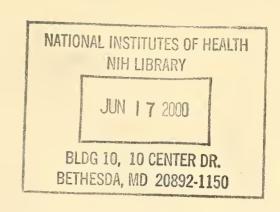BIOMEDICAL ENGINEERING AND INSTRUMENTATION BRANCH

DIVISION OF RESEARCH SERVICES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FY 1984

Dr. Murray Eden, Chief

BIOMEDICAL ENGINEERING AND INSTRUMENTATION BRANCH

DIVISION OF RESEARCH SERVICES

NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FY 1984

Dr. Murray Eden, Chief

BEIB Research Projects (ZO1 RS)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10001-16 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
R.L. Dedrick   Chief, ChES   BEIB, DRS

Others:
F. Farris	Guest Worker	BEIB, DRS
C. Daniels	Chemical Engineer	BEIB, DRS
R. J. Lutz	Chemical Engineer	BEIB, DRS
F. King	Chemical Engineer	BEIB, DRS
P. F. Morrison	Physical Scientist	BEIB, DRS

COOPERATING UNITS *(if any)*
CPB, NCI (J.M. Collins); LMCB-NCI (C.L. Litterst); DR-CC (J.L. Doppman); CP-CC (G. Hook); NTP-NIEHS (H.B. Matthews); SNB-NINCDS (E.H. Oldfield, C. Clark); University of Maryland (M.Egorin; M.F. Flessner).

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Pharmacokinetic models are developed for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and man. They provide a plausible set of equations that can be used to extrapolate data from animals to man and thereby improve chemotherapy, and risk assessment. Emphasis has been placed on regional drug administration, and this has led to the devleopment of spatially distributed models of drug transport in tissue. These analyses have provided considerable insight into the penetration depths of drugs administered intraperitoneally or by infusion into the brain. The penetration of cis-dichlorodiammine-platinum (II) (DDP) into peritoneal and subperitoneal tissue is being examined experimentally with an electron probe and the results compared with a reaction-diffusion equation of the process. A lumped model of DDP pharmacokinetics has been developed to include both metabolism to a mobile species and covalent binding to macromolecules. Pharmacokinetic theory which was developed for intra-arterial drug administration combined with hemoperfusion of vascular drainage had been validated experimentally in monkeys. Clinical trials further demonstrated the pharmacokinetic theory. Systemic exposure to BCNU was reduced by 56-87% compared with intra-arterial administration of the same dose. Studies with intracarotid infusion of indocyanine green have demonstrated the ability to remove a large fraction of the infused blood by pumping from the ipsilateral jugular vein.

OBJECTIVES: Improve and extend mathematical models for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and man to:

(1)   Account for species differences in drug distribution.

(2)   Provide a rational basis for extrapolating toxicity from animals to man.

(3)   In conjunction with pharmacodynamics, provide a basis for optimizing cancer chemotherapy.

(4)   Enable rational transfer of in vitro thermodynamic and kinetic data to in vivo cases.

(5)   Predict effective dose schedules of anticancer drugs in individual patients with particular emphasis on intraperitoneal drug administration and also drug delivery to the brain.

METHODS EMPLOYED: Mathematical models are developed from physiochemical, physiological, and anatomical information and the principles of chemical reaction engineering. Resulting ordinary or partial differential equations are solved analytically or numerically and compared with experimental data. Uncertainties are clarified by additional experiments and model modification.

MAJOR FINDINGS: (1) A physiologic pharmacokinetic model has been developed to describe the distribution, metabolism, and elimination of cis-dichlorodiammineplatinum (DDP) in the tumored rat. The kinetic model consists of a complex set of reactions whereby DDP reacts to form mobile metabolites or reacts with macromolecules to form fixed species. The primary site of elimination of platinum is the kidney which clears DDP at a rate similar to GFR. The bound metabolites remain fixed until the macromolecules are catabolized to form the mobile species which can circulate until they are eliminated by the kidneys. Platinum tissue release rates approximate published protein turnover rates. DDP reaction rates are tissue specific and exceed the aquation rate.

The DDP model is being applied to determine the distribtion of platinum in other species. The animal data are being correlated with body weight to determine if there is a systematic variation. The animal data will then be used to predict human pharmacokinetics.

(2) Peritoneal transport studies were conducted on rats in order to uncover the principal mechanisms involved in the movement of drugs across of peritoneal membrane.

Sprague-Drawley rats were injected intraperitoneally with cisplatin at 25 mg/kg and 35 mg/kg. All drug was allowed to be absorbed over a twenty-four hr. period, the drug remaining in the tissues after this time being that bound to proteins. Tissues containing the peritoneal layer were then removed from the liver, intestines. kidney, and anterior abdominal wall. Frozen tissue sections were prepared for histology and x-ray microanalysis using the electron microprobe.

The microprobe was used to determine cisplatin concentration profiles as a function of distance from the peritoneal surface. Exponentially decreasing profiles were expected if transport occured primarily by diffusion through a relatively homogeneous capillary bed. Albumin/cisplatin standards covering the theoretical concentration range showed that detectability was within the capability of the wave-length spectrometer with the probe operating at 40 kV on the M line of Pt. Ten hour overnight imaging revealed a non-exponential profile of Pt in the anterior abdominal wall, all detectable Pt being confined to the 20 um peritoneal-mesenchynal layer. Initial results show a flat profile over this 20 um layer both for animals dosed at 35 mg/kg as well as for earlier animals dosed at 150 mg/kg. Also shown was the need to correct final images for phosphorous peak overlap of the Pt line.

(3) The pharmacokinetic advantage of intra-arterial drug administration can be improved if blood from the infused region is perfused through a suitable extracorporeal device. The extent of improvement depends upon the blood flow to the device, the fraction of the vascular drainage that can be obtained. and the drug extraction by the device. A relatively simple equation is derived to assess the pharmacokinetic advantage and to define the governing parameters. A hemoperfusion column (XR-010, Extracorporeal Medical Specilaties) was shown to remove BCNU quantitatively from sheep blood flowing at 316 ml/min when the drug was infused at 13 mg/min for 30 min. Under general anesthesia, adult rhesus monkeys underwent catheterization of the internal carotid artery and placement of a catheter in the jugular vein at its junction with the sigmoid sinus. Ten mg/kg BCNU was infused over 20 min. while blood was pumped from the jugular vein through a samll column and back into the inferior vena cava. The procedure reduced systemic exposure by 46-84% compared with intravenous infusion of the same dose. Brain-to-systemic exposure ratios ranged from 18 to 87:1 depending on the pump flow rate and the theoretical basis of calculation. Four patients with malignant gliomas received 220 mg/m^2 BCNU over 45 min into the internal carotid artery while ipsilateral jugular venous blood was pumped through a hemoperfusion column at 300 ml/min. Systemic exposure was reduced by 56-87% compared with intra-arterial administration of the same dose.

(4) A major determinant of the success of extracorporeal treatement of venous drainage is the fraction of infused blood that can be removed. Indocyanine green was infused into the internal carotid artery of three rhesus monkeys while blood was pumped at various flow rates from the ipsilateral jugular vein. Recovery of the dye was generally consistent with pumping from a well mixed venous pool, and very high fraction were obtained at flow rates approaching the extimated total brain blood flow.

SIGNIFICANCE: Drugs and other chemicals are tested for effect in animals, with the aim of extrapolating results to man. At issue are both the risk associated with environmental contaminants and optimization of therapy.

PROPOSED COURSE: Continued pharmacokinetic modeling with consideration of pharmacodynamic and cytokinetic events and drug interactions. Continued clinical emphasis through support of regional procedures and other measures to overcome drug resistance. Research designed to investigate distribution and metabolism of environmetnal contaminants and on methods for incorporating pharmacokkinetics in models of risk assesssment. Investigation of use in in vitro assays of chemical metabolsim in conjunction with pharmacokinetic models for quantitative prediction of metabolism in vivo. Extension of distributed models for description of drug movement through tissue.

PUBLICATIONS

D.S. Zaharko and R.L. Dedrick. Pharmacokinetics of Methtrexate in Animals and Man in F.M. Sirotnak, J.J. Burchall, W.D. Ensminger and J.A. Montgomery (eds.), Folate Antagonists as Therapeutic Agents, Vol. II, Academic Press, New York, 1984.

H.B. Matthews and R.L. Dedrick, Pharmacokinetics of PCBs, Ann. Rev. Pharmacol. Toxicol. 24:85-103, 1984.

G.A. Curt, J.J. Grygiel, B.J. Corden, R.F. Ozols, R.B. Weiss, D. Tell, C.E. Myers. J.M. Collins. A phase I and pharmacokinetic study of carboplatinum (CBDCA) NSC 241240. Cancer Res. 43:4470-4473, 1983.

E.H. Oldfield, R.L. Dedrick, D.C. Chatterji, R.L. Yeager, M.E. Girton, J.M. Collins, P.L. Kornblith and J.L. Doppman. Reduced Systemic Drug Exposure by Combining Intracarotid Chemotherapy with Hemoperfusion of Jugular Drainage, Surgical Forum 34:535-537. 1983.

R.J. Lutz, R.L. Dedrick, D.Tuey, I.G. Sipes, M.W. Anderson, H.B. Matthews, Comparison of the pharmacokinetics of several polychlorinated biphenyls in mouse rat, dog and monkey by means of a physiological pharmacokinetic model. Drug Metab. Disp. in press.

R.L. Dedrick, E.H. Oldfield and J.M. Collins. Arterial drug infusion with extracorporeal removal. I. Theoretic basis with particular reference to the brain. Cancer Treat. Rep. 68:373-380, 1984.

M.F. Flessner, R.L. Dedrick and J.S. Schultz. A distributed model of peritoneal-plasma transport: Theoretical considerations. Am. J. Physiol. 246, R597-R607. 1984.

R.L. Dedrick, M.F. Flessner, J.M. Collins and J.S. Schultz. A distributed model of peritoneal transport. Proc. III. Int. Symp. Peritoneal Dialysis . in press.

M.F. Flessner, R.L. Dedrick, J.D. Fenstermacher, R.G. Blasberg and S.Sieber. Peritoneal Absorption of macromolecules. Proc. III. Symp. Peritoneal Diaylsis. in press.

R.J. Lutz and R.L. Dedrick. Physiological pharmacokinetics: Relevance to human risk assessment in New Approaches in Toxicity Testing and their Application to Human Risk Assessment, A.G.E. Wilson (ed.), Raven Press New York in press.

R.L. Dedrick, Application of model systems in pharmacokinetics. In New Directions for Human Risk Assessment, Vol. 2 Toxicokinetics in Risk Assessment, A. Silvers and G.W. Newell (eds), Electric Power Research Institute, Palo Alto, in press.

R.L. Dedrick, Application of model systems in Pharmacokinetics in Proc. Banbury Conf. Human Risk Estimation, Cold Spring Harbor Laboratory. N.Y., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-RS-10002-19-BEI

PERIOD COVERED
Oct.1, 1983 to Sept. 1984

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

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

John W. Boretos, Physical Scientist, BEIB, DRS

John Doppman, M.D.	Radiologist	CR, CC, NIH
Edward Oldfield, M.D.	Neurosurgeon	NINCDS, NIH
F.T. Hambrecht, M.D.	Health Science Adm.	NINCDS, NIH
William S. Pierce, M.D.	Professor	Penn State Univ.
Murray Eden, Ph.D.	Physical Scientist	BEIB, DRS, NIH

COOPERATING UNITS *(if any)*
CR, CC, NIH; NS, NINCDS, NIH;
FNP, NINCDS, NIH; Penn State Univ., Hershey, PA;
BEIB, DRS, NIH.

LAB/BRANCH
Biomedical Engineering Instrumentation

SECTION
Chemical Engineering

INSTITUTE AND LOCATION
DRS, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to elucidate the interaction of biomaterials used for specific implants with the physiological environment and to explore specially prepared biomaterials and design features with respect to their suitability and performance in a variety of contexts. Polyurethanes are an important class of elastomers for use in catheters, heart assist pumps, electrode insulation and similar implant applications. Variations in the basic chemical structure of these polymers as well as physically induced stress can severely reduce their effectiveness for long-term use as a surgical device. Previous studies undertaken by this project have shown a relationship between the molecular chain structure in resisting hydrolytic forces. Recent evidence suggests that physical forces such as stress induced during fabrication can promote a form of stress corrosion. In vitro test data and SEM photomicrographs of surgical explants of various polyurethane classes show that premature failure is often the result of a combination of forces acting on the polymer at stress risers. A strong correlation exists between these in vitro and in vivo observations over short and long term periods of study.

A radiopaque polymer made from a polyol/diisocyanate resin and finely divided tantalum was developed that exhibits good adhesion to polyurthanes and excellent visibility under fluoroscopy. The substance is intended as a marker for catheters and other indwelling devices whose location must be constantly observed.

PHS 6040 (Rev. 11/84)

OBJECTIVES: Elucidate the interaction of polymers. metals, and ceramics used for specific implants with the physiological environment; explore specially prepared polymers and design features with physiological environment; explore specially prepared polymers and design features with respect to their suitability and performance in a variety of contexts.

METHODS EMPLOYED: Basic composition of biomaterials is carefully controlled, and modifications are employed to enhance acceptability by the living system. After removal, implants are examined for lipid absorption, protein and/or calcium deposition, changes of surface-free energy and alteration of physical properties. Observation techniques include SEM, infrared spectroscopy. contact angle measurements, energy dispersive x-ray analysis, and atomic absorption spectroscopy. Electronic implants are examined periodically in vivo for changes in threshold levels, corrosion, and tissue activity. In vitro studies of the aforementioned are designed to accelerate fatigue testing and methods for improvement of components undergoing stress.

MAJOR FINDINGS: Polyurethanes are a complex and varied class of polymers whose full potential for medical use has not been achieved. Characterization of its behavior under conditions of use is necessary to project its safety and efficiency as a surgical implant. Variations in the basic chemical structure of these polymers as well as physically induced stress can severely reduce their usefulness. Previous studies undertaken by their project have shown a direct relationship between the molecular structure of the polymeric chain and its ability to resist hydrolysis. Recent evidence indicates that physical forces such as stress induced during "hot" processing can accelerate a type of stress corrosion once implanted in the body; whereas this condition seems to exist only minimally for implants fabricated from "solvent cast systems". Dimensional stability can also be adversely effected by improper processing. By controlling the "annealing" sequence during manufacture, these adverse conditions can be reduced and physical properties increased for a stronger and more reliable product. Scanning electron photomicrographs of epidural and pacemaker electrodes taken from animals and patients along with in vitro physical properties measurements before and after "annealing" substantiate that these are influential factors in limiting longevity for the devices in which the polymers are used.

A specially prepared composite of a polyol/diisocyanate and finely divided tantalum powder was developed that showed good radiopacity in thin sections (i.e., less than 400 microns) under conventional flouroscopy. The substance can be used, for example, as a marker for observing the position of various indwelling devices. Viscosity, with thixotropoic properties, of the material was designed for application to verticle surfaces. Reliable adhesion to polyurethanes was observed before and after gas sterilization.

SIGNIFICANCE: Physiologically compatible polymers with enduring strength are needed for surgical implants, indwelling catheters, and subcutaneous uses.

PROPOSED COURSE: Extend experimental studies to further characterize the surface and bulk properties of biomaterials and, more specifically, to determine their interactions with blood and subcutaneous tissue to facilitate development of better surgical implants.

PUBLICATIONS

Boretos, J.W.: Correlation of Long-term Data for Segmented Polyurthanes.
Proceedings of the conference on "Polyurethanes in Medical Techniques",
Stuttgart, Germany (in press).

Boretos, J. and Eden, M.:Contemporary Biomaterials, Material and Host
Response, Clinical Applications, New Technology, and Legal Aspects, Noyes
Publications, Park Ridge, N.J. 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RS 10015-09 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Development of Toposcopic Catheter for Clinical Vascular Use

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

PI: D.R. Shook, Biomedical Engineer,   BEIB, DRS
J.L. Doppman,   Chief,   DRD, CC
E.H. Oldfield Senior, Staff Neurosurgeon, SNB, NINCDS

COOPERATING UNITS *(if any)*
Diagnostic Radiology, CC (A.G. Krudy, N.J. Patronas, D.L. Miller);   Surgical
Neurology, NINCDS (C. Clark)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 0.5	OTHER: 0.8

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

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

Toposcopy has been shown to be a reliable, clinical, means to catheterize long, small diameter, and highly tortuous blood vessels, inaccessible by previous techniques.   A toposcopic element everts from the tip of a conventional catheter. This extremely flexible polyurethane element has been fabricated in 3, 4 and 5 French sixes mated with 5, 6 and 7 French catheters, respectively, and is capable of eversion lengths in excess of 40 cm.

The toposcopic catheter has been applied clinically for the local delivery of chemotherapy to brain tumors.   Treatment is provided by: positioning the conventional catheter in the internal carotid artery from a femoral entry; everting the toposcopic element through the carotid sinus, beyond the ophthalmic artery to avert retinal toxicity; and perfusing the tumor through the middle and/or anterior cerebral arteries.   Note that a conventional catheter cannot safely negotiate the tortuosity of the carotid sinus.

The catheter has been extensively used clinically in its present prototype form and production techniques for its fabrication are being transferred to private industry to provide for a ready source of catheters for future studies.

OBJECTIVES: Develop techniques for selective toposcopic catheterization of small, tortuous blood vessels and optimize appartus for varied diagnostic and therapeutic applications.

MAJOR FINDINGS: Eversion, infusion, and retraction of the catheter have occurred without incident in over twenty clinical chemotherapeutic treatments. Implementation of existing techniques to direct catheter eversion is required in some difficult anatomical situations.

SIGNIFICANCE: By atraumatically negotiating small, highly tortuous blood vessels, the catheter reaches here-to-fore inaccessable regions for both diagnostic and therapeutic application. Thus, routine fluoroscopic examinations are readily augmented by selective treatment modalities for tumors. aneurysms, arterio-venous malformations, and other lesions which previously required major surgical interventions.

PROPOSED COURSE: Optimize catheter system for diverse clinical applications. Transfer production technology to private industry for ready source of catheters. Investigate significance of infusate/blood mixing efficiency at tip of catheter. Develop wire guiding, remote steering and arterial dilatation methods. Develop related devices and explore additional uses for the catheter.

Related Project Number:

ZO1-RS-10001-15 BEIB
ZO1-RS-10   -01 BEIB (GI use)
ZO1-RS-10   -01 BEIB (Polymer Processing)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10018-09 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P.M. Bungay, Chemical Engineer, BEIB, DRS

COOPERATING UNITS *(if any)*
Department of Mathematics, University College, London, England
North Atlantic Treaty Organization, Brussels, Belgium

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

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

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

Mathematical models are being developed to describe passive membrane transport through pores or intercellular junctions. The Taylor-Aris dispersion analysis is extended to treat combined Brownian motion and convection in a single pore. The solute particle dimension is assumed to be large compared to that of the solvent molecules and also appreciable in size compared to the lateral pore dimension. The latter condition implies strong hindered diffusion and related solute-membrane interaction effects. A key aspect of the analysis is a generalized Einstein relation for predicting axial and radial components of the diffusivity tensor from hydrodynamics solutions for resistance coefficients. Perturbation techniques are used to obtain asymptotic solutions to the hydrodynamic equations, and the method of moments is employed to analyze the solute continuity equation. Related hydrodynamic problems are also being considered, such as flow through constricted vessels.

The hydrodynamic results in combination with an analysis derived from irreversible thermodynamics, provide a predictive theory for simultaneous coupled convective and diffusive transport across porous membranes - either biological or synthetic.

A review of the theoretical approaches to transport in porous membranes is included in the Proceedings of the North Atlantic Treaty Organization Advanced Study Institute on Synthetic Membranes held June 26-July 8, 1983, and directed by the principal investigator.

OTHER INVESTIGATORS:

M.E. O'Neill Reader, Department of Mathematics, University College, London, England

OBJECTIVES: The objective of this project is to provide the basis for a rigorous, predictive continuum theory for passive transport phenomena in porous membrances, including such observations as "hindered diffusion". The development of solutions to hydrodynamic problems of interest in other areas of the biological and physical sciences is also considered.

