mutans streptococci and Lactobacillus were evaluated in relation to dental caries. Children with mutans counts of zero CFU/ml of salivary rinse had less than half as many DMFS as those with any detectable mutans. The level of caries rose with increases in mutans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00475-05 ASDSB

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.)*

Utilization, Treatment Needs, Cost, and Dental Disease in Veterans

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

Brown, L. Jackson	Director	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Veterans Administration Outpatient Clinic, Tufts University, and Harvard University, Boston, Massachusetts

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.41	0.36	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Veterans Administration in 1963 initiated an interdisciplinary and longitudinal investigation of the normal aging process. Participants consisted of 2,400 men with stable living and work conditions in the Boston, Massachusetts area. From this panel, 1221 self-selected subjects between the ages of 25 and 75 volunteered for the Dental Longitudinal Study in 1968. These persons have received a complete dental examination every three years since 1968. The triennial examinations include a radiographic survey and a comprehensive clinical examination documenting dental caries, periodontal status, missing teeth, and oral hygiene.

This project is supplementing these clinical data with detailed utilization data from the dental offices visited by the panel members over the past ten years. The data collection is complete and the information has been integrated with the clinical data. The full dataset is being used as a source of previously unavailable longitudinal information as well as cross sectional information. Findings on episodes of use and nonuse of dental services were presented at the AADR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00496-04 ASDSB

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.)*

Determinants of Permanent Tooth Loss in Connecticut and North Carolina

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

Brown, L. Jackson	Director	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Albertini, Tullio F.	Sp Asst for Prog Mgt	EODPP, NIDR

COOPERATING UNITS *(if any)*

University of Connecticut, Farmington, Connecticut

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.38	0.28	.10

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this study are to measure permanent tooth loss and the factors which influence it. The study will be conducted in two (2) phases. The first phase will be independent of the second and will be a complete study without the second phase. Specific aims of Phase I are to describe 1) the biological condition of extracted teeth, 2) the sociodemographic, attitudinal, economic, and dental care-seeking characteristics of individuals who have extractions, and 3) selected characteristics of the dental providers who perform the extractions. Phase II will be conducted after the first phase and will collect information on patients whose teeth were treated with dental services that are alternatives to extraction for given biological conditions. These teeth will be controls for the extracted teeth and will allow the estimation of a model which explains the factors which influence the choice between extraction and its alternatives. The same practices will be used for both phases. Data from both Phases will be used to develop a more complete explanation of the relative significance of these factors for tooth loss.

This year the data collection instruments (patient questionnaire and patient examination form) and the sampling strategy have been finalized. The instruments underwent several rounds of focus group pretesting and pilot testing. The sampling strategy was revised from a "quota" sampling approach to one using random probability sampling to improve inference to the general population. In addition, in vivo/in vitro validation studies were carried out to determine the comparability of measurements (e.g. pocket depth) across cases and controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00497-04 ASDSB

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.)*

Forecasting Dental Health and Utilization Using A Microsimulation Model

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

Brown, L. Jackson	Director	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Cornell University, Department of Sociology, Ithica, New York and University of Michigan, Ann Arbor, Michigan

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.22	0.17	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Branch is developing a computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the U.S. These forecasts will be developed in considerable sociodemographic details. Cornell University was awarded the contract to develop the model on May 15, 1990. Dr. Caldwell, a microsimulation specialist, is principal investigator. Dr. Stephen Eklund of the University of Michigan is assisting in the development of the oral disease and conditions portion of the model. Several noted dental specialists and modeling experts are consultants to the project. Development of the model and the production of initial forecasts has been underway for two years. Programming and testing of the sociodemographic and dental portions of the model are complete. Over the next year NIDR staff will be aligning the model. Microsimulation is the approach being used. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. Policy experiments with the full model are planned both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model will provide a vehicle for carrying out experiments in which the latest dental research can be applied consistently and systematically to key dental policy issues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00515-03 ASDSB

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 Periodontal Health in Adult Americans

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

Brown, L. Jackson	Director	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Beck, James	Special Expert	EODPP, NIDR

COOPERATING UNITS *(if any)*

University of Minnesota, School of Dentistry, Minneapolis, Minnesota

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.40	0.40	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 periodontal health of employed U.S. adults is being studied in collaboration with university researchers. The collaboration has resulted in several publications and will result in several more. An overall description of periodontal conditions is complete and published. An analysis of the association between sociodemographic variables and periodontal status has also been completed and published. A study developing a model to predict groups at high risk to have periodontal destruction is also complete. Profiles containing sociodemographic characteristics of persons with rapidly progressing periodontitis are being constructed. Distributions of these profiles between different groups of adults are being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00517-02 ASDSB

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 Alveolar Bone Loss and Aging Among Healthy U.S. Males

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

Brown, L. Jackson Director EODPP, NIDR

COOPERATING UNITS *(if any)*

Veterans Administration and Tufts University, Boston, Massachusetts

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.02	0.02	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The project is a cross-sectional and longitudinal analysis of alveolar bone loss (ABL) in aging men. Study subjects are 700 adult male participants in the VA Dental Longitudinal Study (VADLS). Bone loss is determined using existing intraoral periapical radiographs.

The VA-DLS has the most extensive longitudinal radiographic data base available with which to examine the factors associated with progressive alveolar bone loss. The existence of sequential radiographs permitted longitudinal analysis of the actual ABL experienced over a twenty year period at three year intervals. Full-mouth series of intra-oral periapical radiographs, obtained at three-year intervals, were computer digitized and used to measure ABL over time.

Percent remaining bone was measured at all interproximal sites at each of six time points. Over 87% of subjects had at least one site that had experienced a rate of ABL ≥10% over 15 years. 47% of subjects had more than 5 sites and approximately 20% had more than 11 sites with a rate of ABL ≥10% over the 15 years. Distribution of sites having more severe ABL was similarly skewed, with a relatively small number of subjects accounting for the majority of sites with severe ABL. The project will yield much improved estimates of the rate of ABL condition on putatively important explanatory variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00540-02 ASDSB

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.)*

Tobacco Use and Oral Health

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

Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Swango, Philip	Chief, FSS	EODPP, NIDR
Kingman, Albert	Chief Statistician	EODPP, NIDR

COOPERATING UNITS *(if any)*

Epidemiology, OSH, Center of Disease Control, Atlanta, Georgia

LAB/BRANCH

Analytical Studies and Decision Systems Branch and Health Assessment Branch

SECTION

Analytical Studies Section and Field Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.30	0.25	0.05

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Epidemiologic evidence has been accumulating that cigarette smoking is causally related to cancers of the larynx, oral cavity, and esophagus in both men and women. The mortality ratios for these cancers are similar for smokers of cigarettes, pipes, or cigars. A strong dose-response relationship exists. Compared with those who continue smoking, the risk of cancer decreases for those who quit smoking. Alcohol consumption is also an important risk factor for oral, pharyngeal, laryngeal, and esophageal cancer. The combination of smoking and alcohol acts synergistically to increase risk of these cancers.

There is mounting concern about the oral health consequences of the recent resurgence of smokeless tobacco use among teenage boys in the United States. While the evidence is strongest that smokeless tobacco causes cancer of the oral cavity, there is also evidence that the use of smokeless tobacco increases the risk of cancer of the pharynx, larynx, and esophagus. Smokeless tobacco also causes a variety of noncancerous and precancerous oral conditions, the most important of which is oral leukoplakia (other less serious oral conditions associated with the use of smokeless tobacco include gum recession and tooth loss).