METHODS EMPLOYED: The essence of the approach to membrane transport is an extension of the Einstein continuum analysis for the Brownian motion of spherical molecules in dilute solutions. Einstein derived his predictive relation for the diffusion coefficient from the theoretical expression for the hydrodynamic resistance to translation of a rigid sphere through a homogenous viscous fluid of infinite extent. The continuum analysis for porous membranes begins with a single solute molecule in a single pore and assumes that the form of Einstein's relationship between the diffusion and resistance coefficients remains valid. However, the presence of the rigid pore wall, in general, increases the hydrodynamic resistance to translation and rotation of the solute relative to the fluid. The diffusivity is thereby decreased in magnitude until, in the limit, as the solute dimension becomes equal to the lateral pore dimension, the diffusion coefficient falls to zero. Where there is, in addition to diffusion, net movement of the fluid through the pore, the hydrodynamic interaction similarly affects the solute flux relative to the solvent flux. The project is concerned with deriving the requisite expressions for the resistance coefficients from hydrodynamic theory as well as developing analyses for diffusive and convective porous membrane transport.

The primary theoretical tools used in the hydrodynamic problems are regular and singular perturbation techniques (typically using the ratio of solute to pore dimensions as the asymptotic expansion parameter) and collocation techniques of the type developed by Weinbaum and Pfeffer.

Two approaches are considered for the prediction of transmembrane transport coefficients for the solute and the solvent. One approach is the Brenner-Gaydos extension of the Taylor-Aris type dispersion analysis. The approach uses the method of moments for deriving expressions for the coefficients without directly solving the complete solute continuity equation (convective-diffusion equation). The second approach is the utilization of the hydrodynamic resistance coefficients in the rigorous irreversible thermodynamic framework of the type developed by Lewellyn, Lightfoot, and Stewart.

MAJOR FINDINGS: The proceedings of the NATO Advanced Study Institute on Synthetic Membranes were edited and prepared for publication, along with a chapter reviewing "Transport in Porous Membranes." The Institute (held in Alcabideche, Portugal, June 26- July 8, 1983 and directed by the principal investigator) was an intensive course on the science and technology of synthetic membranes and their utilization.

SIGNIFICANCE: Channels (pores slitlike gap junctions) represent one important type of transmembrane transport in biological systems. A rigorous conceptual and predictive framework for pore theory would be useful in clarifying relevant biological transport and would find wide applicability in engineering and physical science work pertaining to synthetic membranes.

PROPOSED COURSE: In addition to the models presently under study, it would be desirable to examine a situation in which the solute is a nonspherical body in order to determine how to handle partical orientation and rotational Brownian motion effects. An ellipsodial solute would be the likely choice in terms of posing theoretically tractable problems. Another direction to pursue, which would greatly extend the range of applications for the theory, would be to incorporate into the present models nonhydrodynamic solute-membrane interactions such as electrostatic and Van der Waals forces.

PUBLICATIONS:

Bungay, P.M.: Transport in Porous Membranes. In Bungay, P.M., Lonsdale, H.K., Pinho, M.N.C. (Eds.): <u>Synthetic Membranes.</u> Dordrecht, D. Reidel, 1984 (to appear).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	ZO1 RS 10034-07 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984.

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

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

S.B. Leighton, Mechanical Engineer, BEIB, DRS
A.M. Kuzirian, Neuroanatomist, LB, NINCDS

COOPERATING UNITS *(if any)*
LB NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205.

TOTAL MAN-YEARS: .5	PROFESSIONAL: .45	OTHER: .05

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

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

A semi-automatic system for acquisition of three-dimensional structural information about histological material is being developed. The system should have significant speed and reliability advantages over present techniques using serial sections, although resolution may be limited. In brief, an embedded tissue block will be fixed relative to a scanning electron microscope imaging system, the surface of the block will be imaged and the image stored, and successive slices will be removed by a built-in microtome. Handling and registration of thin sections will thus be eliminated. Human and computer pattern recognition will transform the resulting set of images into a three-dimensional reconstruction. Oxygen plasma etching has been found to give sufficient topographic relief that the resolution of the images is now limited by the SEM and not by the preparation technique. The images of Hermissenda Crassicornis obtained by this technique correlate well with TEM images of the same tissue, indicating that the lack of artifact is adequate for the contemplated studies.

OBJECTIVES: (1) To facilitate making schematic diagrams of neural networks. (2) To facilitate developmental studies of small organs and organisms. (3) To do three-dimensional reconstruction of biological structures.

METHODS EMPLOYED: A miniature microtome has been built to function within the vacuum chamber of a scanning electron microscope. The microtome has cut sections as thin as 0.5 microns. Epoxy has been tried as a embedding medium, and oxygen plasma as an etching means.

MAJOR FINDINGS: The microtome knife and specimen feed mechanisms have been rebuilt for all electronic operation to facilitate interfacing with a computer and with a new SEM, and to remove the risk of fluid leaks into the SEM. The new specimen drive should be capable of 1000 Angstrom section thickness if contact feedback is implemented between the knife and specimen as planned. A miniature gold gun (2.5 x .6 cm) has been successfully tested. This in situ evaporation source can apply a 200 Angstrom gold layer in 30 seconds and can apply approximately 200 layers before requiring refilling. Total time per image (cut, etch, coat. view) is expected to be less than 7 minutes. The system has been integrated with a new SEM in LB, NINCDS.

SIGNIFICANCE: Neuroanatomists may be able to trace significant neural nets with sufficient ease to allow a statistically significant number of samples. Other biological studies may be materially aided.

PROPOSED COURSE: Feedback will be used to try to reduce the section thickness obtained with the microtome to 0 .1 micron. Parameters such as etching time and intensity will be optimized. Computer control will be planned.

PUBLICATIONS

Kuzirian, A.M. and Leighton, S.B. Oxygen Plasma etching of entire block faces improves the resolution and usefulness of serial scanning electron microscopic images. Scanning Electron Microscopy/1983/IV/pp. 1877-1885

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10039-07 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

Marc S. Lewis, Research Chemist, BEIB/DRS
Thomas R. Clem, Electronics Engineer, BEIB/DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Microanalysis

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

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

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

The project is designed to develop new instrumentation and methodology or improve existing instrumentation and methodology for characterization of biological macromolecules and for studying their interactions. Analytical ultracentrifugation, the techniques ancillary to it, and methods of data analysis using mathematical modeling appropriate for these techniques are the major areas of interest.

Improved precision and optimal efficiency in ultracentrifugal analysis requires improved methods of data acquisition. A microprocessor controlled system for the direct acquisition of data from the photomultiplier tube of the ultraviolet absorption scanner of the ultracentrifuge and for the control of that acquisition has been developed. The software requisite for making this system operational is presently in a developmental stage. When complete, this system will permit direct acquisition of data in digital form while the ultracentrifuge is operating and then permit preliminary data processing followed by transmission of the data to the DEC-10 computer for detailed analysis. This will result in significantly improved precision and enhanced facility in data acquisition and analysis. thus effecting a marked increase in efficiency of research as well as permitting studies where the precision of current methods has not been adequate, such as discriminating between two different models of macromolecular association which have very similar but not identical concentration distributions in the analytical ultracentrifuge.

MLAB, operating on the DEC-10 computer, has been used for mathematical modeling studies for the analysis of various types of protein interactions. These studies have been applied to ultracentrifugal studies of binding and of protein self-association. Such studies are described in the annual report entitled Physical Chemistry of Biological Macromolecules.

Z01 RS 10039-07 BEI

OBJECTIVES: To develop data acquisition systems for analytical ultracentrifuges and for ancillary equipment such as plate or film readers and densimeters, to develop appropriate software to use with the acquisition systems, and to study means of utilizing MLAB more effectively for the analysis of ultracentrifuge data by mathematical modeling techniques.

METHODS EMPLOYED: A microprocessor controlled instrument has been developed for the direct acquisition and storage of data from the photomultiplier tube of the ultraviolet absorption optical scanning system of the analytical ultracentrifuge. This instrument is coupled to a Columbia Data Systems desk-top minicomputer which provides (1) temporary data storage in the computer as well as permanent storage on floppy discs; (2) preliminary processing, graphic display and editing of the data; and (3) communication with the DEC-10 computer permitting transfer of the data to be processed to the DEC-10, control of data analysis using the MLAB system on the DEC-10 and finally, the transfer of the results of these analyses back to the laboratory. Software is being developed for optimizing the data acquisition for different modes of acquisition and for data editing prior to transmission to the DEC-10.

MAJOR FINDINGS: Systems for the direct acquisition of data from the analytical ultracentrifuge have been demonstrated to have significant advantages in speed and accuracy when compared to the manual digitization of analog data and the manual entering of this data in the computer for analysis. Programs are being developed for the reduction of data acquired in different acquisition modes and for editing it prior to transmission to the DEC-10 computer for analysis. Improvement in the methods for the analysis of interacting systems of macromolecules by mathematical modeling utilizing MLAB on the DEC-10 computer have been continued. Systems of a variety of possible models of protein-protein interactions have been developed, including systems where binding is complicated by self-association of one or more of the components involved. A new method of writing mathematical models has been developed where the physical properties of the system being studied are incorporated as implicit constraints on the model. This avoids the needs of applying external constraints to make the fitting parameters physically meaningful and also results in more efficient computation and more meaningful results.   This method has been applied to work described in the annual report entitled Physical Chemistry of Biological Macromolecules.

SIGNIFICANCE: Since data acquisition has been the major limiting factor in terms of the qualitative and quantitative aspects of ultracentrifugal research, it is expected that the continuing development of data acquisition systems as described above will significantly facilitate such research. The improved methods of data analysis by mathematical modeling also contribute significantly to the quality and the time and cost effectiveness of this research.

PROPOSED COURSE: Continue the developments outlined above

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RS 10043-07 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

John I. Peterson, Chemist, BEIB/DRS
Randolph E. Patterson H IR CB

COOPERATING UNITS *(if any)*
H IR CB
H IR OD

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

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

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

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

A suitable device is needed for the direct measurement of oxygen partial pressure (PO_2) in blood and tissue for both clinical and research applications. Methods currently available for measuring PO_2 lack convenience, reliability, speed, and relevance to many situations of interest. Efforts to develop electrical sensors have not been successful. It is desirable to have a very small PO_2 sensor which can be inserted into a blood vessel of tissue with little disturbance, and which will provide instantaneous and current PO_2 monitoring for either short or extended periods of time. A fiber optic sensor is ideal for this application, with the advantages, for physiological use, of very small size and flexibility, safety, and low cost. A PO_2 sensor has been developed, based upon the principle of fluorescence quenching by oxygen. In the previous year the feasibility of the sensor was demonstrated and its performance evaluated with in vitro and animal tests. Current work is oriented toward converting the sensor to a needle form for experimental use, improving the instrumentation and probe construction, and solving some remaining problems with the sensor system to increase its utility.

OBJECTIVES: Develop an oxygen sensor for physiological implantation in the blood stream and tissues, to be used in studies of oxygen transport during exercise and clinical PO_2 measurements.

METHODS EMPLOYED: A fiber optic measurement of dye-indicator response to oxygen by fluorescence quenching.

SIGNIFICANCE: PO_2 measurements are fundamental to understanding and control of oxygen transport in research and clinical investigations. Measurements on withdrawn blood samples lack convenience, reliability, and relevance to many situations of interest. Indirect estimation of PO_2 using spectrophotometric measurements of hemoglobin oxygenation and the concentration-pressure transfer function (blood oxygen saturation curve) is subject to too many uncertain variables. Development of a fiber optic PO_2 probe would represent a significant advance in the ability to directly and continuously measure blood and tissue oxygen. A satisfactory electrode for general use has never been developed, and the fiber optic approach offers some distinct advantages in small size, flexibility, and safety.

MAJOR FINDINGS and PROPOSED COURSE: The previous year, development and feasibility testing of a fiber optic PO_2 probe was completed, and its satisfactory performance with in vitro and animal tests was demonstrated. Current work is directed toward the following goals in order to allow the practical experimental use of the probe.

   1. Convert the sensor to a needle form for insertion into heart muscle, for measuring the PO_2 of the tissue: This was one of the initial objectives in developing the sensor, to provide a tool for ischemia related studies, and to find whether direct PO_2 measurement is of value in preservation of the tissue during surgery. The previously developed pH sensor was made in a needle form and used for measurements in heart tissue, and it is expected that the PO_2 sensor will have a similar form. Methods of probe construction have to be worked out and tested, and it is desirable to make it smaller that the original model.

   2. Redesign of the instrument to allow construction of single fiber sensors: This will allow smaller sensors to be made, and it is expected that faster response can be achieved.

   3. Solution of two problems with the current sensor system: The sensor has a short storage life. As a result, it is necessary to make it within a week or so of use, which is impractical. Also, the indicator system used is subject to analytical interference by the halogenated anesthetics. This is not a problem for some applications, but the sensor would be more generally useful if this did not occur.

Three papers based on this work were published in this period: a description of the PO_2 sensor; a description of a method of making small diameter tubing of porous film, which is used in the sensor but has other applications; and a general review of the state of development of fiber optic sensors in the biomedical field. Also, publications appeared which were based on previous work on fiber optic sensors.

PUBLICATIONS

"Fiber Optic PO_2 Sensor for Physiological Use", J.I. Peterson, R.V., Fitzgerald, and D.K. Buckhold, Analytical Chemistry 56: 62 (1984).

"Method of Making Small Diameter Tubing from Porous Film", J.I. Peterson and J.V. Sullivan, Review of Scientific Instruments 54: 1792 (1983).

"Fiber Optic Blood Gas Sensors", J.I. Peterson, in "Proceedings: Physiological Sensors in Medicine: A Forum" IVAC Corp., San Diego, Ca.

"Transmural pH Gradient in Canine Myocardial Ischemia", R.M. Watson, D.R., Markle, Y.M. Ro, S.R. Goldstein, D.A. McGuire,and R.E. Patterson, American Journal of Physiology 246 H232 (1984).

"The Toposcopic Catheter and the Fiberoptic pH Probe - Two Medical Instruments of Potential Use to Gastroenterologists", S.R. Goldstein, D.R. Shook, D.R. Markle, J.L. Doppman, R.E. Patterson, and J. Dooley, Gastrointestinal Endoscopy 29: 236 (1983).

"Fiber Optic Sensors for Biomedical Applications", (a review), J.I. Peterson and G.G. Vurek, Science 224: 123 (1984).

Fiber Optic Chemical Sensors - A View from the Past to the Future", J.I. Peterson, Proceedings of the National Science Foundation Symposium on Biosensors, Los Angeles, Sept. 15-17, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10053-06 BEI

PERIOD COVERED
October 1, 1983 to September 30. 1984

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Membrane Based Sampling Systems for In Vivo and In Vitro Kinetic Studies

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

P.M., Bungay, Chemical Engineer, BEIB,DRS

COOPERATING UNITS *(if any)*
Laboratory of Chemical Pharmacology, DCT

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

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

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

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

Synthetic membranes are being utilized in kinetics studies to provide a means
for continuous sampling of the liquid phases from systems in which a dispersed
particulate phase is suspended in the liquid phase. In one application a
study of the mammalian blood-brain-barrier permeability is being aided by the
development of an apparatus incorporating a sampler in an arteriovenous ex
vivo shunt. In this plasmapheresis application, pooling of the plasma
filtrate yields a single sample from which the plasma concentration-times-time
integral can be evaluated for a chemical administered to the animal. Such
sampling systems can be useful for the study of the kinetics of other fluid
phase systems for which a membrane can be found that is permeable to one pool
of the chemical of interest but impermeable to other pools or another
necessary reagent. Thus, other applications might be found in the areas of
enzyme kinetics, pharmacokinetics, and the membrane transport of vesicle and
cell suspensions.

PROFESSIONAL PERSONNAL

Dr. P.M. Bungay, Chemical Engineer, BEIB/DRS
Dr. Joseph Fenstermacher, Laboratory of Chemical Pharmacology, DCT,
and Department of Neurosurgery, State University of New York, Stony Brook

OBJECTIVES: The principal objective is the development of the capability for fluid sampling based upon synthetic membrane technology. In many potential applications sampling by filtration or ultrafiltration may be more appropriate than alternative sampling techniques. Ultrafiltration membranes allow the formation of samples representative of the free concentration of small soluble substances. These membranes will retain within the system under sutdy macromolecules and those substances which are bound to them, as well as colloidal or cellular components of the system. Other applications may call for the use of larger pore diameter membranes if, for example, macromolecules are to be sampled as well.

METHODS EMPLOYED: The sampling system generally consists of three elements: (1) a module or modules containing sampling membrances, (2) sample collection equipment, and (3) a means for controlling the rate of production of sample. The membrane module is designed so that the membrane forms a part of the wall of the channel through which the liquid to be sampled flows. Only a small fraction of the liquid is diverted across the membrane to form the sample. The sample is produced as a consequence of a difference in pressure imposed across the membrane. The rate of production of the sample is regulated either by controlling the transmembrane pressure difference or through use of a sample metering pump.

SIGNIFICANCE: One application concerns in vivo studies of exchange of substances betweenthe blood and brain tissue across the blood-brain barrier. The objective of developing a membrane sampling scheme in this application is to permit the determination of transport coefficients for the exchange process in cases for which alternative sampling techniques are not applicable or would be less accurate or less convenient. The transport coefficient, like permeability, depends upon the chemical substance employed. A particular category of substances for which membrane sampling might be attractive is the group of substances which are sequestered intracellularly in the brain, for example, potassium ion. Within this grouping there are three subsets for which alternative sampling techniques can present specific measurement uncertainties. One subset consists of substances with intermediate rates of equilibration between plasma and blood cells. Blood sampling techniques require an additional step of separating the plasma from the blood cells subsequent to withdrawing. Such a two step process for obtaining plasma samples could yield inaccurate plasma concentrations if significant exchange between plasma and blood cells occurs before the separation step is effected. A second subset consists of substances for which the distribution kinetics are very rapid. Here continuous sampling techniques, like membrane sampling and continuous blood withdrawal would be preferable to discrete sampling techniques, like serial blood sampling. A third subset consists of substances which are subject to significant binding to plasma proteins. In order to follow free plasma concentrations in the animal as a function of time, the equilibrium binding curve has to be known and possibly also the kinetics of binding and dissociation. With membrane sampling this may be obviated by use of an ultrafiltration membrane for production of protein-free plasma sample.

The membrane sampling technique can be applied to other acute pharmacokinetic studies which require the determination of the plasma concentration versus time integral.

MAJOR FINDINGS: As repored previously, validation experiments produced results by membrane sampling which were comparable to those obtained by continuous blood withdrawal and serial blood sampling for labeled marker compounds such as sucrose, urea and water. The kinetics of distribution of these compounds are such that it was reasonable to expect that the three sampling techniques would each give valid results. We searched for non-metabolizing marker substances with plasma-to-blood cell equilibration kinetics such that the traditional whole blood sampling techniques might yield artifacts because of redistribution of the marker between the plasma and cellular phases of the blood samples after sampling. We measured the distribution kinetics of several substances, but only cycloleucine displayed kinetics which seemed to satisfy the criteria we set. Experiments were conducted in which cycloleucine was administered to rabbits and the plasma concentration-time integral was determined by the same three sampling techniques. Again, the three techniques gave comparable results. Further analysis indicated that the expected artifacts for the whole blood sampling techniques tend to be self-compensating in so far as the determination of the integral is concerned.

PROPOSED COURSE: In the next fiscal year we expect to begin a collaboration with Dr. Clifford Patlak of the Theoretical Statistics and Mathematics Branch, NIMH, and Dr. Louis Sokoloff of the Laboratory of Cerebral Metabolism, NIMH. The objective of this effort will be to determine if the membrane sampling approach could be adapted for use in the measurement of local cerebral glucose utilization rates. The current measurement protocol involves serial blood sampling following the administration of 2-deoxyglucose to test animals. The investigators believe that an alternative procedure for measuring the plasma-concentration-time integral of 2-deoxyglucose could promote glucose utilization rate measurements within the neurosciences research community.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 RS 10062-05 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
IEEE-488 General Purpose Interface Bus Program Development

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

T.R. Clem, Sr., Electronic Engineer, EEES, BEIB, DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.2	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 increased availability and reduced cost of the small desk-top computer
has created increased interest in automating data acquisition and process or
experiment control in areas where such things were not feasible before due to
cost or complexity. With these changes also came a significant increase in the
use of the IEEE-488 GPIB by instrument makers. By combining the two,
sophisticated instrumentation and data acquisition systems can be assembled
quickly and inexpensively. The BEIB is continuing to develop the expertise to
provide guidance and assistance to requirements where this approach provides
the optimum solution. This capability is further assisted by the BEIB SERP
specifying the IEEE-488 interface on new equipment acquisitions whenever
possible. The increase at the NIH, in the numbers and use of the IBM PC has
made this capability of great value to the NIH Intramural Research program.

OBJECTIVE: Continue to develop expertise in the Branch in the use of IEEE-488 GPIB equipment, instruments, and controllers and recommend bus-compatible instruments for acquisitions by the SERP in order to be prepared to respond quickly to a request for an instrumentation system.

METHODS EMPLOYED: Generally an instrumentation system will be assembled around the IEEE-488 GPIB for one of four reasons: 1) a prototype system is desired to prove feasibility before a dedicated system is designed and built, 2) a temporary system is needed to satisfy an urgent, short term need, such as replacing a dedicated system that is out of operation for maintenance or repair purposes, 3) to expedite data acquisition into a computer system for further analysis, or 4) to produce a system which is inherently flexible enough to adapt to a wide range of potential future needs.

SIGNIFICANCE: The capability of assembling an instrumentation system with a controller and GPIB-controlled instruments allows the EEES to provide a rapid response to an investigator's call for instrumentation. By assembling a system with "off-the-shelf" instruments from the SERP, the cost of special-purpose measurement or control systems can be kept quite low. If the experiment is a short-term project the instruments can be returned to the SERP with virtually no expenditures, by the investigator, for equipment.

PROPOSED COURSE: Continue to maintain state-of-the-art capability in the field of bus-compatible instruments and controllers. Seek to make this capability better known and understood around NIH. Expand capability to include IBM PC computers as controllers and integrating systems with other types of PC-based I/O hardware.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10064-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Indirect Blood Pressure Measurements in Laboratory Animals Using Oscillometry

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

E.C. Walker, Mechanical Engineer, ACES, BEIB

COOPERATING UNITS *(if any)*
SLAMS OD NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.75	PROFESSIONAL: 0.75	OTHER:

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

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

Presently there are no reliable indirect methods of measuring blood pressure in dogs. Traditional techniques, used in humans, are unsatisfactory primarily because they require the placement of a transducer over the artery being monitored.

Because of the unreliability of traditional techniques we have been investigating a technique called oscillometry. Oscillometry is the method of measuring blood pressure by analyzing the pulse pattern of the cuff pressure oscillations.

In practice, a cuff is placed around a limb and inflated to a pressure above systolic and then slowly deflated. While the cuff is being deflated the amplitude of oscillation in cuff pressure, produced by the arterial pulse beneath the cuff, if monitored. Systolic and diastolic transitions in the oscillometric waveform are then used to indicate the indirect systolic and diastolic pressures respectively.

OBJECTIVES: The primary objective of this project is to develop an instrument to indirectly measure peripheral arterial blood pressure in laboratory animals.

METHODS EMPLOYED: A model of the oscillometric process is currently being employed to develop and test an automated oscillometric instrument. The model consists of a simulated artery, cuff, sinusoidal pressure generator, and a Tektronix 4052 computer.

SIGNIFICANCE: Animal surgery labs and other laboratories or veterinarian offices could use an indirect device on a daily basis. For various laboratory studies monitoring blood pressure in awake animals is very desirable, particularly with protocols involving atherogenic diets, and drug therapy. Another area of application would be in screening animals for hypertension research. For surgical procedures, and indirect technique will allow blood pressure monitoring before and during induction of anesthesia.

MAJOR DEVELOPMENTS: In addition to having the capability of measuring the systolic and diastolic pressures, oscillometry is also used to measure the mean arterial pressure. In order to incorporate a mean arterial measurement we have studied the relationship of the maximum cuff pressure oscillation to the mean arterial pressure. Our studies have shown that under the proper conditions the maximum pressure oscillation can be used to measure the mean arterial pressure. The studies have also shown that the maximum cuff pressure oscillation correlates very well with the sum of the diastolic pressure and the arterial collapse pressure. Thus, although the maximum cuff pressure oscillation can be used to measure the MAP it appears to be an intrinsic measurement of the sum of the diastolic pressure and the pressure required to collapse the artery at the diastolic point.

Due to the unique anatomy of the dog we have developed our own blood pressure cuffs that are also quite useful in humans. The cuffs are ultra light, very thin, washable, and strong. Additionally, the cuffs are adaptable to a variety of anatomical locations. Our design has resulted in an approved NIH patent application.

Using the results of in vivo and in vitro studies and algorithm has been designed to oscillometrically measure systolic and diastolic pressures in the dog.

PROPOSED COURSE: Future efforts will be directed toward completing a prototype instrument and testing.

PUBLICATIONS

Walker, E.C., Pierce, J.E. "An in vitro system for studying the relationship between indirect pressure oscillations and the direct arterial pressure," Proceedings AAMI 17th Annual Conference, May 1982.

Walker, E.E. "Oscillometry: Theory for systolic and diastolic pressures, "Proceedings 35th ACEMB, September 1982.

Walker, E.C. Pierce, J.E. "Oscillometry: Systolic and diastolic pressures in the dog," Proceedings 35   ACEMB, September 1982.

Walker, E.C., "The Relationship of M.A.P. To Maximum Oscillation" Proceedings 36th ACEMB, September 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10065-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Transient Response of Micro-Calorimeter Using R-C Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboretory, and institute affilietion)*

C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
R.L. Berger, Physicist, LTD, NHLBI

COOPERATING UNITS *(if any)*
LTD, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.20	.20	.

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

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

In earlier work, we developed an R-C model which enabled us to predict calorimeter performance within 5%. The model revealed several sources of error in the current calorimeter design. With this information, we re-designed the calorimeter to reduce these errors and to offer increased performance and flexibility. The re-design (a) eliminates the air gap, (b) requires only one sensor, (c) can accept all three currently used cell sizes 0.3, 0.5 and 1.0 ml, and (d) increases sensitivity with no significant change in rise-time.

During FY83, this design was implemented and evaluated. All of the above features were realized. However, with the larger cells, the magnitude of the mixing artifact increased to the point that it negated the advantage of using the large cell volume. With the large 1 ml cell, the mixing artifact due to the rotation of the cell averaged 900 micro-joules with a large variability of ± 700 micro-joules.

The major cause of this artifact was found to be a small temperature gradient (4m°C) within the constant temperature chamber. The larger cell volumes were much more sensitive to this gradient than the smaller cells.

The gradient was reduced to less than 0.5m°C by shunting different currents through the heating pads. This reduced the artifact to approximately 100 ±30 micro-joules.

OBJECTIVES: To implement into hardware a re-design of the existing heat conduction calorimeter. An R-C model of the calorimeter is used to evaluate the re-design and predict its performance.

SIGNIFICANCE: This new design of the batch calorimeter allows the use of 3 different cell volumes in the same instrument. The sensitivity (by chemical calibration) is 2.07 joules/volt second and is independent of the cell size used. The rise-time and mixing artifact are dependent on the cell size as shown below:

Cell Volume	Rise-Time	Mixing Artifact
0.3 ml	150 seconds	50 ± 20 micro-joules
0.5 ml	270 seconds	60 ± 25 micro-joules
1.0 ml	490 seconds	100 ± 30 micro-joules

This design allows the operator the choice of different cell volumes for different types of reactions. When reagents are plentiful and inexpensive, the use of the larger cell volume yields the best signal to noise ratio, whereas the smaller cell can be used when the amount of reagent is limited with only a modest decrease in the signal to noise ratio.

PROPOSED COURSE: The calorimeter will be evaluated in the Laboratory of Technical Development in a series of experiments involving the cleavage of acid-base pairs in DNA. We anticipate that this is the final version of the batch type caloriment to be developed under this project. Since the magnitude of the mixing artifact is now the limit on the useful sensitivity of this instrument, future efforts will be directed toward transferring these design improvements to the development a stopped flow calorimeter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10066-04 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Egyptian Training Project

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

C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
H. Metz, Chief, RIS, BEIB, DRS

COOPERATING UNITS *(if any)*

RIS, BEIB, DRS

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

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

As part of a larger project to develop an instrument repair facility in Egypt, a series of lessons in basic electronics was developed. This series was divided into three parts which covered: (1) discrete components (active and passive), (2) digital devices and techniques, and (3) linear devices and techniques. The series consists of 60 lessons with a lab experiment for each lesson. The lessons and experiments were developed in close cooperation with two Egyptian instructors who will be presenting the course in Egypt.

OBJECTIVES: To develop an extensive series of lessons on basic electronics with a strong emphasis on practical applications and experiments. The lessons must be developed to re-orient the Egyptian engineer from a heavily theoretical background to a practical one in which actual device characteristics are studied. This course is intended to be a pre-requisite to a practical trouble-shooting course in which more complicated circuits are studied in complete instruments.

SIGNIFICANCE: At the conclusion of this project, the Egyptian Facility must be capable of operating independently from the NIH.

PROPOSED COURSE: Condensed, preliminary versions of the training courses were presented in Egypt in March-April and June of 1982. The trial courses were presented in order to determine: (a) the suitability of the course material, (b) an appropriate mechanism for the presentation of the material, (c) an appropriate laboratory evaluation technique for the students and (d) identification of possible trainees for the Egyptian presentation of the course.

During the period October 1982 to December 1982, we developed the course in basic electronics and trained three Egyptian instructors to present the course in Egypt. In March 1983, the first version of the course was started in Egypt with the instructors. NIH personnel observed the beginning of the course and administered a final exam in June 1983 to evaluate the students, instructors, and course material. The evaluation revealed the course to be successful overall but indicated the need for additional material at the end of the course and a slight reorganization.

The complete version of the lessons has been developed and prepared for presentation in Egypt sometime during the fall of 1984. The course will be taught by the Egyptian instructors in Arabic. NIH will monitor the beginning of the course and will administer a final exam to the students at the conclusion of the course.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10073-05 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

L. Kelner. Visiting Scientists, BEIB

COOPERATING UNITS (if any)
LC, NHLBI and LCS, NIMH

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
DRS, National Institutes of Health, Bethesda, MD 20205

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

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

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

During this fiscal year a few modifications have been made in order to improve
the performance and versatility of the SEMS instrument. These modifications
included: 1) Cs+ ion gun for comparative study of the secondary ion formation
under bombardment by particles of different nature; 2) Faraday cup to measure
the intensity of primary ion beam more precisely; 3) conversion dinode
multiplier and immersion lens to improve secondary ion detection; 4)
differential vacuum system for the MS/MS portion of the instrument.

Experimental studies of the particle desorption ionization of labile compounds
continued on the instrument, including studies of the primary beam parameters
in relation to the resulting mass spectra of the target material. Some of
recent biological applications included demonstrating the utility of the SEMS
system for biological extract analysis in studies involving the drug induced
Parkinsonism in primates and humans.

A new direction in the instrument application has been undertaken this year:
an application as a molecular microscope - a device which will detect the
spatial distribution of organic substances on the surfaces of biological
specimens. The instrument design was presented at the Annual Conference on
Mass Spectrometry and Allied Topics on June 1, 1984 in San-Antonio, Texas.

PUBLICATIONS:

L. Kelner, S.P. Markey, P.A. Cole, and C.K. Crawford: Ion Sources for Mass Spectrometry. II, Filaments for Low Temperature Ion Sources. Int. J. Mass Spectrom. Ion Physics. 51: 215-223, 1983.

L. Kelner and S.P. Markey: Energy Distribution of Secondary Organic Ions. Int. J. Mass Spectrom. Ion Processes. 59:157-167, 1984.

L. Kelner, S.P. Markey, H.M. Fales, and T. Lundquist: Secondary Ion Mass Spectrometer - Application and Evaluation. Int. J. Mass Spectrom. Ion Processes. Accepted for Publication, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10096-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Light Scattering Method for Evaluation of Platelets

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

R. F. Bonner Physicist BEIB DRS

Other Investigators:
T.R. Clem	Elect. Engineer	BEIB DRS
S.B. Leighton	Mech. Engineer	BEIB DRS

COOPERATING UNITS *(if any)*
Bureau of Biologics, FDA
Surgery Br., NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

TOTAL MAN-YEARS: 0.4	PROFESSIONAL: 0.4	OTHER:

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

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

Assessment of the functional status of platelets for transfusion is confounded by the inherent complexity of the cell, as well as the intricate requirements of sample preparation. A correlation between discoid shape and the functional integrity of the platelet has been established. We have developed a complex automatic machine based on previous experiments with a simple prototype which measures the fraction of the platelets that are discoid and the optical (volume) concentration of unaggregated platelets in standard blood bank platelet concentrate units within their bags. The mircroprocessor-based instrument automatically determines the volume concentration of unaggregated platelets and the fraction that are fully viable (discoid) during a 3-minute measurement sequence following simple insertion of the blood storage bag in the instrument. This process is noninvasive (sterile) and nondestructive. Thus it allows frequent measurements on the platelets prior to transfusion in order to optimize the quality and quantity of transfused platelets given patients. It also allows accurate investigations for optimizing storage and preparation methods. The instrument has been intensively studied with respect to invasive measurements of concentration and morphology of platelets and has proved to be highly accurate. In order to test the clinical significance of these measurements, a clinical protocol has begun in collaboration with Surgery Branch, NHLBI to test the functional qualilty of tested units transfused into patients following cardiac surgery.

ASSOCIATE INVESTIGATORS:

J. Fratatoni	Dir. Blood Bank Products	BB FDA
B. Poindexter	Biologist	BB FDA
D. Underhill	Clinical Fellow	SB NHLBI
W. Clark	Chief	SB NHLBI

OBJECTIVES: Develop an optical method to evaluate platelets in standard blood storage bags and design and construct a practical clinical instrument using this noninvasive optical method. Develop and test optimal methods for the routine use of this technology in blood banking and tranfusion.

METHODS EMPLOYED: Measurement of a large number of platelet concentrates on a simple prototype optical instrument in parallel with biochemical and visual grading providing a basis for the evaluation of the light scattering method. Light scattering theory applied to this data base led to the understanding of effects of the platelet number density and fraction that are discoid. This theoretical understanding provided the basis for the design parameters of the automatic instrument as well as its ability to automatically convert the raw optical signals into the desired concentration and viability fraction. It allows the use of the instrument with all the different types of commercial blood storage bags.

MAJOR FINDINGS: The automatic instrument can be used routinely by relatively unskilled technicians to evaluate quickly, noninvasively and nondestructively the quality and quantity of platelets in any standard storage bag. In preliminary tests the accuracy appears to be as good or better than that of the present invasive measurements not routinely allowed for a product that is transfused.

A newly developed multiple scattering theory very accurately predicts the optical scattering effects, which suggests further application to many type of concentrated cell suspensions.

SIGNIFICANCE: This method and instrument allow for the first time the optimal utilization of the blood bank product (platelet concentrate) for transfusion. It allows continuous quality control of the preparation and storage of the platelet concentrates at blood banks and hospitals. It may prove to be very useful in assessing new methods of preparing and storing the blood product for tranfusion.

PUBLICATIONS

Fratantoni, J., Poindexter, B. and Bonner, R.F. J. Lab Clin. Med. 103; 620-631 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10097-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

R.S. Chadwick, Biomedical Engineer, BEIB, DRS
D. McGuire,   Mathematician,   BEIB
R. Patterson,   Chief   EPPS, CB, NHLBI

COOPERATING UNITS *(if any)*
NHLBI- Cardiology Branch

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.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.)*

Theoretical calculations of the contraction of the left ventricle against an arterial system with propagating pulse waves have been undertaken. The dependence of aortic input impedance on frequency, the time course of ventricular and aortic pressure, and ventricular pressure - volume loops are computed using the theory. A model of arteriolar contraction and lumen regulation including the effect of endothelial cells has also been developed.

OBJECTIVES: To develop a quantitative theory of mechanical and hemodynamic events in the cardiovascular system that can be used to evaluate performance characteristics.

METHODS EMPLOYED: A time-varying elastance model of the left ventricle is coupled to a model of arterial load that is based on a description of the arterial system as a network of flexible vessels supporting wave propagation. The coupled system is solved iteratively on the computer.

MAJOR FINDINGS: Preliminary calculations indicate that the numerical algorithm is efficient and that realistic pressure and flow waveforms have been obtained with physiological parameter settings.

SIGNIFICANCE: The model is capable of parameter estimation from experimental data.

PROPOSED COURSE: Venous return and the pulmonary circulation will be included, as well as control and reflex phenomena. We also plan to document the model in a report to supplement a user-friendly computer program.

PUBLICATIONS

Chadwick R.S. and McGuire D.A: Contraction of the left ventricle against an arterial system with propagating pulse waves. To appear in Proceedings of the VIth International Conference and Workshop of the Cardiovascular System Dynamics Society. Philadelphia, 1984.

Chadwick, R.S: Mechanics of arteriole contraction. To appear in Proceedings of the 4th International Conference on Mechanics in Medicine and Biology, Buffalo, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10098-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Laser Instrumentation for Vitreous & Cardiovascular Microsurgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
R.F. Bonner Physicist BEIB DRS

P.D. Smith Physicist BEIB, DRS

COOPERATING UNITS *(if any)*
Clinical Branch, NEI
Cardiology Branch & Surgery Branch, NHLBI.

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 1.3	PROFESSIONAL: 1.3	OTHER:

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

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

This study entails the development and methodology of use pulsed carbon dioxide laser systems (one was a prototype pulsed system with fiber optics delivery and the other was a modification of a commercial medical carbon dioxide laser), pulsed Nd:YAG laser coupled to a slit lamp, Q-switched Excimer lasers and high-power CW Argon laser with fiber-optic delivery systems. These laser systems are being tested in a series of animal experiments to test the efficacy and safety of cutting vitreal membranes and of removal of atherosclerotic plaque from the intima of arteries. For the CO_2 and Nd:YAG laser systems systematic studies of animal vitrecomies were performed in order to characterize laser pulse characteristics necessary to transect vitreal membranes. Additional studies of retinal damage as a function of pulse characteristics and distance of cutting site from the retina clarified the potential for use of these laser systems close to the retina. For all laser systems systematic studies of tissue damage on human coronary arteries are in progress to characterize the feasibility (and optimal system design) of laser angioplasty. Evaluation of prototype fiber-optic angioscopes in pigs is being carried out in order to fully explore all facets of technology necessary for clinical laser angioplasty.

Preliminary data reveal that the carbon dioxide laser can cut experimentally created membranes in rabbits for virtually any condition of the clarity of the optical media. The Nd:YAG laser pulses can cut vitreal membranes when power densities exceeding 1 GW/cm^2 are achieved at the target. Moderate to severe turbidity of the optical media greatly decreases the ability to cut vitreal membranes with the Nd:YAG laser and creates the potential for increased retinal damage.

ASSOCIATE INVESTIGATORS

M. Leon   Sr. Investigator   CB, NHLBI
D. Underhill   Clinical Fellow   SB, NHLBI
R. Nossal   Sr. Res. Physicist   PSL, DCRT
Gaasterland, D.E.   Chief, Glaucoma Section   CB, NEI

OBJECTIVES: This study is designed to develop and test the efficacy and safety of a carbon dioxide laser instrument for use in vitreous surgery. Additionally the safety and efficacy of Q-switched and mode-locked Nd:YAG laser systems for vitreous surgery are examined. Preliminary studies of a large variety of laser systems for laser angioplasty are being directed towards optimizing laser/fiber optic system for this experimental surgery and other cardiovascular microsurgery.

METHODS EMPLOYED: Two carbon dioxide lasers with special delivery systems adapted for use in vitreous surgery were developed in previous years.   The safety and efficacy of the prototype units as well as an experimental prototype pulsed Nd:YAG system have been tested in rabbits and monkeys with vitreal membranes.   Retinal damage mechanism are systematically examined in normal eyes as well.   These systems and pulsed Excimer (UV) laser systems are being used to systematically study the efficacy of laser pulses (pulse energy density, wavelength, duration, and power density) to remove tissue from the intima of coronary arteries while minimizing damage to underlying artery wall.