The purpose of this study is to re-examine the relationships between tobacco use (cigarette smoking and smokeless tobacco) and oral health using recent large national data sets and examine the possible physiologic mechanisms through which tobacco acts on oral tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00542-02 ASDSB

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.)*

Analysis of Trends in and Risk Factors for Permanent Tooth Loss

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

Brown, L. Jackson	Director	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.13	0.08	0.05

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The objectives of this study are to characterize the trends in tooth loss in the U.S. population and major sociodemographics using data from repeated cross-sectional surveys and to model risk factors for tooth loss using longitudinal data from panel studies. Data from Sri Lanka and Norway were used to develop appropriate measures and test analytical strategies. Plans call for further analyses of the VA longitudinal data base and repeated cross-sectional surveys of the U.S. population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00543-02 ASDSB

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.)*

Optimizing Throughout on an IBM RISC 6000 System

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

Zion, Gary	Computer Programmer	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Scientific Programming Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.09	0.09	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A tremendous amount of dental research survey data has been collected over the past quarter of a century. The computer programs that perform statistics and mathematical computations on the data in order to present it to researchers in a useful format require extensive use of computer resources. CPU power, memory, and disk storage are three critical resources that impact the speed with which a job runs. When resources are scarce jobs run slowly. Granting more resources to a job than it requires is wasteful, and leaves then unavailable for other jobs. The intent of this project is to determine the optimal configuration of an IBM RISC, model 320H, and the optimal combination of resources to grant to typical statistical programs to maximize throughout on the RISC system and fully utilize its resources. The findings of this study shall be implemented on the program's RISC system, and a paper shall be written describing the approach and results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00544-02 ASDSB

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 Periodontal Health in Adolescent Americans

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

Brown, L. Jackson	Director	EODPP, NIDR
Löe, Harald	Director	NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Brunelle, Janet	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.66	0.66	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 prevalence of early onset periodontitis in U.S. children aged 14 to 17 years has been completed and was published in October, 1991. In a national survey, the total number of adolescents affected by LJP was about 70,000. Seventeen thousand were estimated to have GJP and another 212,000 adolescents had incidental LA (≥3mm on 1 or more teeth). Blacks were at greater risk of all forms of early onset periodontitis than whites. Males were more likely (4.3 to 1) to have GJP than females when other variables were statistically controlled.

A major contract to relocate, re-examine and collect risk factor information on these children began in October, 1991. Research objectives are to: a) assess the progression of periodontal destruction among the cases of early onset periodontitis, b) characterize the microbial ecology of the sub-gingival plaque among persons with early onset periodontitis, c) describe periodontal destruction and the presence of biologic and non-biologic putative risk markers among the probands' similar aged siblings, and d) compare the presence and concentration of selected putative pathogens and high-resistance factors among individuals with early onset periodontitis to controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00545-02 ASDSB

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.)*

Longitudinal Studies of Periodontal Health in Norwegians and Sri Lankans

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

Brown, L. Jackson	Director	EODPP, NIDR
Löe, Harald	Director	NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR
Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.26	0.26	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 the progression of periodontal conditions from longitudinal data on subjects from Norway and Sri Lanka are ongoing. Recession and tooth loss due to the progression of periodontal destruction are being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00565-01 ASDSB

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.)*

Comparison of Mutans Counts from Three Selective Media

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

Brunelle, Janet A.	Statistician (Health)	EODPP, NIDR
Little, Wayne A.	Microbiologist	PIRS, OD, NIDR

COOPERATING UNITS *(if any)*

Division of Research Grants, NIH

LAB/BRANCH

Analytical Studies and Decisions Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20897

TOTAL STAFF 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.)*

Over two thousand salivary rinse samples were collected during the 1986-87 national survey of U.S. schoolchildren. Specimens collected with this technique were plated on three selective media: MSB, GSTB and TSY20B. Counts of mutans streptococci were determined on all three media. Mean DMFS were computed for all children aged 7 to 17 who had rinse samples. 1109 specimens had readable plate counts for mutans on all three media. In over one-fifth of the samples from each media, no CFU of mutans were detected.