MAJOR FINDINGS: Data reveal that the carbon dioxide laser can cut experimentally created membranes in rabbits for virtually any condition of the clarity of the optical media.   The Nd:YAG laser pulses can cut vitreal membranes when power densities exceeding 1 GW/cm^2 are achieved at the target. Moderate to severe turbidity of the optical media greatly decreases the ability to cut vitreal membranes with the Nd:YAG laser and creates the potential for increased retinal damage.   The Nd:YAG laser can not be used within 2 mm of the retina without significant risk of retinal damage due to the absorption of radiation by the outer retina.   The CO_2 laser systems can be used within 2 mm of the retina safely only when short duration pulses are used at a slow repetition rate in order to prevent the establishment of convective currents of hot vitreous.   The CO_2 laser is appropriate for cutting vascular membranes due to thermal coagulation of blood vessels accompanying the cutting action, whereas the use of the Nd:YAG laser in these cases without previous coagulation of vessels would result in vitreal hemorrhage.   A prototype CO_2 laser system of the above design is currently being use in human clincial trials at the Clevend Clinic (S. Meyers).   Preliminary results on human coronary arteries in vitro suggest that only highly absorbed radiation in submillisecond pulses (e.g., pulsed CO_2 lasser or Q-switched KrF laser) offers the potential for such highly specific (local) laser microsurgery.

SIGNIFICANCE: Although the present mechanical vitrectomy instruments perform well in most cases, there is a risk of intraoperative complications (retinal tears and hemorrhage) when vitreal membranes are cut, especially if the membranes are taut and have a strong adhesion to the retina. "Tension" on the membranes is increased as the "cutter" of the vitrectomy instrument or the vitreous scissors cuts the tissue.   This tension is transmitted to the vitreoretinal adhesion and surrounding retina predisposing this area to hemorrhage and retinal tears.   If the vitreal membrane is vascularized, this tension may cause bleeding.   The carbon dioxide laser vitrectomy may decrease the incidence of these intraoperative complications and increase facility in cutting selected vitreal membranes.

The fiber optic delivery system for the CO_2 energy as well as the specific laser pulse pattern developed for optimal cutting of vitreous bands without damaging surrounding eye tissues may be generally applicable to all types of CO_2 laser surgery. The combined illumination-irrigating 20 gauge probes have improved the currently available instrumentation available for vitreous surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 RS 10099-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cochlear Mechanics and Hair Cell Transduction

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

R.S Chadwick, Biomedical Engineer BEIB, DRS
J. Rinzel,   Chief   MRB, NIADDK
S. Shamma,   Staff Fellow,   MRB, NIADDK
J. Wilbur,   Staff Fellow,   MRD, NIADDK

COOPERATING UNITS *(if any)*
NIADDK, Mathematical Research Branch

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.25	PROFESSIONAL: 0.25	OTHER:

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

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

This study is concerned with a theoretical analysis of the propagation of mechanical waves in the cochlea, the traduction process in the cochlear hair cells, and the subsequent propagation of electrical impulses in the auditory nerve. In auditory physiology, both mechanical and neural processes play important roles. This study is intended to clarify their relative roles and interaction.

OBJECTIVES: To calculate the electrical signals being transmitted to the brain via the 8th auditory nerve in response to sound input in the cochlea.

METHODS EMPLOYED: The appropriate equations of fluid mechanics, solid mechanics, and neurophysiology are solved in a cascaded model of the basilar membrane, fluid-cilia coupling and hair cell.

MAJOR FINDINGS: The processing of a single tone has been computed. The calculated dependence of AC and DC hair cell potentials on frequency and stapes amplitude is in general agreement with reported experimental findings. We found that the DC hair cell tuning curve is sharpened by the nonlinear cilia displacement - conductance relation for the hair cell.

SIGNIFICANCE: This is one of the first attempts to coordinate what has been until now two distinct areas of investigation: cochlear mechanics and auditory neurophysiology. Such a combined approach will help to answer some fundamental questions in auditory physiology, and in the design of cochlear prostheses such as the relative importance and interaction of mechanical and neural events in auditory analysis.

PROPOSED COURSE: We plan to extend the applicability of the model to higher frequency and analyze the processing of more complex input signals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10103-04 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Triple Laser-Multi Parameter Flow Cytometry System for Study of Tumor Cell Kinetics

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

W. Schuette, Chief, ACES. BEIB, DRS
S. Shackney NCI
J. Dvorak LPD-NIAD

COOPERATING UNITS (if any)

DRS-NCI
LPD-NIAD

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

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

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

A triple laser flow cytometry has been developed so that various immuno-fluorescent labeling techniques may be employed for the investigation of cell kinetics. Three laser beams at different wave lengths are made to intersect a tumor cell flow stream passing through a quartz cuvette so that multi-parameter signals may be obtained. These signals are processed by specialized electronics and then analyzed by means of a PDP 11 computer. Simplified optics have increased light detection efficiency by an order of magnitude. The system is currently being used for the investigation of a unicellular protozon known as trypanosoma cruzi, the causative agent of Chagas disease.

PUBLICATIONS

Shackney S.E., Schuette, W.H.:   Multicompartment Analysis of Cell Proliferation and Cell Migration in the Sezary Syndrome", Hematological Oncology 1: 31-48, 1983.

Jakesz R., Smith C.A., Aitken S., Huff K., Schuette W., Shackney S., Lippman M: "Influence of Cell Proliferation and Cell Cycle Phase on Expression of Estrogen Receptor in MCF-7 Breast Cancer Cells," Cancer REsearch 44: 619-625, February 1984.

Shackney S.E., Levine A.M., Fisher R.I., Nichols P., Jaffe E., Schuette W.H., Simon R., Smith C.A., Occhipinti S.J., Parker J.W., Cossman J., Young R.C. and Lukes R.J.: The Biology of Tumor Growth in the Non-Hodgkins Lymphomas, A Dual Parameter Flow Cytometry Study of 220 Cases.   J. Clin Invest, Vol 73, 1201-1214, April 1984.

Jakes R., Smith C.A., Aitken S., Schuette W.H., Shackney S and Lippman M.E.: McF-7 Cells Secrete Factors Which Stimulate Proliferation and Down Regulate Estrogen Receptor, presented at Endocrince Annual Meeting, San Antonio, Texas, June 8-10, 1983.

Schuette W.H., Shackney S.E., Plowman F.A., Smith C.A., Tipton H.W. and MacCollum M.A.: A High Efficiency Optical Detection System for use with Multi Beam Flow Cytometry. Presented at Analytical Cytology X Pacific Grove CA. June, 1984.

Shackney, S.E., Levine, A.M., Fischer R.I., Schuette, W.H., Smith C.A., Katz A., and Lukes R.J.: The Differential Effects of Aneuploidy and S Fractions on Patient Survival and Complete Response Rate in Non-Hodgkins Lymphomas of the B and T Cell Type. Presented at Analytical Cytology X Pacific Grove CA. June, 1984.

Shackney S.E., Chen S.S., Ochipiuti S.J., Schuette W.H. and Ritch P.S.: Multi-Parameter Image Analysis Studies of Feulgen Stained Radioautographs in the Non-Hodgkin's Lymphomas. Presented at Analytical Cytology X Pacific Grove CA. June. 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10109-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

R.L. Levin, Biomedical Engineer, BEIB, DRS
M. Hagmann, Senior Staff Fellow, BEIB, DRS
J-L Guerquin-Kern, Fogarty Fellow, BEIB, DRS
M. Maxwell, Staff Fellow, BEIB, DRS
A. Zabel, Physician, ROB, NCI
E.J. Glatstein, Chief, ROB, NCI

COOPERATING UNITS *(if any)*
Radiation Oncology Branch, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 3.0	PROFESSIONAL: 3.0	OTHER

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

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

The purpose of this project is to facilitate the development of adjunct
hyperthermia modalities for cancer treatment by theoretically and
experimentally studying the spatial and temporal variation in the temperature
field of tissues subjected to microwave and radio-frequency electromagnetic
radiation. Currently, we are (1) experimentally measuring the patterns of
energy deposition within extremity phantoms produced by various types of
helical coil applicators: (2) theoretically describing the electromagnetic
interaction of a limb with a helical coil in terms of its design parameters;
and (3) theoretically describing the transient thermal profiles within limbs
produced by helical coils. We are also performing electromagnetic and thermal
modeling of the unwanted non-local energy deposition occuring outside of the
hyperthermia applicator.

CONCLUSIONS: Our experimental results for the helical coil indicate that in general (1) the axial electric field varies sinusoidally with axial position and is relatively independent of radial and azimuthal positions; (2) the axial electric field is the dominant component of the resultant electric field; and (3) the helical coil is inherently an efficient applicator.

Our RF modeling studies indicate that appreciable optimization of the pattern of energy deposition could be achieved by a suitable choice of helical coil design parameters. The position of the arms and legs with respect to the torso, however, are critical in the minimization of non-local energy deposition effects.

Our thermal modeling studies indicate that (1) the resultant thermal and dose profiles are significantly affected by autoregulation of the blood supply and the presence of "thermally significant" major blood vessels. (2) the temperature of the bone does not significantly lag behind the temperature of the surroundings muscle even though much more energy is being absorbed by the muscle as compared to the bone; and (3) the regions of highest thermal dose do not necessarily correlate with the areas of highest energy absorption and/or highest instantaneous temperature.

OBJECTIVES:

(1) To optimize the radiation fields generated by a "helical coil" applicator.

(2) To measure the spatial and temporal variation of the temperature field generated by the "helical coil" applicator in various types of "phantoms".

(3) To develop a generalized mathematical model which will predict the spatial and temporal variation of the temperature field within a tissue or organ subjected to electromagnetic irradiation.

(4) To develop optimal adjunct hyperthermia modalities.

METHODS EMPLOYED:

(1) Mathematical modeling of the radio-frequency and thermal characteristics of various types of phantoms and human extremities subjected to RF irradiation from various types of helical coil applicators will be accomplished through the use of ROB's VAX-11/750 computing system and its associated graphics hardware.

(2) Experimental measurements of the electric field and thermal profiles of various types of phantoms subjected to RF irradiation from various types of applicators will be accomplished through the use of MES/BEIB's PDP-11/34 computing system and its associated data acquisition system.

SIGNIFICANCE: At present, the heat treatment of cancerous cells when combined with conventional radiotherapy and chemotherapy shows considerable promise in the management of cancer. Nevertheless, there still remain numerous important problems that must be resolved. Of paramount importance is the problem of generating and controlling uniform temperature fields within tissues. This study will therefore attempt to facilitate the development of optimum hyperthermia modalities by theoretically and experimentally studying the temperature fields within tissues subjected to radio frequency EM radiation.

MAJOR FINDINGS:

(1) Experimental:

    a) The axial electric field is the dominant component of the resultant electric field for a self-resonant helical coil.

    b) The axial electric field varies sinusoidally with axial position and is relatively independent of radial and azimuthal positions for self-resonant helical coils.

(2) Theoretical:

    a) The resultant thermal and dose profiles are significantly affected by autoregulation of the blood perfusion rate and the presence of major blood vessels.

    b) The regions of highest thermal dose do not necessarily correlate with the regions of highest energy deposition and/or highest instantaneous temperature.

    c) The positions of the arms and the legs with respect to the torso significantly affect the patterns of non-local energy deposition.

PROPOSED COURSE:

(1) To continue the electromagnetic and thermal characterization of various helical coil/phantom combinations using non-perturbing probes.

(2) To begin characterization of: Mini-Amular Phased Array system which is being obtained from BSD Medical Corp.

(3) To begin work on a computerized treatment planning system.

PUBLICATIONS

Levin, R.L., "The Helical Coil: An Effective Hyperthermia Applicator". 1983 Advs. in Bioengineering (1983).

Hagmann, M.J. "Propagation on a sheath helix in a coaxially-layered lossy Dielectric Medium "IEEE Transactions on Microwave Theory and Techniques, MTT-32:122-126 (1984).

Hagmann, M.J. and Levin, R.L., "Analysis of the Helix as an RF Applicator for Hyperthermia". Electronics Letters 20:337-338 (1984).

Hagmann, M.J. and Levin, R.L., "Non-Local Energy Deposition: A Problem in Regional R-F Hyperthermia". Proceedings of the 1984 NAHG Meeting (1984).

Levin, R.L. and Hagmann, M.J. "A Heat and Mass Transfer Model of Human Extremities during Hyperthermia" Proceedings of the 1984 NAHG Meeting (1984).

Guerquin-Kern, J-L Hagmann, M.J. and Levin, R.L. "R-F and Thermal Characterization of Helical Coil Hyperthermia Applicators". Proceedings of the 4th Intl. Symp on Hyperthermic Oncology (1984).

Levin, R.L. and Hagmann, M.J. "A Heat and Mass Transfer Model of Human Extremities during Hyperthermia" Medical Physics (1984). In press.

Hagmann, M.J. and Levin. R.L. "Coupling Efficiency of Helical Coils Hyperthermia Applications " IEEE Trans., Biomedical Eng. (1984). In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10110-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Dual 3-Dimensional Position Monitor For Speech Analysis: MOD. II

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

E.C. Walker, Mechanical Engineer, ACES BEIB

Other Investigators
H.W. Tipton, Mechanical Engineering Tech. ACES BEIB
T.L. Talbot, Mechanical Engineer ACES BEIB
C.L. Ludlow, LCD NINCDS
M. Dorn-Quine, Guest Researcher NINCDS

COOPERATING UNITS *(if any)*
LCD-NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

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

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

Several speech monitoring instruments have been designed to study the etiology of normal and abnormal articular movements in humans. The first system, which is in current use, provided a unique method of monitoring 3-D lip and jaw movements. The second system, MOD II, an improved version of the first. The device consists of two, mirror image, transducers mounted on a common head frame. Each transducer, which can be individually adjusted, is capable of measuring the movement of a point in three orthogonal planes. The transducers have been structurally and operationally redesigned to provide increased linearity, resolution, and reduced weight. Additionally, the head frame has been totally redesigned to provide improved wearing comfort and reduced weight. The improved system will facilitate studying a broader patient population such as children and the elderly.

Design and fabrication of MOD II has been completed. Results of bench tests indicate that the system performs well, within its specified range. Future efforts will be directed toward patient testing.

Following system entracement, patient testing will be scheduled for the upcoming fiscal year.

PUBLICATIONS

Walker, E.C., Tipton, T.W., Ludlow, C.L.; A photographic method of producing variable transmittance optical disks for position monitoring. Advances In Bioengineering, November 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	ZO1 RS 10112-04 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Microcirculatory Blood Flow by Laser Doppler Scattering

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

R.F. Bonner, Physicist, BEIB, DRS

T.R. Clem, Elect. Engineer, BEIB, DRS

COOPERATING UNITS *(if any)*
Hypertension Br., NHLBI; Lab of Clinical Investigation, NIAID; Lab of Chemical Biology, NIADDK; Allergy and Rheumatogoly Depts., Walter Reed Army Medical Center; MED Pacific, Inc. Seattle, Washington.

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 1.4	PROFESSIONAL: 1.4	OTHER·

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
The purpose of this project is the development of a clinical, non-invasive monitor of tissue blood flow by analysis of the spectrum of Doppler-scattered laser light. The NIH Laser Doppler Blood Flow Monitor has been demonstrated to be highly portable and clinically convenient with sterilizable, rugged flexible 4m fiber optic probes and portable photodiode detection system. The linearity of the flow analysis processor has been demonstrated in a variety of tissues and clearly resolves physiologic flow changes including pulsatile flow in the microcirculation. Muscle blood flow in over 50 patients with neuromuscular disease has been studied and data suggest that post occlusive reactive hyperemia responses may be primary or secondary indicators of disease state. Measurements of local muscle blood flow dynamics in patients with neuromuscular diseases indicate abnormalities distinct to different disease types. Nasal blood flow has been shown to be a quantitative measure of the physiologic response of the nose to drug challenges. Scleroderma patients' fingertip blood flow appears to fall in three easily-discernable classes associated with the severity of the disease and a simple measurement protocol may elucidate efficacy of drug therapy. Studies show a characteristic local oscillatory flow pattern in a capillary microcirculation of the skin in sickle cell patients which appears to correlate with severity of disease and its response to drug therapy. Similar oscillations presumably due to myogenic arterioler smooth muscle vasomotion are characteristic of several hypertensive patients, particularly those on durgs inducing peripheral vasocontriction. Preliminary studies on patients with Type I diabetes have shown abnormalities potentially related to the etiology of the microvascular component of long-term type I diabetes. In summary numerousongoing clinical studies seek to characterize microvascular functional abnormalities and their role in a number of diseases with microvascular components.

ASSOCIATE INVESTIGATORS

M. Kaliner	Section Chief LCI NIAID
H. Druce	Clinical Assoc. LCI NIAID
G. Rogers	Clinical Assoc. LCI NIADDK
M. Roy	Visiting Scientist NEI
D. Goldstein	Sr. Invest. HB NHLBI
N. Kaiser	Chief HB NHLBI

OBJECTIVES: Ongoing clinical applications include muscle blood flow at open muscle biopsy in muscular dystrophy patients, skin blood flow in normal, hypertension, diabetes, sickle cell disease, cold urticaria and scleroderma patients and nasal blood flow responses to drug and antigen challenge in normals and rhinitics. Specific objective at this stage is the application of the instrument and technique in the above variety of clinical and experimental problems. Assistance to the commercial manufacturer (Med Pacific) is being provided under the NIH Patent license agreement to insure a high quality commercial instrument.

METHODS EMPLOYED: The present form of the apparatus has demonstrated its clinical convenience and portability. Minor modifications to probe tip are made for specific applications. Methodologies for clinical diagnosis are developed in collaboration with clinicians. The modifications suggested and implemented in the commercial instrument design are based on spectral noise analysis and theoretical analysis of the method.

MAJOR FINDINGS: (1) The commerical models have been dramatically upgraded to performance comparable to NIH prototype. The mean frequency detection scheme and fiber optic system developed at NIH has been implemented in all commercial devices. A new blood volume measuring circuit has been implemented in the NIH prototype and is undergoing tests. (2) A rigorous scattering theory substantiates the validity of our analysis method as do empirical correlations with alternative techniques. The instrument output conversion of 1 volt - 15 ml/min/100gm tissue holds with small variations for very different tissue types. (3) Measurements of local muscle blood flow dynamics in patients with neuromuscular diseases indicate abnormalties distinct to different disease types. (4) Scleroderma patients fingertip blood flow appears to fall in three easily-discernable classes associated with the severity of the disease and a simple measurement protocol may elucidate efficacy of drug therapy. (5) Nasal blood flow has been shown to be sensitive to allergic state and various nasal challenges. (6) Studies show a characteristic local oscillatory flow pattern in a capillary microcirculation of the skin in sickle cell patients. (7) Studies in selected hypertensive patients have provided additional evidence for rhythmic fluctuatious in myogenic tone of anteriolar smooth muscle as a physiologic regulator of microciruloatory flow.

PROPOSED COURSE: Pursue clincial trials to establish the instrument as a useful clinical and experimental tool.

SIGNIFICANCE: The NIH Laser Doppler Blood Flow Monitor is an instrument which holds great promise for study of the local tissue microcirculation. It has potential applications in the research laboratory, and in the clinical study of scleroderma, sickle cell disease, hypertension, diabetes, peripheral vascular disease, allergy testing, screening of vaso-active drugs, and the monitoring of patients with burns and skin grafts.

PUBLICATIONS:

Druce, H.M., Bonner, R.F., Choo, P., Patow, C., Summers, R., and Kariner, M.A.: Response of nasal blood flow to neurohormones as measured by laser-doppler velocimetry. J. Appl. Physiol., in press.

Rodgers, G.P., Schechter, A.N., Nogucli, C.T., Klein, H.G., Nienhius, A.W. and Bonner, R.F.: Periodic Microciculatory Flow in Patients with Sickle Cell Disease. New Eng. J. of Med., in press.

Goldstein, D.S., Bonner, R.F., Zahn, T.P., Cannon, R.O., Rosing, D.R., Stull, R. and Keiser, H.R.: Indices of Sympathetic Vascular Innervation in a Patient with a Lumbar Sympathetectomy. Am. J. Cardiology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10114-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Phasic Aortic Pressure Control System for Awake Dogs

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

S.R. Goldstein, Chief,   MES, BEIB, DRS
M. Maxwell,   Staff Fellow, BEIB, DRS,
R. Patterson,   Chief,   EPPS, CB,   NHLBI

COOPERATING UNITS (if any)
CB, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER.
.75	.65	.1

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

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

A system for manipulating phasic aortic blood pressure in closed chest experimental dogs has been under development. An intra-aortic balloon residing in the descending aorta and controlled by feedback from a catheter tip pressure tranducer is phasically inflated or deflated by a piston type actuator to vary the pressure to conform with a predetermined desired pressure waveform. While still under servo control, the balloon is reset (either filled or deflated) every few beats due to the action of a roller pump which bypasses the systemic circulation in order to withdraw (or infuse) blood into the aorta. In this way it was hoped to control both the average pressure level and the exact waveform so that the effects on the myocardium of various drug interventions could be evaluated independent of their effects on systemic blood pressure.