Mean mutans counts (CFU/ml of salivary rinse) were approximately 1.5×10^4 for MSB, 1.2×10^4 for TSY20B and 1.7×10^4 for GSTB. Repeated measures ANOVA indicated significant differences ($p<.0001$) between counts on different media. However, correlations between media were high and differences were normally in the same direction with GSTB highest and TSY20B lowest.

Associations with dental caries were measured for all three media. Mean CFU for those with zero DMFS were 30 to 40% lower than for children with 1 or more DMFS. Mean DMFS scores were 50% lower for groups with zero mutans counts versus those with any mutans for all three media. (approximately 2 DMFS for 0 mutans counts and 4 DMFS for those with mutans). Odds ratios for the association between mutans and caries were approximately 2.2 for all three media, i.e. children with mutans were about twice as likely to have caries as those with no mutans ($p<.0001$).

Counts from all three media were able to differentiate between groups of children with or without dental caries reflecting the strong association between dental caries and mutans and making the salivary rinse a viable collection technique.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00566-01 ASDSB

PERIOD COVERED

June 1, 1991 to September 30, 1992

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

Utilization as a Risk Factor of Adult Periodontitis

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

Marcus, Stephen E.	Senior Epidemiologist	EODPP, NIDR
Brown, L. Jackson	Director	EODPP, NIDR
Oldakowski, Richard J.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.26	0.26	

CHECK APPROPRIATE BOX(ES)

☐ (a) 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 role that the utilization of dental services plays in modifying the progression of periodontal disease in adults is not known. Increased access to dental services may have paradoxical effects; it may be associated with an **increase** in disease. These effects may be due to the so-called "discovery effect", where increased utilization translates into increased detection of previously undiagnosed disease. On the other hand, to the extent that periodontal services are effective, we would expect that increased service use would **decrease** the prevalence or the severity and extent of periodontal disease (or slow the progression of the disease). In response to an invited presentation at a conference on risk factors of periodontitis (to be held in 1993), NIDR staff is examining the effects utilization has on various measures of periodontal disease using several cross-sectional and longitudinal data bases. Preliminary analyses (on the VA longitudinal data base) and an extensive literature review have been conducted in 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00568-01 ASDSB

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 Cost Curve for Community Water Fluoridation Based on Water Usage Information

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

Brown, L. Jackson	Director	EODPP, NIDR
Zion, Gary R.	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Florida Health Department, Tallahassee, Florida

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.46	0.21	0.25

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Previous data relating to the cost of water fluoridation have frequently been limited to variable costs such as chemicals and other materials. The purpose of this study was to measure opportunity costs and to estimate a cost curve for water fluoridation. Data were collected from 44 Florida communities which had initiated community water fluoridation between 1981 and 1989. Equipment installation and engineering costs were derived from actual invoices and adjusted to 1988 dollars. Output of the water systems was measured by water usage and community population.

For large systems, average costs approached an asymptote at $0.21 per person per year when population was used as a measure of output and $1,199 per year to fluoridate one million gallons of water daily. Most of the economies of scale were exhausted with water systems serving moderate sized towns with 10,000 to 50,000 people. While water usage is conceptually the preferred output measure, results indicate that population can serve as a very good proxy when water usage is not available. L shaped average cost curves were very good models of fluoridation. Long range average cost curves exhibited economies of scale.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00569-01 ASDSB

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.)*

Adapting Survey Data Analysis Software to a RISC/6000 Scientific Workstation

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

Zion, Gary R. Computer Programmer EODPP, NIDR

COOPERATING UNITS *(if any)*

Research Triangle Institute, Raleigh/Durham, North Carolina

LAB/BRANCH

Analytical Studies and Decision Systems Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	

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.)*

SUDAAN is a software package developed by the Research Triangle Institute which uses advanced statistical methodology to analyze data obtained from surveys with complex sample designs. This software has not previously been available for use on the IBM RISC/6000 advanced scientific workstation, one of which was purchased by EODPP in 1991.

Under a new type of agreement with RTI, EODPP was able to bring the SUDAAN software to the RISC/6000 at no cost. Rather than funding RTI to port the software at an estimated cost of $30,000, EODPP signed a nondisclosure agreement with RTI which gave EODPP staff access to the source code for SUDAAN so that they could port the software themselves. The new version of the software remains the property of RTI and EODPP has signed a license agreement for use of the software at a cost equal to that of other versions of SUDAAN. RTI benefits by having a new version of their software which they are free to license to other users.

EODPP staff are currently extending the usefulness of SUDAAN by designing a link between that software and SAS, which is a widely used statistical and data manipulation software package.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00070-20 DPHP

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.)*

Combined self-applied fluorides and sealants for caries prevention

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

Driscoll, William S.	Chief, DP Section, DPHPB	EODPP, NIDR
Selwitz, Robert H.	Research Dentist	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.46	.45	.01

CHECK APPROPRIATE BOX(ES)

☒ (a) 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 self-administered dental health program was initiated in Nelson County, VA., a fluoride-deficient community in October 1972. Children in the County's schools, under teacher supervision, chewed and ingested daily a 1 mg F tablet and rinsed weekly with a 0.2% NaF solution. A fluoride dentifrice was provided for ad libitum use at home. Baseline DMFS examinations were made of 2,138 children in the County's elementary, junior high, and senior high schools. Follow-up DMFS examinations were conducted at two-to-three year intervals. Final examinations were conducted in 1983 when the full effectiveness of the fluoride program could be assessed.

In the fall of 1983, a sealant program was added to the ongoing fluoride program. Children who were 6, 7, 12, and 13 were eligible to have pit-and-fissure sealants applied. An initial screening to identify those tooth surfaces to be sealed was made in December 1983. Caries data (DMFS) from the September 1983 dental examination served as a baseline for those children who participated in the sealant phase of the study. In succeeding years, new groups of 6 and 12 year olds were enrolled. Treatments continued for four years. Interim dental examinations took place at the start of the third year of the study (September 1985) and final examinations were made in September 1987. Descriptive analysis is complete and a manuscript is in review.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00439-06 DPHP

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.)*

Eval. of different approaches to prev. gingivitis in teenage children

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

Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Driscoll, William S.	Acting Chief, DPHP Branch	EODPP, NIDR
Kingman, Albert	Statistician (Health)	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.25	.24	.01

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Four hundred and ninety three ninth and tenth graders participated in a study in York County, Virginia designed to compare the effectiveness of traditional plaque control methods with self-assessment of gingival bleeding as approaches to the prevention of gingivitis in teenagers. At baseline examination in April 1987 participants were examined for periodontal health, DMFS, and gingival recession. Questionnaires regarding oral hygiene methods and professional care practices were completed. Subjects were then randomly assigned by grade to a positive control plaque group or to a test self-assessment of bleeding group.

Dental hygienists provided appropriate instruction using manuals outlining procedures for the identification of plaque or the self assessment of gingival bleeding. Two weeks following this classroom based instruction, individual instruction was held to reinforce each message. One year later each participant had individual instruction with the dental hygienist and the opportunity to have an oral prophylaxis.