During the past year the system was evaluated both in dogs, and in an elaborate in vitro model of the aorta and systemic circulation. A number of problems were identified which could only be solved by placing constraints on the system which ultimately proved to be mutually incompatible -resulting in termination of the overall effort. The fundamental problem which could not be overcome arose from the large phase shift and lack of attenuation with increasing frequency between actuator position and aortic pressure which arises due to the distributed compliance and low wave speed of the aorta. This undesirable open loop characteristic precluded attempts to design suitable servo compensation, resulting in phasic waveforms that did not faithfully reproduce the desired waveform. This situation could be partially mitigated only by making the balloon small- which resulted in a need to reset the balloon too often for the system to function properly.

OBJECTIVE: To control pressure downstream of the heart in experimental dogs so that this variable is either a) changed in an arbitrary manner to assess the effect on ventricular performance, or b) is maintained during other interventions e.g. drugs, so that effects of the intervention are not obscured by changes in downstream pressure (and coronary perfusion).

SIGNIFICANCE: The circulatory system is extraordinarily complex with a multiplicity of feedback mechanisms which prevent unequivocal interpretation of the effects of various interventions commonly performed in its study. Decoupling of the downstream load pressure (and coronary perfusion pressure) should greatly facilitate the interpretation of drug interventions where at present, drugs that alter contractility also alter systemic pressure. Additionally, the ability to arbitrarily change load pressure should provide a means of validating mathematical models of ventricular function where load pressure is a key parameter. Similarly, control of end diastolic pressure in conjunction with downstream pressure in an intact animal should give investigators great flexibility in the design of experiments that elucidate the inherent contractile properties of the myocardium.

METHODS EMPLOYED: Aortic pressure is manipulated by a rapidly responding gas filled intra-aortic balloon which resides in the descending aorta and is used in conjunction (when necessary) with a speed controlled roller pump which bypasses the peripheral circulation with catheters. Aortic pressure is measured with a catheter tip pressure transducer and fed back to an electronic controller which drives a linear motor to position a bellophragm that fills or deflates the balloon catheter. The roller pump provides the DC and low frequency part of the control and the balloon pump provides the high frequency control.

MAJOR FINDINGS: Preliminary in vitro tests of the balloon servo indicated that large phase shifts between actuator motion and "aortic" pressure combined with resonances and lack of attenuation at high frequency would severely limit the attainable servo performance. These results were confirmed in-vivo with the balloon in the descending aorta. Additionally, changes in venous return produced by the roller pump (required to reset the balloon) cause changes in cardiac output which potentially could compromise the experiments unless the end diastolic filling pressure or volume is clamped which is beyond the scope of the effort at present.

To better understand the sources of the above mentioned phase shift, extensive tests were performed using a life size silicon rubber model of the aortic tree which had an appropriate non-linear compliance approximating that of a dog. It was determined that the phase shift resulted from the distributed compliance of the aorta and balloon combination. The only way to mitigate this (somewhat) was to use a very short and therefore small volume balloon.

However, a small balloon has to be reset too often for the system to function effectively. Utilizing the balloon to partially block off the aorta and modulate resistance to flow was also found to be impractical due to poor gain phase characteristics.

PROPOSED COURSE: Due to the impossibility of meeting mutually incompatible constraints imposed by the physiology and anatomy of the dog, work in this project has been terminated.

PUBLICATIONS

Goldstein, S.R.: A Servo System Designed to Control Phasic Aortic Root BLood Pressures in a Conscious Closed Chest dog. In Barter, D. (Ed.): 1983 Advances in Bioengineering, American Society of Mechanical Engineers, New York, N.Y. 1983 pp. 74-75.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10116-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Modeling of Arterial Pulse Waves

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

R.S. Chadwick,   Biomedical Engineer, BEIB,DRS
D. McGuire,   Mathematician,   BEIB
D. Goldstein ,   Senior Investigator EHL, NHLBI
H. Kaiser,   Chief EHL,   NHLBI

COOPERATING UNITS (if any)
NHLBI, Endocrine-Hypertension Laboratory

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.55	.25	.3

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

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

A new theory was developed to compute the propagation of the pressure wave in a branching system. The theory includes cross sectional area and stiffness variation, viscous and viscoelastic effects, and side branch flow. The theory was then used in an analytical model of the brachial artery system which can reproduce the phasic pressure waveforms as measured in normal and hypertensive subjects. Particular emphasis was directed toward an understanding of the genesis of the peripheral dicrotic wave, and its modulation by vasoactive drugs.

OBJECTIVES: To establish the mechanisms that are responsible for the clinically observed reduced dicrotic wave modulation of young hypertensive subjects in response to vasoactive drugs.

METHODS EMPLOYED: A new theory was developed to deal with arterial wave propagative in a branching system. The present theory included the effects of area variation, wave speed variation, viscosity of blood, viscoelasticity of blood vessel wall, and side branch flow. Asymptotic methods are used to solve the differential equation for pressure propagation in the frequency domain. Periodic waveforms are computed using Fourier synthesis.

MAJOR FINDINGS: We found that very realistic pressure waveforms can be computed with this theory. To simulate the clinically observed modulation of the dicrotic wave, it is necessary to change both the main branch wavespeed and the side branch network resistance and compliance.

SIGNIFICANCE: The theoretical work described here leads to the suggestion that defective modulation of the dicrotic wave with age and high blood pressure result from a combination of increased large vessel stiffness and decreased vasodilator capacity. Such a finding helps to identify both the sites and modes of action of vasoactive drugs as well as clarifying the fundamental processes in the control of blood pressure. The approach used in the present study represents one of the first attempts to use a circulatory model to explain abnormalities in the contour of the pressure waveform in hypertension.

PROPOSED COURSE: Measurements of brachial artery blood flow and pulse wave velocity are planned to further validate our present views on dicrotic wave modulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10122-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Micro-computer Controlled Fermentation System

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

T.R. Clem, Sr., Electronic Engineer, EEES, BEIB, DRS

Other Investigators:
Y. Shiloach	Chief, Pilot Plant	LCDB NIADDK
A. LeRoy	Chemical Engineer	BEIB, DRS

COOPERATING UNITS *(if any)*
LCDB - NIADDK

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

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

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

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

A process control system using a small desktop computer as the central control element is continuing to be developed for the NIH Fermentation Pilot Plant. The various monitoring transducers and controlled elements (pumps, valves, etc.) are connected to the computer via the IEEE-488 General Purpose Interface (GPIB). For maximum versatility most components used are commercially available items (instruments, meters, etc.). The GPIB-based design allows changes in the parameters measured or controlled, or scaling to different size-vessels to be accomplished relatively quickly and easily. Some instruments, though, are connected to the controller through direct hardware I/O connections. Utilizing the computational capabilities of the computer/controller allows initial selection of the operating parameters and dynamic alteration of these parameters as the process continues, thus allowing optimization of yields or detailed study of the process parameters.

OBJECTIVE: Increase efficiency and productivity of the Pilot Plant Unit, expand fermentation process capability and improve methods whereby research into the fermentation process can be performed by implementing a computer-controlled process control system.

METHODS EMPLOYED: A small and inexpensive desktop computer and an electronic interface system are connected to both new and previously-owned laboratory instruments and to a small fermentation vessel via the IEEE-488 GPIB. The interface scheme is versatile enough to allow changes to be made to the system rather easily. The control programs are written ina high level languages, such as BASIC or PASCAL, which facilitate understanding and modification by the user.

SIGNIFICANCE: Computer monitoring and controlling of the fermentation process produces several significant advantages over manual methods. By using the computer to make decisions based on what is occurring in the fermentation process, parameters can automatically be altered to either produce an increased yield of a particular product, produce a purer form of the product, or study the effects on a particular product of the alteration of selected parameters. The computer can also perform some of the "housekeeping" tasks associated with running a fermentation process that would normally occupy an operator.

PROPOSED COURSE: To continue expanding and refining the system to provide additional functions, monitor and control additional parameters, and implement more sophisticated control programs to expand operation of the system. The system has outgrown the capabilities of the HP-85 computer that was being used, so everything is being converted over to an IBM PC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10126-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Trace Analysis and Elemental Microanalysis in Biological Materials

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

A.F. LeRoy, Chief, Analytical Methods Sect., BEIB, DRS
M. Linnoila M.D., Ph.D., CPB, NIMH
S. Tsai, Ph.D., LCM, NHLBI
P. Galle, Prof. of Biophysics. Medical Faculty, Creteil, France
G. Mathe, Director, ICIG, Villejuif, France
B. Hecquet, Ph.D., Centre Oscar Lambret, Lille, France
P. Parsons, Ph.D., BEIB, DRS

COOPERATING UNITS (if any)
CPB-NIMH; LCM-NHLBI; LCP-NIAMDD, LMP-NCI; ICIG-Villejuif, France;
Biophysics Department, Medical Faculty-Creteil, France;
Centre Anticancereux "Oscar Lambret", Lille, France

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Analytical Methods

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	1.25	.50

CHECK APPROPRIATE BOX(ES)

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

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

Atomic absorption spectrophotometry using electrothermal atomization and neutron activation analysis have been used for the quantitation of platinum, vanadium, nickel, cobalt, calcium, and iron and some other elements in biological tissues and fluids. Electrochemical techniques are being developed for analysis of some elements. The analyses are important in clinical biochemical, pharmacokinetic and binding studies. The analytical techniques must be very sensitive because in most instances these elements are present in trace concentrations in the samples typically of interest e.g. part-per-million (10^{-6}g/g), part-per-billion (10^{-9}g/g), and in some cases even the part-per-trillion (10^{-12}g/g) while sample volume is also often small (typically 0.5 ml or less). Various separation techniques such as chromatography, solvent extraction, and electrophoresis have been used to purify samples and fractionate and concentrate chemical species for analysis and reduce or eliminate interferences.

Microprobe techniques using instruments such as the electron probe microanalyzer have also been used to localize elements on the microscopic scale in sub-cellular structures in different tissues.

OBJECTIVES: To improve techniques of identifying, characterizing and quantitating elements (especially metals and their complexes) and other chemical species in often complex biological samples. The emphasis has been on the quantitation of elements of biological importance such as platinum, vanadium, nickel, cobalt, calcium, and iron. Some of these elements are found in trace level concentrations in normal biological tissues; they may also be present in abnormal concentrations in some disease states. Others may be present as a result of therapeutic intervention.

METHODS EMPLOYED: Ion exchange chromatography is used as a method to resolve metal-ion complexes in in vitro studies in well defined media at concentration levels similar to those obtaining in the clinical uses of a drug such as cisplatin in cancer chemotherapy. Each of the chemical species of platinum is selectively removed from the column by integral elution with an appropriate eluant and quantitation of each species is then performed using electrothermal atomization and atomic absorption spectrophotometry. Collection of fractions of the eluate permit determining the elution characteristics of each of the species; collection of large numbers of samples necessary for thorough characterization can present significant problems in analysis. Few analytical techniques offer the sensitivity required when dealing with the small sample sizes obtaining (typically < 0.1 ml) and concentrations in the ranges typically of interest e.g. the part-per-million (10^{-6} g/g), part-per-billion (10^{-9} g/g), and in some cases even the part-per-trillion (10^{-12} g/g) range, making detailed studies of kinetic and thermodynamic constants difficult by the sheer number of determinations necessary. These physico-chemical constants are important for establishing the chemical species present and their proportions and determining the optimal delivery conditions for the drug. Techniques of electrochemistry appear to have the requisite sensitivity but have presented difficulties to other investigators. Preliminary results in our laboratory using amperometry show good promise; polaragraphic techniques are now being evaluated.

MAJOR FINDINGS: Analysis of hemolysate samples and cerebro-spinal fluid samples from patients for vanadium have shown the presence of concentrations in the low part-per-billion (10^{-9} g/g) range in sample volumes of approximately 20 l. The sensitivity of the technique for vanadium is approximately 15×10^{-12} g. Considerable effort has been made to increase the precision of these measurements. The levels found in the CSF samples appear to fall below the present level of detectability. Our efforts have been aimed at obtaining improved detectability for this very refractory element and reducing the untoward effects of the biological matrix on the determination and the variability introduced by it.

SIGNIFICANCE: Identification, characterization, and quantitation of elements and chemical species at trace levels and below in biological samples which are themselves often small in size, represents an important and often difficult task in the conduct of biomedical research. Such quantitation is frequently essential to an understanding of drug action or other biochemical interactions. The physico-chemical methods being developed offer quantitation for stable elements and obviate the need for radiolabeling which may not be possible or practical in may studies.

PROPOSED COURSE: Develop improved sensitivity of techniques for quantitation of elements and chemical species available for a wide range of biological samples; simplify and minimize preliminary treatment of samples. Extend range of Standard Reference Materials available for comparison and referee analyses with biological matrices.

PUBLICATIONS

"Red Blood Cell Membrane Adenosine Triphosphatases in Patients With Major Affective Disorders." Linnoila, M., MacDonald, E., Reinila, M., LeRoy. A.F. Rubinow, D.R., and Goodwin, F.K. Archives of General Psychiatry 40 1021-(1983)

"Pharmacokinetics of Cisplatin in CSF of Man." Armand, J.P., Macquet, J.P., and Le Roy, A.F. p. 142- in "Platinum Coordination Complexes in Cancer Chemotherapy" Hacker, M.P., Douple, E.B. & Krakoff, I.H. (Editors), Martinus Nijhoff Publishing, Boston, 1984.

"Platinum and Iron Concentration as a Function of Time in Kidneys of Rats Treated with Cisplatin" LeRoy, A.F., Berry, J.P., Brille, P., Gouveia, Y., Ribaud, P., Galle, P., and Mathe. G. p. 204 in "Platinum Coordination Complexes in Cancer Chemotherapy" Hacker, M.P., Douple, E.B. & Krakoff, I.H. (Editors), Martinus Nijhoff Publishing, Boston, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10132-03 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Image Processing and Cell Classification

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

J. R. Ellis, Expert, BEIB/DRS

Other Personnel
C. C. Gibson Electronics Engineer BEIB/DRS
M. A. Greenwood SNB/NINCDS
   J. Edwards SNB/NINCDS
   T. Baginski SNB/NINCDS

COOPERATING UNITS *(if any)*

SNB/NINCDS

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Office of the Chief

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.45	0.25	0.2

CHECK APPROPRIATE BOX(ES)

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

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

In vitro cell culture is an effective method for the study of the effects of chemotherapeutic agents on neurological tumors. Quantitation of all but the most rudimentary of these effects is generally beyond the ability of human observers. Thus, an automated system for acquisition of image data, extraction of parameters of interest, and statistical processing of results is necessary for any quantitative effectiveness.

Historically, this field has been qualitative. Thus, moving toward quantitative evaluations has involved a very broad spectrum of discussions about methods, limitations of methods, desired results, and significance of results. Specifically, it is necessary to prepare cultures and micrographs differently for human visual inspection and for reliable machine processing. In addition, it has been necessary to clarify the strong and weak points of human and automated image processing.

This has been especially important in this project because the system originally purchased was ill-suited to the tasks envisioned and support from the manufacturer was totally inadequate. Thanks largely to the efforts of C. C. Gibson of BEIB, the system has been substantially rebuilt and made much more reliable and capable. As a result, in vitro cytotoxicity assays of the effects of AZQ, BCNU, and other drugs on Glioma tumor cell lines using cell counts over titer plates have become a reliable production operation.

Morphology studies using size, aspect ratio, and a shape factor were begun on micrographs of granules and mitochondria. These studies could be continued in the future. However, the laboratory has been reorganized following certain personnel changes. As a result, studies other than cell counting are allowed to the extent that they do not interfere with this production operation.

OBJECTIVES: Quantitative analysis of morphological properties of in vitro Glioma cell cultures under varied conditions and courses of treatment.

METHODS EMPLOYED: Cell lines are taken from surgical biopsies and cultured by standard methods. Sample cultures are subjected to selected therapeutic procedures. Light microscopic and electron microscopic images of both control and treated cell populations are analyzed by a Bausch and Lomb Omnicon Feature Analysis System.

This system derives measurements consisting of several parameters of thresholded images - including area, length, breadth, perimeter, etc. - and their algebraic combinations.

MAJOR FINDINGS: Meaningful quantitative results can be obtained in this environment. Although the system available is not nearly as powerful as it was represented to be by the manufacturer's representative, it can be used for worthwhile operations.

More importantly, even though desired features cannot always be recognized automatically, it has been found that use of intermediary representations, such as overlays, can be a useful match between the complex human visual recognition system and the detailed computational world of the machine.

Morphological studies using such overlays have been started employing a BASIC program which was finally written successfully around the numerous bugs in the Omnicon FAS. Size, length-to-breadth ratio, and perimeter-to-(square root of area) ratio have been measured on micrographs of granules and mitochondria. Initial results are consistent with qualitive observations of drug effects -e.g., the effects of AZQ on mitochondria.

SIGNIFICANCE: Reliable, consistent. quantitative processing of image data concerning neurological tumors must be done by machine. The history of operator fatigue and boredom in these tasks is well known. Further, humans can seldom make any quantitative measurements beyond cell counts. The program of study underway here can widen the range of feasibility of investigations far beyond the qualitative arena which now exists.

PROPOSED COURSE: We have some confidence that studies of cell morphology under chemotherapeutic treatment can now be pursued with reasonable efficiency. This was our original intent when this project was initiated. It is now the responsibility of the cell culture organization to make appropriate use of the more than adequate engineering and computer facilities provided them.

However, since Dr. Barry Smith's departure, the laboratory has been reorganized by Dr. William Meyer. The effect on this project has been that cell counting studies have been declared dominant, and other studies are tolerated to the extent that they do not interfere with this production operation. Substantial progress is unlikely to occur in areas of interest to us with this value system.

PUBLICATIONS

M. A. Oberc-Greenwood, B. H. Smith, C. Cooke, J. R. Ellis, P. L. Kornblith, and P. E. McKeever. "Mitochondrial Toxicity of 2,5 Diaziridynl-3-6-bis(Carboethoxyamino)-1,4 Benzoquinone (AZQ)". Journal of the National Cancer Institute, Vol. 71, pp. 723-733 (1983).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10135-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Fluid Shear on Cultured Endothelial Cells

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

R.J. Lutz   Chemical Engineer, BEIB/DRS

P.M. Bungay Chemical Engineer, BEIB/DRS

COOPERATING UNITS *(if any)*
American Red Cross
FDA

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

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

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

Many pathophysiological processes in the cardiovascular system such as thrombosis, vessel wall injury, and atherosclerosis occur in the presence of fluid shear forces. These shear forces have a direct mechanical effect on the vessel wall and can also indirectly affect wall properties by mediating the interactions of blood elements with the lumenal surface. The normal integrity of a vessel wall is maintained by a delicate monolayer of endothelial cells grown in monolayer culture. Morphological, cytoskeletal, and metabolic changes in the endothelial cells are being investigated as a function of fluid shear stresses on the cell surface. A parallel plate flow chamber was specially designed to accommodate Thermanox circular cover slips upon which human umbilical cord endothelial cells are cultured. The flow patterns and shear stress values within the chamber have been characterized using electrochemical shear measurements. flow visualization techniques, and laser doppler anemometry during steady flow. We have tested the chamber at shear stresses up to 20 dynes/cm^2. The rate of development of cell orientation in the monolayer was observed as a function of shear stress from no flow to 16 dynes/cm^2. Radio-immunoassays were developed to measure levels of von Willibrand factor, Factor VIII:R, and prostacyclin in the culture medium associated with release/production by the endothelial cells. The utility of these assays to follow the appearance of the substances as functions of time and shear stress intensity will be improved by increasing the proportion of monolayer area to culture medium volume. Alternatives to the parallel plate flow chamber with higher area-to-volume ratios were examined. A cup-and-bob viscometric system was chosen for more detailed evaluation. One option being explored is to use the viscometer to shear suspensions of cross-linked dextran microcarrier beads coated with the endothelial cell monolayers.