Four interim examinations to assess periodontal health were conducted. The final examination, in April 1989, assessed DMFS, gingival recession and periodontal status. A dramatic decrease in the mean number of bleeding sites in both the plaque control and self assessment of gingival bleeding groups was found for the 356 remaining subjects. Data analyses are completed and manuscripts are in review.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00310-12 DPHP

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.)*

Eval. fluoride mouthrinse & fluoride tablets used separate & combined combine

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

Driscoll, William S.	DP Section, DPHP Branch	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR
Selwitz, Robert H.	Research Dentist	EODPP, NIDR
Li, Shou-Hua	Statistician (Health)	EODPP, NIDR
Gregg, Mary J.	Statistical Assistant	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.76	.51	.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.)*

In September 1981, a study designed to compare the caries-preventive effects of a combined regimen of weekly fluoride rinsing and daily fluoride tablets with those of each procedures used alone was begun in Springfield, Ohio, a fluoride-deficient community. The approximately 1700 children attending 20 public and non-public elementary schools were randomly assigned to one of three treatment groups. The children in Group I dissolved and ingested daily a 1 mg F tablet; the children in Group III rinsed weekly with a 0.2% NaF solution; and Group II carried out both procedures. The assigned treatments were self-administered under the supervision of classroom teachers who received in-service training.

Before the procedures were started, baseline DMFS examinations were conducted. First and second follow-up examinations were conducted in October 1983 and November 1986 respectively. Final DMFS examinations were conducted in May 1989. Data from this exam have been analyzed and a report has been published. Follow-up examinations for dental fluorosis were conducted in February 1992. Following completion of data analysis, a final report will be prepared.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00523-03 DPHP

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.)*

Prev./Dental Caries & Dental Fluorosis in Relation to Water Fluoride C

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

Driscoll, William S.	DP Section, DPHP Branch	EODPP, NIDR
Selwitz, Robert H.	Research Dentist	EODPP, NIDR
Kingman, Albert	Chief Statistician	EODPP, NIDR
Nowjack-Raymer, Ruth E.	Health Research Specialist	EODPP, NIDR

COOPERATING UNITS *(if any)*

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.84	.69	.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.)*

This study is a follow-up of previous studies conducted by the NIDR in Illinois in 1980 and 1985 and in Iowa in 1982 to assess the prevalence of dental fluorosis and dental caries among schoolchildren exposed to different concentrations of fluoride in their drinking water. Both Illinois surveys were carried out in the same communities, which had naturally occurring fluorides in their water supplies at concentrations of approximately one, two, three and four times that recommended as optimal for those areas. In contrast, the Iowa communities had negligible concentrations of fluoride in their drinking water.

The need to monitor for possible changes in the prevalence of dental fluorosis continues to receive high priority. Additional research also is needed to further elucidate and define current interrelationships between dental caries, dental fluorosis, and various concentrations of fluoride in drinking water. The continued availability of the same communities in Illinois and the identification of two communities in Nebraska with negligible concentrations of water-borne fluoride have afforded an excellent opportunity to address these research needs. Examinations were completed in April 1990 on approximately 300 schoolchildren ages seven through 16 in the two Nebraska communities. Each child was examined for dental caries using the DMFS index and for dental fluorosis using both Dean's Index and the Tooth Surface Index of Fluorosis. Examinations, using the same indices, were conducted in October 1990 in Illinois. Data from all study sites currently are being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00530-02 DPHP

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.)*

Second. Data Analyses Oral Disease Prev./Health Promotion Strategies

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

Gift, Helen	Sociologist	EODPP, NIDR
Horowitz, Alice	Education Specialist	EODPP, NIDR
Larach, Dina	Research Specialist	EODPP, NIDR
Redford, Maryann	Public Health Specialist	EODPP, NIDR
Oldakowski, Richard	Computer Programmer	EODPP, NIDR

COOPERATING UNITS *(if any)*

Corbin, Stephen	Disease Prev. Policy Anal.	CDC

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.1	.5

CHECK APPROPRIATE BOX(ES)

☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

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

Existing national data sets, including 1986 NHIS, 1989 NHIS, 1990 NHIS, 1981 HRSA, National Adolescent Student Health Survey, current state and local surveys are being analyzed to improve understanding of disease prevention, health promotion, oral complications of systemic conditions, risk factors for minority and issues of specific concern for women. These data sets and extensive literature reviews have been analyzed for policy analysis, program planning and research publication.