PROFESSIONAL PERSONNEL

S. Hilbert   Pathologist   FDA
A.R. Koslow   Research Fellow   American Red Cross
R. Stromberg   American Red Cross

OBJECTIVES: BEIB is involvement in this project is focused on the fluid mechanical and other engineering aspects. This includes particularly, characterizing the flow systems in a manner which will describe the mechanical stress to which the biological specimens are subjected in a given experiment. The overall aim of the project is to elucidate the role of mechanical stress and other hemorheological factors in the interaction between plasma, platelets, endothelial cells and basement membrane, with emphasis on the mechanisms involved in platelet adhesion and aggregation on the basement membrane. The presence of Factor VIII·R is believed to be necessary for adhesion of platelets to basement membrane. Prostacyclin is a potent inhibitor of platelet aggregation. Both of these substances are synthesized and secreted by endothelial cells. The in vitro systems developed for this project will be used in a variety of studies. In one study, secretion of Factor VIII:R and prostacyclin will be measured as a function of time for various levels of shear stress on the endothelial cells in the absence of other factors affecting secretion. Other studies will focus on the processes of platelet adhesion and aggregation on simulated basement membrane in the presence and the absence of endothelial cells in the controlled flow environments of the in vitro system.

METHODS EMPLOYED: A parallel plate flow chamber has been designed and prototypes have been constructed and tested. The chamber consists of a parallel arrangement of plexiglass plates approximately four inches wide and ten inches long separated by a thin (.08 cm) silicon gasket. The gasket is cut out so as to form a 10° diverging section leading to a parallel section where two circular Thermanox cover slips are positioned flush with the inner surface of the bottom parallel plate. The endothelial cells grow in monolayer on the cover slips. Variable fluid shear stresses are exerted on the endothelial cells by the regulated flow rate of nutrient media through the chamber. The cover slips can be removed from the flow system at various time intervals for examination of the endothelial cells for ultrastructure changes by TEM and SEM. Biochemical assays can be performed on samples of withdrawn media at various times to look for von Willebrand's factor, Factor VIII·R and prostacyclin that have been produced by the cells in response to the shear stress. Immunoassays will be used to measure these factors. The usefulness of the biochemical assays would be enhanced by minimizing the proportion of sheared culture medium to exposed surface area of endothelial cells. This is particularly true for short time periods after the initiation of mechanical stress. Alternative flow schemes have been considered. One such scheme is being tested for suitability. The alternative would utilize an available Kaltec Hercules Hi-Shear viscometer. The normal configuration for this viscometer consists of a staionary right cylindrical cup and a rotary inner bob (cylindrical with a conical bottom). The sample to be sheared fills the narrow gap between the cup and bob and amounts to, at most, 3.6 ml in volume

when the gap width is 0.05 cm. One approach to culturing the endothelial cells in a manner convenient for introducing into the viscometer would be to employ cross-linked dextran microcarrier beads as a solid support to which the cells attach and organize into confluent monolayers. Aliquoting a portion of the endothelium-coated bead suspension would provide a large number of replicated samples for a variety of experiments.

MAJOR FINDINGS: For the parallel plate test chamber, the flow remains laminar in the test region over the cover slips. Laser Doppler velocimetry has verified the characteristic parabolic velocity profiles. Both the velocity profiles and the measured shear agree to within 10% of theoretical values for parallel plate flow. With no flow in the chamber, the endothelial cells form a random pattern of cobblestone cells with no orientation. Little or no orientation is observed at shear stress from 4 to 7 dynes/cm^2 up to 72 hours. At 8 to 12 dynes/cm^2, cell orientation is observed by 48 to 72 hours. At shear stresses from 12 to 16 dynes/cm^2, the cells have oriented within 24 hours.

Fluid mechanics analyses have been performed to assist in evaluating the suitability of the viscometer/microcarrier bead approach. The instantaneous shear stress distribution over the surface of single, isolated bead in linear shear flow has been predicted from Lamb's general solution for a spherical particle in an infinite Newtonian fluid. Modifications to bead behavior due to the presence of the viscometer walls and to interaction with other beads have been preliminarily examined.

SIGNIFICANCE: One theory of atherosclerosis holds that the disease progresses from endothelial cell damage through a series of steps to an atheroma. To understand atherogenesis, it is necessary to identify the initial processes and to develop means of measuring the effects of drugs, blood components, and flow on the endothelial lining. Endothelial insult may change the properties of the endothelium to promote atherogenesis. The advent of improved methods for culturing endothelial cells affords a unique opportunity to study these cells under controlled conditions of chemical environment and flow in the newly designed flow chamber.

PROPOSED COURSE: The adoption of an in vitro system with a significantly lower proportion of culture medium volume to exposed endothelium area has a higher priority within the present workscope. Any given cell on the surface of a microcarrier bead within the viscometer will not experience a shear stress which is constant in magnitude or direction. The significance of the time dependence of the stress, the consequences of secondary flows, bead-bead encounters, and possible nonuniform distribution of beads within the gap region are among the aspects to be considered.

PUBLICATION

Lutz, R.J., Hsu, L., Menawat, A., Zrubek, J., Edwards, K., "Comparison of Steady and Pulsatile Flow in a Double Branching Arterial Model." J. Biomechanics, 16: 753-766, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10136-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Imaging in Positron-Emission Tomography

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

J. R. Ellis Expert BEIB/DRS

Other Personnel:
R. M. Kessler NM/CC
M. Eden   Chief BEIB/DRS

COOPERATING UNITS (if any)
NM/CC

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Office of the Chief

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	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.)

Positron-Emission Tomography (PET) and emission computed axial tomography (ECAT) offer unprecedented opportunities to visualize and measure in-vivo organ metabolism. Our current interest centers on the brain, perhaps the least accessible region of the body to non-invasive diagnostic probes.

One desirable goal of image processing in this field of interest is determination of volumetric metabolic activity from collections of scan plane data. Our work on data spreading and attenuation due to finite object size (partial voluming) has made specification of object recovery as a function of system resolution possible. Thus, axial sampling can be chosen to maintain visibility of, or activity recovery from, objects of a selected minimum size through a set of scan planes.

With such choices. it is possible to relate scan plane data to a three dimensional model of the brain robustly. Use of such a model can allow meaningful comparisons of data taken from subjects with different orientations and locations relative to the PET or ECAT frame of reference.

Another, more ambitious, goal is to make a dynamic model of brain activity. Then, one can use time-series PET or ECAT data to estimate biological quantities in functional compartments. Further analysis allows estimates of the parameters of the kinetic rate equations relating them.

Work has been underway on these topics.

ZO1 RS 10136-03 BEI

OBJECTIVES: There are currently four main objectives: 1) Find a good model for the system excitation-response function. 2) Determine limits of diagnostic performance of PET systems from their imaging parameters. 3) Develop a good model for geometrical description of significant regions of the brain. 4) Develop useful biochemical kinetic functional models of the brain.

METHODS EMPLOYED: Measurements of system response to positron emitting phantoms are made. Models are analyzed mathematically. The point spread function has been modelled as a normalized Gaussian with Full Width at Half Maximum (FWHM) as a single free parameter. Values of relevant parameters, such as pixel counts, associated with the models are calcu lated and compared with measurements made on phantoms.

Predictions of system response are made from models, especially image parameters expected for particular brain structures. Finally, these predictions are compared with experiments or with data obtained in the normal course of clinical investigation.

MAJOR FINDINGS: The PET and ECAT system responses are generally accepted to be Gaussian. An analytical solution was found for spherical regions of a three-dimensional Gaussian point spread function, both on- and off-center.

This Gaussian model has been found to match phantom measurements made by Dr. R. Kessler on the Nuclear Medicine ECAT machine very well. The solution has also been found to match several well known "rules of thumb" for PET system performances well. This essentially satisfies objective number 1 above.

A paper describing phantom results, analytical results, and comparisons between experiment and theory has been published.

SIGNIFICANCE: The accepted model can be used for detailed calculation and prediction with minimal computational load. Phantom results can be generalized and compared much more effectively than previously thought.

Axial response curves calculated here have been used to specify axial sampling criteria to guarantee the appearance of structures of a selected size and activity on at least one of a set of scans.

PROPOSED COURSE: We plan to use existing results to evaluate the capabilities of existing and proposed systems to image selected combinations of physiological and functional structures. It is also desirable to make some of the complexities and important variables of these systems better known to the community of users.

A three-dimensional model of brain structure has been proposed to make more robust use of information from sets of tomographic slices. Functional biochemical modelling is also planned. The interaction between the functional and 3-D models should give intriguing insights into in-vivo brain function and morphology.

PUBLICATIONS

R. M. Kessler, J. R. Ellis. and M. Eden. "Analysis of Emission Tomographic Scan Data: Limitations Imposed by Resolution and Background". Journal of Computer Assisted Tomography, vol. 8, pp. 514-522 (1984).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10137-03 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Processing of Electron Microscope Images

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

J. R. Ellis, Expert, BEIB/DRS
T.G. Pun, Visiting Fellow, BEIB/DRS
C.E. Fiori, Physical Scientist BEIB/DRS
R.D. Leapman, Visiting Scientist. BEIB/DRS
C.R. Swyt, Physicist. BEIB/DRS
G. Hook, Staff Fellow, BEIB/DRS

COOPERATING UNITS *(if any)*

CSL/DCRT

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Office of the Chief

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

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

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

An overall goal of the DRS-BEIB/DCRT-CSL Electron Beam Imaging and Microspectroscopy (EBIM) Project is to obtain chemical elemental maps of biological samples with resolution in the sub-micron range which are subjectively satisfying and objectively meaningful. Our image processing work associated with this project has developed with essentially four foci consistent with this broad target area: 1) Quality enhancement, 2) pixel value and uncertainty estimation for normalized functions of the raw data, 3) controlled parameter image generation, and 4) provision of a sufficiently friendly user interface for a user to employ tools developed to satisfy the first three points.

Our definition of quality enhancement for a set of images with dissimilar characteristics includes making them compatible for comparison using overlays and other forms of composition by expansion, contraction and smoothing. It also involves construction of collages or montages from several images, background subtraction, region edge enhancement, and matching of desired signal ranges to the eye's response.

Significant new analytical and simulation results have been obtained concerning the statistical variations to be expected for EEL elemental edges and X-Ray elemental peak-to-background ratios. Work on improved pixel and region value estimation for normalized functions is in progress.

Synthetic images with controlled parameters can be used very effectively in resolving questions of object visibility and artifact generation in this project.

OTHER PERSONNEL:

K. E. Gorlen   CSL/DCRT
S. Orlow   Consultant   CSL/DCRT
J. Del Priore   CSL/DCRT

OBJECTIVES: Our image processing work associated with this project has has essentially four goals for processing chemical elemental maps of biological samples with resolution in the sub-micron range which are subjectively satisfying and objectively meaningful. These are the following: overall image quality enhancement, estimation of value and uncertainty of functions of raw data for local pixels and regions, plausible artifact recognition, and provision of a sufficiently friendly user interface to enable a user to employ tools developed to meet the goals of the first three points.

METHODS EMPLOYED: Enhancement of images includes controlled loading, expansion, and smoothing of images derived from the available signals to allow visual overlay and separate comparisons of data having varied sampling lattices, relative registration, and statistical properties. The wide variety of signals include electron energy loss (EEL) spectra, X-Ray Emission spectra from energy and wavelength dispersive detectors, and bright field and dark field current from the Hitachi 7000 STEM; as well as signals from the Cameca Microprobe.

Further capabilities include copying data to different locations, display lattice compression, image arithmetic, and exponential and differential transforms. These allow construction of collages or montages from several images, background subtraction, matching of desired signal ranges to the eye's response and region edge enhancement.

A set of programs has been developed which can generate synthetic images with controlled parameters that are virtually indistinguishable from real images with similar parameters. They have been used very effectively in resolving questions of object visibility and artifact generation.

Our programs emphasize parameter control and repeatability. The image processing software supplied by CSL/DCRT emphasizes interaction and speed.

MAJOR FINDINGS: Analytical and simulation results concerning the uncertainties of general case measurements have been applied to specific EEL and X-Ray signals with background correction. These results, in conjunction with appropriate synthetic image simulations, have given significant new insight into the statistical variations to be expected for EEL elemental edges and X-Ray peak-to-background elemental maps. Specifically, we have found that under 'normal' experimental conditions the errors are considerably greater than expected, and that background estimation and extrapolation are the principal sources of error.

Work on object visibility, especially in a color display environment, is very important in this microscopic world where human visual intuition is relatively unreliable.

This is especially true where powerful image processing tools, such as those mentioned above and the complementary set implemented on this system by personnel from CSL/DCRT, make it possible to generate very plausible artifacts from data not having the desired structure.

PROPOSED COURSE: We will continue to provide consultative expertise for this project. In the past we have put significant effort into specific software designed to implement ideas understood by other workers on this project. Our substantial efforts will be more general and theoretical in the future. Such a plan is consistent with the orientation of the personnel on this project and with the resources available for image acquisition and processing.

PUBLICATIONS

T. Pun, J. R. Ellis, and M. Eden. "Optimized Acquisition Parameters and Statistical Detection Limit in Quantitative EELS", Accepted for publication in Journal of Microscopy.

T. Pun and J. R. Ellis. "Application of Simulated Poisson Statistical Processes to STEM Imaging", Submitted for publication.

T. Pun, J. R. Ellis, and M. Eden. "Weighted Least Squares Estimation of Background in EELS Imaging", Accepted for publication in Journal of Microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10143-03 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Interaction of Body Temperature and Sleep Rhythms

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

T.L. Talbot, ACES, BEIB, DRS
Wallace Mendelson, Psychobiology, NIMH
M. MacCollum ACES, BEIB, DRS

COOPERATING UNITS *(if any)*

Psychobiology Lab-NIMH

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

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

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

Clinical evidence suggests a correlation between core body temperature and the frequency of sleep episodes. An artificial thermoregulatory system is completed which will enable core body temperature manipulation. This device permits the evaluation of the efficacy of thermal regulation in the treatment of sleep disorders. Clinical trials are now performed on a regular basis. Simultaneous sleep recordings are obtained during both a non-manipulated and manipulated 24 hour period.

Clinical studies are now underway and the data obtained so far suggests no interaction between sleep rhythms and body temperature. More studies are being performed to substantiate conclusively these findings.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10146-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

S. B. Leighton, Mechanical Engineer, BEIB, DRS,
Marston Linehan, M.D., DCT, NCI
Stevenen Scoog, M.D. WRAMC

COOPERATING UNITS *(if any)*
DCT, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

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

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

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

A number of techniques are used for treating urinary incontinence, including a
number of artificial prosthetic sphincters. The present work concerns an
entirely intraurethral artificial sphincter that can be implanted without
surgery. Concepts are being explored which would allow the device to be used
in situations in which surgery is contraindicated, and would also presumeably
lower the cost. The valve would be appropriately matched to urethral
dimensions, pressures, and flowrates. The valve would be potentially useful
in cases of nonopening normal valves as well as in cases of non-closing
valves.

OBJECTIVES: To develop and test and artificial prosthetic urethral sphincter that can be introduced non-surgically into the urethra.

METHODS: A large scale model of an artificial valve with appropriate pressure flow characteristics has been designed. It will be bench tested and miniaturized. After thorough and successful bench testing, clinical tests will be designed to verify function and safety.

MAJOR FINDINGS: A physical noon has been built and tested. A prototype has been designed.

SIGNIFICANCE: Urinary incontinence affects about 10% of the population over 65, and sigtnificant numbers of younger people due4 to paralysis, infection, and other causes. A simpler, less expensive treatment technique would presumeably be useful for at least a reasonable fraction of these cases.

PROPOSED COURSE: Design and bench testing of various valve designs will proceed. Clinical tests will be designed to test proper function and safe operation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10147-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

Aydin Tozeren, Visiting Scientist, BEIB, DRS

COOPERATING UNITS *(if any)*
Bioengineering Institute, Columbia University, N.Y.
Shu Chien, M.D., Ph.D.
Richard Skalak, Ph.D., K.L.P. Sung, Ph.D.

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.30	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.)*

The erythrocyte membrane is modelled as a two dimensional viscoelastic continuum that evolves under the application of stress. The present analysis on the erythrocyte membrane is motivated by the recent development of knowledge on its molecular structure and by its complex behavior exhibited in dynamic micropipette testing and in tank treading during shear flow. The proposed constitutive equations have the form similar to that of a two dimensional Kelvin model with a constant area condition. However, the membrane viscosity is made to depend on the rate of strain and the elastic strain tensor is measured from the evolving preferred configuration.

The constitutive equations proposed in the present analysis explain in a consistent manner the data on both the deformation and recovery phases of the micropipette experiment. The rheological equations of the present study are applied in a later section to the analysis of a plane membrane deformation that is quantitatively similar to the tank-treading motion of the erythrocytes in a shear field. The computations yield useful information on how the membrane viscosity becomes a more dominant feature in tank-treading motion. The present model reflects the microstructure of the erythrocyte membrane. A membrane composed of a lipid bilayer may be idealized as a viscous membrane with a constant area condition. The network of protein molecules embedded in and attached to the lipid bilayer of the erythrocyte membrane serves as a storage medium for the elastic strain in the membrane. The molecular organization of this network evolves continuously during a prolonged deformation. The material constants appearing in the proposed constitutive equations may be useful indicators of the biochemical state of the membrane in health and disease.

OBJECTIVE: To develop a continuum model of erythrocyte membrane based on the structure of its molecular organization.

MAJOR FINDINGS: The constitutive equations proposed in the present analysis explain in a consistent manner the data on both the deformation and recovery phases of micropipette experiments. The rheological equations of the present study are applied to the analysis of a plane membrane deformation that is quantitatively similar to the tank treading motion of the erythrocytes in a shear field. The computations yield useful information on how the membrane viscosity becomes a more dominant feature in tank treading motion. Present theory takes into account the elasticity of the network of protein macromolecules embedded in the lipid bilayer. The short time constant of membrane deformation observed in micropipette experiments is considered to be due to the structure of the lipid bilayer. The longer time constant observed in a prolonged deformation is explained by the evolution of the molecular organization of the protein network.

SIGNIFICANCE: Membrane viscoelasticity plays an important role in various cell deformation processes such as cell division, response of endothelial cells to stress and blood rheology. Viscoelastic properties of the erythrocyte membrane are of particular interest for the study of blood in health and disease.

PUBLICATIONS

1. A Tozeren, R. Skalak, B. Fedorciw, K.L.P. Sung, and S. Chien: constitutive equations of erythrocyte membrane incorporating evolving preferred configuration. Biophys. J. 45: 541-549, 1984.

2. H. Tozeren, S. Chien and A. Tozeren: Estimation of viscous dissipation inside an erythrocyte during aspirational entry into a micropipette. Biophys. J. 45: 1179-1189, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10148-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of the Coupling Between Left Ventricle and Vascular System

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

Aydin Tozeren, Visiting Scientist, BEIB, DRS

COOPERATING UNITS *(if any)*
Dr. Shu Chien, Department of Physiology, Columbia University, New York

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.30	0.30	

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

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

The aim of this study is to develop equations governing left ventricular pressure and volume during a cardiac cycle. It is assumed that the stress in the myocardium is composed of an isotropic tissue pressure term and fiber tension. Fiber tension T depends on the stretching and rate of stretching of cardiac fibers as well as an internal variable c that describes the degree of electrochemical activation of the muscle fiber. Using mechancial equations of equilibrium and the constitutive equations mentioned above, a time dependent pressure-volume relation (P-V) is obtained for the left ventricle. Hydraulic characteristics of the large arteries are modelled by a three parameter windkessel model. It is assumed that the aortic pressure is equal to the ventricular pressure P when the aortic valve is open. Mechanical events of the cardiac cycle are considered as a function of heart rate by changing one of the following parameters: end diastolic volume EDV, contractility c_0, time constant of contraction w, and resistance R and compliance C of the large arteries. For given EDV, an increase in HR leads to increases of both systolic pressure P_s and diastolic pressure P_d with a decrease of pulse pressure, and a decrease of ejection fraction, and a biphasic change in cardiac output CO, which increases at first to reach a maximum and then decreases. When w is doubled, the rate of pressure rise and maximum flow rate are approximately doubled, but there is very little change in stroke volume SV, P_s and P_d for moderate HR. At higher HR levels, cardiac output CO increases with w because of the lengthening of diastolic phase; aortic valve opens at an earlier time. An increase in c_0 increases CO as well as P_s and P_d, regardless of HR level. SV and CO vary inversely with R and directly with the slope of the isovolumetric P-V curve.

OBJECTIVES: To develop equations govening ventricular volume and pressure during a cardiac cycle and to study the interaction of the left ventricle with the vascular system.

METHODS EMPLOYED: Large deformation analysis of mathematical theory of elasticity is employed to study the deformation of the left ventricle during a cardiac cycle. Myocardium is idealized as a fiber reinforced continuum, and fiber tension is assumed to depend on stretching and rate of stretching of the fibers as well as on an internal variable c describing the degree of activation. Computations involve the solution of a set of nonlinear differential equations with free boundary conditions. An iterative procedure is developed to determine the time at which aortic valve opens as a function of the heart rate.

SIGNIFICANCE: The proposed model of the cardiac cycle may be helpful in the assessment of the influence of drugs on the various components of the cardiovascular system. The proposed model is also useful in describing explicitly the dependence of various contractility indices of the heart on the preload and afterload.

MAJOR FINDINGS: Functional relationships have been explored between the parameters identifying the contractility of the left ventricle, the resistance and compliance of the vascular system and experimentally observed quantities such as pressure, flow rate, cardiac output, etc. Cardiac output is predicted to decrease significantly with increasing vascular resistance. An increase in the slope of the isovolumetric pressure-volume curve has the opposite influence on cardiac output provided that the end-diastolic volume remains the same. The theory also indicates that the cardiac output increases in proportion with increasing end diastolic volume. These findings are in close agreement with the experimental results of Sagawa and his coworkers. For given EDV, an increase in HR leads to increases of both systolic pressure P_s and diastolic pressure P_d with a decrease of pulse pressure, and a decrease of ejection fraction. When w is doubled, the rate of pressure rise and maximum flow rate are approximately doubled, but there is very little change in stroke volume SV P_s and P_{dE} for moderate HR. At higher HR levels, CO increases with w because of the lengthening of diastolic phase; aortic valve opens at an earlier time.

PROPOSED COURSE: The present analysis is considered to be a first step in mathematical modeling of the cardiovascular system in health and disease. The determination of the end-diastolic volume as part of the solution and the analysis of the influences of exercise and various drugs on the cardiovascular system are subjects of further investigation.

PUBLICATIONS

Tozeren, A.. Elastic properties of large arteries and their influence on the cardiovascular system. J., Biomech. Engin.a 106:182-185, 1984

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10149-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Constitutive Equations of Skeletal Muscle Based on Cross-Bridge Mechanism

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

Aydin Tozeren, Visiting Scientist,   BEIB
Mark Shoenberg, Senior Investigator, LPB, NIAID
Evan Eisenberg, Section Chief,   IRLC, NHLBI

COOPERATING UNITS *(if any)*
LPB, NIAID
IRLC. NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

TOTAL MAN-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.)*
Statistical mechanics of cross-bridge action are considered in order to develop constitutive equations that express fiber tension as a function of degree of activation and time history of speed of contraction. The kinetic equation of A.F. Huxley (Prog. Biophys. Mol. biol. 7:255,1957) is generalized to apply to the partially activated state. The rate parameters of attachment and detachment and cross-bridge compliance are assumed to be step functions of extension, x, with a finite number of discontinuities. This assumption enables integration of the kinetic equation and its moments with respect to x analytically resulting in equations where x has been eliminated. When the constants in the rate parameters and the force function are chosen such that Hill's force-velocity relation and features of the transient kinetic and tension data can be fitted, the resulting cross-bridge mechanism is quite similar to the one proposed by Podolsky and co-workers (Proc. Natl. Acad. Sci. USA, 64:504, 1969). Because the derived constitutive equations simplify mathematical analysis, they enable the evaluation of the influence of various cross-bridge parameters on the mechanical behavior of muscle fibers. For example, (ix) Instantaneous elastic response $(T_0 - T_1)$ and the magnitude of rapid recovery $(T_2 - T_1)$ after a step length change can be explained well when the rate of attachment is assumed high for positive x. In that case T_2 corresponds to the force generated by cross-bridges in the region of negative x; (ii) Kinetic transients occur as a result of the jumps that exist in the distribution of attached cross-bridges during the isometric state. Because of the hyperbolic nature of the kinetic equation, these jumps propagate in the -x direction causing rapid changes in the speed of contraction. This study is further extended to take into account of multiple action sites and cross-bridge interaction. In the simplest case (transient response after a step length change) the model reduces to set of 14 ordinary differential equations.

OBJECTIVES: To develop a continuum theory of striated muscle contraction incorporating the kinetics of cross-bridge action during relaxed, rigor and activated states.

METHODS EMPLOYED: As a first step muscle tissue is idealized as a fiber reinforced continuum. Fiber tension is defined as the total fiber force divided by the cross-sectional area of the fibers. Fiber tension depends of the stretching and rate of stretching as well as the degree of electrochemical activation. A set of differential equations have been developed to express fiber tension in terms of the variables mentioned above. These equations have been solved numerically to compare the predictions of the model with the existing data in the literature concerning transient tension, isometric and isotonic tension tests.

SIGNIFICANCE: A continuum theory of muscle contraction is essential in the mechanical analysis of some biological organs such as those in the cardiovascular, respiratory and urinary systems. The theory may also provide a new insight into the understanding of muscle contraction at the molecular level.

MAJOR FINDINGS: When the constants in the rate parameters and the force function are chosen such that Hill's force-velocity relation and features of the transient kinetic and tension data can be fitted, the resulting cross bridge mechanism is quite similar to the one proposed by Podolsky and coworkers (Proc. Natl. Acad. Sci. USA, 64:504, 1969). Because the derived constitutive equations simplify mathematical analysis, they enable the evaluation of the influence of various cross-bridge parameters on the mechanical behavior of muscle fibers. For example (i) Instantaneous elastic response (T_0-T_1) and the magnitude of rapid recovery (T_2-T_1) after a step length change can be explained well when the rate of attachment is assumed high for positive x. (ii) Kinetic transients occur as a result of the jumps that exist in the distribution of attached cross bridges during the isometric state. Because of the hyperbolic nature of the kinetic equation, these jumps propagate in the -x direction causing rapid changes in the speed of contraction.

PROPOSED COURSE: To continue the analysis of transient tension experiments by using kinetic models that take into account the presence of two heads at every cross-bridge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10151-02 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Nuclear Magnetic Resonance Imaging

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

Dr. David I. Hoult, Physical Scientist, BEIB, DRS

Other personnel:
Dr. Ching-Nien Chen, Expert
Dr. Victor J. Sank, Expert
Dr. L. Kyle Hedges, Staff fellow
Mr. Michael S. Silver, Student

COOPERATING UNITS *(if any)*

Department of Radiology, Clinical Center

LAB/BRANCH

NMR Imaging Laboratory, Biomedical Engineering and Instrumentation

SECTION

Office of the Chief

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.8	0.4

CHECK APPROPRIATE BOX(ES)

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

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

In the past year, effort in nuclear magnetic resonance imaging has been expanded on several research fronts.

A.   Following the failure of the magnet manufacturer to install successfully the clinical NMR imaging system in the Department of Radiology. Clinical Center, the research group took over the homogenizing of the magnetic field necessary for clinical imaging, and, using a new technique, achieved the specified homogeneity, thereby enabling the Radiology department to produce high quality images.

B. Considerable controversy exists within the NMR image community over the choice of an optimal field strength for imaging. Bench methods were developed for accurately assessing the signal-to-noise ratio from, and radio-frequency power deposition in, the human body at any frequency used for imaging. It is hoped these results will help resolve the matter.

C. A pulse for highly selective spin population inversion has been discovered. The result is of considerable experimental and theoretical importance for it represents only the second known analytical solution of the non-linear differential equations governing the motion of an NMR spin system. Further, above a critical threshold, the inversion is independent of applied power.

D. A so-called "quadrature" probe system has been invented which reduced radio-frequency power dissipation in the body by almost a factor of 2 while improving signal-to-noise ratio by almost 40%.

E. The electronic upper frequency limit for adult head imaging has been pushed from 84 MHz to 130 MHz with the aid of a novel phased-array receiving coil.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10153-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Position Sensor for Computer Modeling

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

H. E. Cascio, Electronic Engineer, BEIB, DRS
P. Smith, Visiting Scientist, BEIB, DRS
S. Leighton, Mechanical Engineer, BEIB, DRS
R. Feldmann, CCB DCRT

COOPERATING UNITS *(if any)*
CCB, DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
National Institutes of Health. Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.45	0.15	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.)*

For the study of macromolecular structure and of molecular interactions. the use of a computer generated display of the molecule is a powerful interpretive tool. In particular, the ability to be able to manipulate in space, i.e. to rotate and translate the molecule(s) to any desired orientation enhances the usefulness of the device. A further degree of sophistication is to provide a feedback mechanism giving an indication of the repulsive or attractive forces (Van der Waals interactions) present as two molecules are fitted together or as a single molecule is twisted to a new conformation. A joy stick apparatus has been designed to provide these features. Two molecules or part of the same molecule can then be rotated and translated in any direction. With each new position, the computer program re-evaluates (on a one millisecond timescale) the appropriate forces to provide the investigator with a feel of the total forces involved.

OBJECTIVES: To produce a device as an instructive aid in the study of macromolecular structures and of molecular interactions; and also to provide the investigator with the feel of repulsive or attractive forces between molecules.

METHODS EMPLOYED: An Apollo computer model DPS80 will be interfaced to a control gantry. The control gantry, placed in front of a display monitor, will have two joy sticks positioned side by side and supported in space with eighteen connecting lines (nine lines per joy stick), with each line terminated on a shaft of one of eighteen motors. A joy stick represents a molecule in space and any change in the position of the joy stick will cause the computer program to cause a corresponding position change to the displayed molecule. Each connecting line to the joy stick will be appropriately placed to provide a rotational and translational information to the computer. The computer program will calculate and then will initiate the proper current to the appropriate motor, thereby exerting a force on the connecting lines of the joy stick. This exerted force on the joy stick provides the investigator with the feel of molecular forces involved.

Two electronic units will contain the interface and control circuitry for the system. The control circuitry will sense the displacement of the joy stick by counting the pulses generated by the motor shift encoders and supply the motor drive current as determined by the eighteen D/A converters.

SIGNIFICANCE: The study of macromolecular and of molecular interaction becomes more informative while observing the molecular orientation and feeling the appropriate forces involved.

PROPOSED COURSE: Test and evaluate the system electronics as fabrication is completed. Test and evaluate the computer program and interface circuitry. Assist in the development of the system software.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10155-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

H.E. Cascio, Electronic Engineer, BEIB, DRS
P. Smith, Visiting Scientist, BEIB, DRS
R. Balaban, Staff Fellow, KE NHLBI

COOPERATING UNITS *(if any)*
KE, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2

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

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

For one dimensional spectral analysis of the fluroescent properties of kidney microtubules, a vidicon detector has been mounted at the image plane of a microscope. The Princeton Applied Research model 1216/1254 detector is ideally suited for this application in that the scanning parameters of the vidicon are readily adjustable to provide the optimum configuration for the viewed image. However, for initial sample set up, and for two dimensional data collection, the lack of a standard video mode is a limitation. A modification has been made to this detector to produce an optional standard composite video signal. The existing preamplifier in the model 1254 detector is a charge sensitive preamplifier which integrates the video signal across each horizontal scan line. This integration causes a loss of resolution in the horizontal direction, when used in the standard video mode. A fast video preamplifier was designed and placed inside the preamplifier housing with the original preamplifier. A command from the computer terminal selects the scanning mode and connects the proper preamplifier into the system.

OBJECTIVES: To produce a detector system for a fluorescence microscope capable of providing both a noomal output suitable for a display monitor and a spatial-wavelength output suitable for determing fluorescent intensity changes as a function of position and time.

METHODS EMPLOYED: A model 1254 B silicon intensified target vidicon detector and a model 1216 controller, both manufactured by Princeton Applied Research, have been interfaced to a Digital Equipment Corporation DECLAB-11/MNC minicomputer. A FORTRAN program has been developed to permit control of the scanning parameters of the vidicon and collect the output data. The program is interactive and allows for changed of parameters with error checking to reduce the possibility of damage to the detector by overscanning a small target area.

The vidicon has been mounted on the fluorescence microscope to permit either normal observation of the image plane or observation of a slit after passing through a monochromator.

A modification has been made to the vidicon controller and an electronic circuit constructed to produce a composite video signal suitable for display monitors or for storage on video tape recorders. Due to the charge coupled integrating preamplifier used in the 1254 detector. an image of low horizontal resolution (about 100 television lines) was obtained. To improve the picture resolution, a fast low noise FET preamplifier was constructed and mounted inside the preamplifier compartment directly above the existing preamplifier. A computer controlled relay, mounted between the two preamplifiers. switches the vidicon output from the original non-standard scan system to the standard video (including the fast preamplifier) scan. To increase the dynamic range of the imaging system, a video processing amplifier was constructed and included in the system. The processing amplifier permits the researcher to adjust the video output signal to a standard video level from the wide range of brightness levels that he may encounter.

SIGNIFICANCE: The ability to switch from a normal composite video signal to a programmed non-standard video scan provides an extra level of sophistication for microscope work. The actual object seen by the vidicon can be observed and aligned to an optimum configuration before switching to the wavelength analysis mode. This is expected to be highly attactive to workers in this field.

PROPOSED COURSE: To establish a satisfactory high resolution image on the monitor display. To analyze the wavelength-position data for accuracy. To provide for time resolved information within the limits of the detector readout time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10156-02 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Differential Scanning Calorimeter

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

C.P. Mudd, Biomedical Engineer, ACES, BEIB, DRS
T. Talbot, Mechanical Engineer, ACES. BEIB, DRS
R.L. Berger, Physicist, LTD, NHLBI
P.D. Ross, Physical Chemist A LMB, NIADDKD

COOPERATING UNITS *(if any)*

LTD, NHLBI
A LMB, NIADDKD

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

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

The transfer of sensor design and modelling techniques to the field of
differential scanning calorimetry from earlier work in differential heat
conduction calorimeter has resulted in two models of differential scanning
calorimeters which should satisfy the sensitivity and scan rate requirements.
One system uses two sensors and takes the difference electronically while the
other system used only one sensor which operates as a null detector in two
match thermal channels.

OBJECTIVES: To scan or change the temperature of a sample and observe small changes in the heat content of the sample which are attributed to physical changes within the sample (e.g. conformation changes in proteins) and not the scanning rate.

SIGNIFICANCE: The increasing use of DSC (differential scanning calorimetry) in biological work is an indication of its usefulness in determining the energy levels involved in the conformation changes in large biological molecules. The sensitivity of current instruments limits their use to applications involving relatively large amounts of heat. If the sensor design developed for the differential batch calorimeter can be successfully implemented into a DSC, the sensitivity should increase by a least a factor of 10.

PROPOSED COURSE: Both of the proposed models offer the promise of increasing the sensitivity. Each model has its own advantages and disadvantages. The single sensor design does not require careful matching of amplifier gains and sensor sensitivities but does require a more carefully matched thermal pathway for the heat flow resulting from the scanning. The two sensor design uses a simple thermal pathway which is easily balanced but requires careful matching of the sensors and amplifier gains.

The next phase will involve implementing these two designs into hardware and evaluating them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10157-02 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Temperature Controlled Chamber for X-ray Diffraction Specimens

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

C.P. Mudd Biomedical Engineer, ACES, BEIB,DRS
H.W. Tipton Mechanical Engineering Tech. ACES, BEIB, DRS
A.V. Parsigian, Research CR,PSL
B.K. Lee, Researcher CR,PSL

COOPERATING UNITS *(if any)*

DCRT, CR-PSL

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Biomedical Engineering and Instrumentation

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Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

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

A proto-type chamber was constructed of lexan with a 1 cm diameter beryllium window to allow the x-ray beam to enter the chamber. The specimen is mounted in a holder next to the beryllium window. The edge of the holder is mounted to the lexan case to preserve the vacuum seal while the center area of the holder is mounted to a Peltier solid state heat pump. The other side of the heat pump is connected to a heat sink. A thermistor mounted next to the specimen in the holder controls the heat pump to keep the specimen at the set temperature. The film plate is mounted inside the chamber on an adjustable bracket. The chamber will hold a vacuum of 0.01 atm while the controller keeps the specimen at any temperature between 4°C and 70°C with a stability of + 0.2°C.

OBJECTIVES: There are two primary objectives:

(1) To reduce the temperature-motion-artifacts of the sample which tend to blur the x-ray image.

(2) To reduce the scattering of the x-rays caused by the air molecules between the sample and the film plate.

SIGNIFICANCE: The control of the sample temperature and reduction in the air scattering should yield a dramatic increase in the reproducibility and sharpness of the diffraction images. In this design there is only one beryllium window between the sample and the film plate.

PROPOSED COURSE: The prototype is now in operation in the investigators lab and is undergoing evolution. No additional development anticipated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10158-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

M.A. MacCollum, Mechanical Engineer, ACES, BEIB, DRS,
Greg Ribakove, M.D. Heart Surgery, NHLBI

COOPERATING UNITS *(if any)*
Surgery Branch, NHLBI

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	.1	1.1

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

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

An apparatus for perfusion of isolated rat hearts has been developed which circulates a buffered blood analog. This device is used to investigate biochemical and physiological parameters of the heart. including blood pressure, pulse pressure, cardiac output, tissue pH and others. The system allows investigators to induce temporary cardioplegia, during which pharamacologic agents may be introduced into the modes of operation: working heart, ischemia (both warm and cold) and Langendorf aortic perfusion. Comparing the pre- and post-plegic parameters allow the investigators to assess the ability of certain drugs and procedures to sustain cellular life through ischemic periods.

The results gained from the isolated heart experiments will be tested in large animals in vivo, using the same parameters along with animal survival after surgery.

Engineering refinements in the perfusion circuit described above are completed and the system is fully operational.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10159-03 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathophysiology of Cachexia in Sarcoma Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*
R.Corsey Electronic Engineer BEIB, DRS

Others:
J.A. Norton Section Chief SURG,NCI
J.F. Moley Research Associate SURG, NCI

COOPERATING UNITS *(if any)*
Anesthesiology Service, Clinical Center, NIH (D.E. Lees)
Nutrition Department, Clinical Center, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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

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

The first phase of this study has demonstrated that patients with metastatic disease have higher levels of energy expenditure than controls, and patients with extremity sarcomas have slightly higher levels of energy expenditure than controls. The next phase will determine if glucose oxidation, whole body protein turnover and potassium 40 levels are different in preoperative sarcoma patients from controls. Sarcomas are useful for understanding the pathophysiology of cachexia in that they usually do not alter the patient's ability to aliment himself, they are metabolically active and patients bearing them have been shown to have an increase in glucose consumption across tumor - bearing limbs. Stable isotopes in the forms of 13C-glucose and 15N-glycine and 13C-leucine are not radioactive and are very safe to administer. The study will determine if any difference demonstrated by measurements of isotopes can be correlated with tumor size or growth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10162-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Wound Healing: Biology and Rheology

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

Thomas L. Talbot, MS, ACES, BEIB
Walter T. Lawrence M.D., C. Surgery, NCI
Lawrence E. Thibault, ScD. Bioengineering Dept, Univ. of Pa

COOPERATING UNITS *(if any)*
DRS, NCI, University of Pa, Philadelphia, Pa

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1	0.2

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

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

Preliminary studies have been completed with swine models. These studies involved stamping an indelible gird (10cmx10cm) on the skin of the swine, and the excision of a 1 cm by 10 cm strip of the skin out of the grid area, and finally approximating the incision edges with silk sutures. Photographs of the grid were taken before excision, after excision, and after suturing. These photographs will be analyzed to determine the impressed strain on the wound closure and eventually relate this information to wound breaking strength (WBS).

Studies have been completed using a rat model which relate biologic and pharmocologic interventions to WBS. Certain groups were pharmacologically intervened during the wound healing process. Significant decrease in WBS was observed in these groups as compared to control groups. Further studies will include the comparison of tumor bearing group to control groups.

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Magnetoencephalographic Localization of Foci of Neurologic Activity

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

P.D. Smith   Visiting Scientist   BEIB, DRS
R.F. Bonner   Physicist   BEIB, DRS
W.S. Friauf   Section Chief   BEIB, DRS
S. Sato   Sr. Investigator   EB NINCDS
R. Porter   Chief   EB NINCDS
M. Nisenoff   Chief   NRL

COOPERATING UNITS (if any)
Epilepsy Branch, NINCDS; Naval Research Laboratory

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD   20205

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2

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

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

A continued collaboration with the Naval Research Laboratories has enabled a SQUID magnetometer to be used to study inter-ictal spike discharges in epileptic patients. Correlation of the MEG activity and the corresponding EEG signal for these discharges permits signal averaging of spikes associated with the same neurological event. These measurements allow a magnetic field map at the surface of the scalp to be obtained from which a prediction of the source of the epileptic focus can be made. Computer and electronic processing techniques have been developed to analyze these MEG and EEG signals with the aim of producing an efficient method of localizing the sources of the epileptic discharges in a selective manner. Several patients have been scanned using these techniques. To enhance the collection of MEG data associated with a single spike discharge, a seven channel array detector has been specified.

OBJECTIVE: The development of a SQUID magnetic field gradiometer and methodology for its use to localize the three dimensional coordinates of the foci of neurologic activity in the brain particularly those associated with epileptic discharges.

METHODS EMPLOYED: Through collaboration with the Naval Research Laboratory, a single channel second order magnetic field gradiometer has been used to study the MEG signals perpendicular to the surface of the scalp arising from spike discharges in epileptic patients. Simultaneous EEG scalp activity has been monitored using a full set of electrodes. Both MEG and EEG signals were recorded for typically 2 hours per session as the SQUID sensor was positioned across the surface of the scalp. A non-magnetic gantry and bed were designed and constructed to facilitate this positioning of the sensor with as little inconvenience to the patient as possible. Correlation of the MEG and EEG signals was performed off-line using both a computer and a digital processing oscilloscope. Based on these analyses, real time processing of the MEG and EEG signal has been instigated.

MAJOR FINDINGS: In trying to localize the source of neurologic activity associated with an epileptic spike discharge, the major limitation in producing a magnetic field map required to achieve this goal is the non-reproducible nature of the neurological event being measured. In fact, there are two aspects to this non-reproducibility: firstly, the signal derived from any given inter-ictal discharge is variable, due either to the recruitment of a variable number of neurons cooperating to produce the spike, or alternatively, to a change in orientation within a neuron grouping. A second factor is that in many patients there exist multiple sites of neurological activity each of which elicit an EEG and MEG signal. For these considerations, and based on findings described below using a single sensor, a multiple channel array has been specified to permit simultaneous position measurements of the MEG signal associated with an individual spike discharge. This array will comprise six 1.5 cm diameter coils arranged in a circle with another single coil at the center; the spacing of the center coil to any of the coils arranged in the circle is 3 cm, and the planes and the coils are arranged on the 10 cm mean radius of the skull. Noise cancellation channels are also to be provided. This detector is at the limit of current sensitivities and represents the maximum number of channels to which present dewar technology can accommodate.

The single channel measurements have been made with the SQUID sensor placed perpendicular and as close as possible to the scalp. In all cases a set of EEG electrodes were present and used as markers of position for the SQUID sensor. A major effort has been to develop a methodology, which is aimed at reducing the time required to scan a patient, to analyze the data so that spikes arising from varied locations can be distinguished; this permits signal averaging techniques to be applied to signals arising from the same location. Cross-correlation and auto correlation techniques were applied to the MEG and selected EEG signals using both a mainframe computer and a digital processing oscilloscope. The main drawback of this type of analysis is that it cannot be performed in real time and thus the investigator is not able to make on-the-spot decisions on the most appropriate head scan to pursue. Where correlation was found to exist, simple multiplication of the MEG and EEG signals gave essentially the same results in terms of demonstrating correlation, as well as being performable in real time. A signal processing module is now being evaluated with this capability; additional functions

available on-line from this module are $(MEG)^2/(EEG)^2$ which gives the ratio of the power associated with each discharge and thus provides a normalization of the signals, and $(EEG)^3$ and $(MEG)^3$ which enhance the spike signal to noise ratio while preserving polarity to permit detection of the spike event. It is felt these techniques will allow the MEG activity to be measured for each neural grouping and provide means of normalizing each spike so that signal averaging can be applied to a unique focus.

PROPOSED COURSE: To compare the results obtained from the signal processing module with more sophisticated analysis using a mainframe computer and to provide additional processing where necessary. To evaluate the prediction of source localization with in-depth cortical electrodes for patients undergoing surgery and based on these results, to refine the mathematical methods used to make to make this prediction.

SIGNIFICANCE: The potential of MEG to localize in three dimensions the source of neurologic discharges noninvasively offers a great advantage over conventional scalp EEG recording which suffers from the diffusion of scalp electrical potentials, due to high skull resistance. The localization of epileptic foci in patients inadequately responding to drug therapy is important in the evaluation of surgical prospects and the direction of surgical procedures. The noninvasive localization of coordinates of normal and abnormal neurologic activity in the brain offers the potential for much greater understanding of the topography of human brain neurologic processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10170-02 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 charecters or less. Titla must fit on one line between the borders.)*
Biological Applications of a Computer Controlled Analytical Electron Microscope

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
R.D. Leapman   Visiting Scientist   BEIB, DRS

Others:
C.E. Fiori	Physical Scientist	BEIB, DRS
C.R. Swyt	Physical Scientist	BEIB, DRS
K.E. Gorlen	Electronic Engineer	CSL, DCRT
C.C. Gibson	Electronic Engineer	BEIB, DCRT
R.L. Ornberg	Senior Staff Fellow	LCBG, NIADDK

COOPERATING UNITS *(if any)*
DCRT, NIADDK

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 2.5	OTHER:

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

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

The computer controlled analytical electron microscope developed jointly by BEIB and CSL, DCRT, provides a unique tool for measuring sub-cellular elemental distributions with a resolution of some 10 nm. Experiments have been carried out to test the capabilities of the instrumentation and to develop further the methodologies for analysis. Nitrogen electron energy loss images have been recorded from cryofixed pancreas beta and adrenol chromaffin cells. Calcium and nitrogen maps have been obtained from rapidly frozen and freeze substituted spinal cord neurons, treated with potassium and sodium glutamate to produce calcium deposits in their mitochrondria. In these images it has been found crucial to model the spectral background correctly and a one-parameter fit, which is often assumed, in general leads to artifacts despite an apparent improvement in signal-to-noise ratio. Ratio maps of nitrogen to phosphorus or sulphur can be obtained from the appropriate x-ray images and these ratios can be related to the biochemical constituents of organelles. New software controlling the DeAnza image display system allows quantitation of the chemical maps. Thus, for example, the ratio image of two energy loss maps eliminates, to first order, the effects of plural scattering. It has been possible to quantitate x-ray microanalysis in thin cryosections by using inelastic electron scattering as measured by EELS to determine the mass per unit area in 100 nm diameter regions at extremely low dose (approximately 100 electrons nm^{-2}). This enables quantitative measurements to be made from hydrated cryosections, including an estimate of the water content. Forthcoming improvements in the electron energy loss spectrometer should provide increased sensitivity and allow annular dark field STEM imaging to be carried out simultaneously. It will also be possible to perform Z-contrast imaging in suitably thin samples. This new contrast mode is predicted to depend only on local mean atomic number and thickness effects are cancelled.

OTHER INVESTIGATORS

A. Boyne   Dept. Pharmacology and Experimental Therapeutics,
                  University of Maryland, Baltimore

OBJECTIVES: To determine how electron energy loss and energy dispersive
x-ray spectroscopy and spectroscopic imaging may be optimized for biological
applications.

METHODS EMPLOYED: The computer controlled Hitachi H700H analytical electron
microscope system built in collaboration with CSL, DCRT. has been developed
further and applied to a number of biological problems. New computer
programs, that use the DeAnza image display system, have permitted
quantitation of digitized elemental maps. A cryotransfer sample stage has
been used to investigate frozen hydrated and dehydrated cryosections, and a
side-looking energy dispersive x-ray spectrometer has enabled x-ray maps to be
obtained with increased detection efficiency.

MAJOR FINDINGS: Great care must be taken in modelling the spectral background
in electron energy loss imaging. Experimental results show that if a
one-parameter fit is assumed, artifacts can be generated because of plural
inelastic scattering, even though the signal-to-noise ratio appears to be
improved. Nitrogen images have been obtained from pancreas alpha and beta
cells and from adrenal chromaffin cells. When combined with phosphorus and
sulphur x-ray images, it is possible to obtain ratios of N:P:S distributions
which can be related to the biochemical constituents in the secretory granules
of these cells. Nitrogen can be mapped with a sensitivity of better than 1
atomic percent. A method of quantifying x-ray spectra has been developed by
which the mass per unit area of a sample is measured from the energy loss
spectrum at very low dose. This allows data to be obtained from frozen
hydrated or dehydrated samples without appreciable mass loss.

SIGNIFICANCE: It has been demonstrated that elemental distributions can be
obtained in STEM with a computer controlled analytical electron microscope.
Several advantage of this system have emerged, especially the importance of
correct subtraction of the spectral background at each pixel.

PROPOSED COURSE: It is planned to install an energy loss spectrometer of
advanced design with correction for all second order aberrations. This should
provide higher energy resolution and collection efficiency. Annular dark
field imaging will extend present capabilities by allowing simultaneous high
contrast structural imaging with elemental mapping. Further experiments will
be carried out with the cryotransfer sample stage. Cryosections are essential
for the study of elemental distributions and such sections are probably best
dehydrated in situ to prevent translocation of moveable ions.

PUBLICATIONS

R.D. Leapman, C.E. Fiori and C.R. Swyt. "Mass Thickness Determination by
Electron Energy Loss for Quantitative X-ray Microanalysis in Biology" J.
Microscopy 133:239-253, 1984.

R.D. Leapman, C.E. Fiori, K.E. Gorlen, C.C. Gibson and C.R. Swyt, "Combined
Elemental and STEM Imaging under Computer Control," Ultramicroscopy
12:281-292, 1984.

R.D. Leapman, "Electron Energy Loss Microspectroscopy and the Characterization of Solids," in Electron Beam Interactions with Solids, eds. D.F. Kyser, H. Niedrig, D.E. Newbury and R. Shimizu. published by SEM Inc. 1984, p217-233.

R.D. Leapman, K.E. Gorlen, and C.R. Swyt, "Processing of Electron Energy Loss Spectra and Images", in Proc. SEM 84, ed. O. Johari. SEM Inc.. 1984 (in press).

R.D. Leapman, K.E. Gorlen, and C.R. Swyt, "Background Subtraction in STEM Energy Loss Mapping," Proc. 42nd EMSA Meeting ed. G.W. Bailey, San Francisco Press, 1984, p. 568-569.

K.E. Gorlen, L.K. Barden, J.S. Del Priore, C.E. Fiori. C.C. Gibson and R.D. Leapman, "A Computerized Analytical Electron Microscope for Elemental Imaging", Rev. Sci. Instrum, 55, 912-921, 1984.

C.R. Swyt and R.D. Leapman, "Removal of Plural Scattering in Electron Energy Loss Spectra: Practical Considerations," Proc. Microbeam Analysis Society, San Francisco Press, 1984 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10182-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders)*
Study of Cartilage Mineralization by Analytical Electron Microscopy

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

R.D. Leapman   Visiting Scientist   BEIB, DRS
E. Livne   Visiting Scientist   LOBP, NIDR
C.E. Fiori   Physical Scientist   BEIB, DRS
C. Oliver   Biologist   LOBP, NIDR

COOPERATING UNITS *(if any)*
LOBP, NIDR

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	

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

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

The distribution of calcium in mouse mandibular condyle was investigated by electron energy loss spectroscopy in order to determine the role of the matrix vesicles in cartilage mineralization. Ultrathin embedded cartilage sections from 1 week, 1 month, and 1 year old animals were analyzed both at the surface of the condyle and at different depths. High calcium concentrations were found inside vesicles near the mineralization but matrix vesicles close to the surface did not contain appreciable amounts of calcium either crystalline or amorphous. This suggests that the matrix vesicles near the surface may be involved in other processes, such as the fibrillation which occurs in non-inflamatory ulceration of cartilage or osteoarthritis.

OBJECTIVES: To determine the extent to which matrix vesicle in mouse mandibular condyle cartilage are involved in mineralization processes.

METHODS EMPLOYED: Plastic embedded sections were investigated in a Hitachi H700H analytical electron microscope. Electron energy loss spectra were recorded from 20 nm diameter regions in the tissue and quantitation was achieved by software written in BEIB.

MAJOR FINDINGS: High calcium concentrations were found in matrix vesicles near the mineralization zone, but vesicles near the surface in mature condyle did not contain appreciable calcium.

SIGNIFICANCE: Mandibular condyle serves as a growth center for bone formation in the young animal. It has been believed that the matrix vesicles are primarly involved in the mineralization, and they are known to provide the primary nucleus for calcification. When the animal matures the condyle does not participate in growth but resembles articular cartilage which does not mineralize. However, the abundance of matrix vesicle containing little calcium in mature condyle suggests that the vesicles may be involved in processes other than mineralization. It is speculated that they may be implicated in the degenerative changes that occur in osteoarthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10183-01 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Elemental Imaging of Nerve Terminals and Cerebellum

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

R.D. Leapman   Visiting Scientist   BEIB DRS

Others:

S.B. Andrews	Special Expert	LN, NINCDS
T.S. Reese	Chief	LN, NINCDS
C.E. Fiori	Physical Scientist	BEIB, DRS
C.R. Swyt	Physical Scientist	BEIB, DRS

COOPERATING UNITS *(if any)*

Computer Systems Laboratory, Division of Research and Technology, NIH (K.E. Gorlen), LN/NINCDS

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

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

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

Distributions of potassium, sodium, calcium, phosphorus and sulphur were obtained from dried cryosections of rapidly frozen synaptosomes and cerebellum. The synaptosomes derived from cholinergic endings of squid optic lobe were imaged by emission of characteristic x-rays with the use of an energy dispersive spectrometer. Specimens were cooled to -120°C in a special cryotransfer sample stage, in order to minimize mass loss by radiation damage. The digitally acquired elemental maps showed a bimodal distribution of potassium and sulphur in the synaptosome population. This result suggests that only a fraction of synaptosomes with high potassium and low sodium are physiologically equivalent to the cholinergic endings of in vivo cells.

In an effort to understand changes in potassium and calcium concentrations that occur in cerebellum trauma, rat cerebellum cryosections were analyzed. Although little contrast, apart from compression artifacts , was visible in the elastic images, potassium maps obtained at a resolution of some 50 nm revealed areas of differing K concentration (60 - 180 mmol). These regions may correspond to dendrites, axons and and glial cells. Regions containing calcium were found with concentrations in the range 20-50 mmol, and these may correspond to organelles involved in calcium regulation.

OBJECTIVES: To obtain elemental distributions in cryosections of nerve terminals (synaptosomes) and cerebellum, and to relate these distributions to intracellular morphology in cryofixed, embedded samples.

METHODS EMPLOYED: Rapidly frozen ultrathin cryosections were investigated in a Hitachi H700H analytical electron microscope controlled by a Digital Equipment Corporation PDP 11/60 computer and an LSI 11/23 satellite. This system, built in collaboration with CSL, DCRT. enables background-corrected digitized elemental images to be acquired. Freeze dried samples were cooled to -120°C to minimize radiation damage and x-ray maps were obtained with a side-looking Tracor Northern energy dispersive x-ray spectrometer.

MAJOR FINDINGS: Potassium and sulphur distributions in synaptosomes were found to be approximately bimodal. Potassium distributions in cerebellum varied between 60 and 180 mmol and regions of calcium some 100 nm in diameter with concentration in the range 20-50 mmol were found. Cryosections show very poor contrast in the conventional elastic image and an important remaining problem is to relate ultrastructure to the chemical maps.

SIGNIFICANCE: The synaptosome results demonstrate that only a fraction of the isolated nerve terminals have K concentrations similar to those in whole cells. This finding is consistent with differing morphology observed in cryofixed embedded stained samples, and may determine which structure are most appropriate in describing neuron terminals. The cerebellum data are the first measurements of subcellular potassium and calcium concentrations in this tissue. Such studies must be carried out on cryosections to preserve chemistry on this scale. The results are significant in understanding how K and Ca concentrations change in trauma.

PROPOSED COURSE: More measurements will be made using a cryotransfer specimen stage to examine hydrated cryosections directly transferred from the microtome to the microscope vacuum at -170°C, so that dehydration can be performed in situ. The possibility of ion translocation is then greatly reduced since dehydrated cryosections are very sensitive to degradation by ambient water vapor. It is planned to analyze a cryosection and then to freeze substitute the remaining block of frozen cells, so that a serial embedded stained section with good morphology can be obtained. Correlation of ultrastructure with chemical composition will then be greatly simplified.

PUBLICATIONS

S.B. Andrews, R.D. Leapman, D.M.D. Landis C.E. Fiori and T.S. Reese, "Elemental Distribution in Rapid-Frozen Cerebellar Cortex." J. Cell Biol (a) in press (1984).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10184-01 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Physical Chemistry of Biological Macromolecules

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

Marc S. Lewis Research Chemist BEIB DRS

COOPERATING UNITS *(if any)*

LVR/NEI, LB, NIDR, SNB/NINCDS

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Microanalysis

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of this project is to study the physical properties of a wide variety of biological macromolecules with the goal of correlating these properties to the structure and function of the macromolecules. The emphasis is on the thermodynamics of the interactions of these macromolecules and on their molecular size and shape.   Analytical ultracentrifugation and mathematical modeling are the principal research techniques used.

The studies on the binding of plasminogen by fibrinogen described in the 1983 report have been published. Studies currently in progress in this area deal with the association of plasminogen with plasmin inhibitor, with the association of plasminogen with the D and E fragments of fibrinogen and with the association of fibrinogen with plasma Factor XIII.

Studies on the association of the A and B chains of reduced ricin indicate that the formaion of the AB complex is reversible and that the complex undergoes further reversible self-association to form polymers up to octomer. The temperature dependence of these reactions indicates that they are entropically driven, suggesting strong hydrophobic interaction between the chains.

Studies on the retinoid-binding protein from the interphotoreceptor matrix of the retina of macaque monkeys demonstrate that this is a significantly asymmetrical, hydrophobic glycoprotein with a molecular weight of 106,000.

ASSOCIATE INVESTIGATORS

G.J. Chader	Laboratory Chief	LVR NEI
S.I. Chung	Research Chemist	LB NIDR
T.M. Redmond	Research Chemist	LVR NEI
B. Wiggert	Research Chemist	LVR NEI
R.J. Youle	Research Chemist	SNB NINCDS

METHODS EMPLOYED: The macromolecules have been isolated and purified from appropriate sources by conventional methods. The associations have been studied by establishing equilibrium gradients in the analytical ultracentrifuge and then analyzing the resultant data by means of mathematical modeling techniques using the MLAB system operating on the DEC-10 computer.

MAJOR FINDINGS: Proteins Involved in Blood Clotting: Studies on the interactions involving some of the various proteins involved in blood clotting and fibrinolysis have indicated that there are specific interactions involved. However, there have been problems with the purity and reactivity of some of the proteins and we have not achieved results with the accuracy and reproducibility that would warrant reporting at this time. There is no reason not to expect that these problems will be overcome.

Ricin: It was demonstrated that neither intact ricin nor the individual A and B chains which were isolated following cleavage of the disulfide bond by reduction exhibited any self-association. Exact equimolar mixtures of ricin A and B chains were prepared by reduction of the disulfide bond between them with beta mercaptoethanol and reoxidation was prevented by the presence of dithiothreitol in the buffer solution. High pressure liquid chromatography demonstrated that the level of reoxidized or intact ricin was less than 1%. The use of exact equimolar mixtures permitted the derivation of a mathematical model for the distributions of the A and B chains and their complexes in the ultracentrifuge cell that had implicit constraints that greatly enhanced both the speed and the accuracy of the computations. It was found that the A and B chains reversibly associated to form an AB complex and that this complex then would undergo reversible self-association to form polymers up to the level of octamer. Determination of the natural logarithms of the equilibrium constants of these reactions as a function of temperature enabled calculation of the associated changes of enthalpy and entropy. Values of 14 kcal/mol/deg and 70 cal/mol/deg respectively were found for the changes of enthalpy and entropy of association. Since the free energy change for association has a negative value, this indicates that the association reaction is entropically driven, suggesting the probability of strong hydrophobic interaction between the chains.

Monkey Interphotoreceptor Retinoid-Binding Protein: This protein has been isolated for the first time from the interphotoreceptor matrix of the retinas of macaque monkeys. It was purified to homogeneity by affinity chromatography, by ion exchange chromatography and by size exclusion chromatography. The protein has been characterized in terms of amino acid composition, carbohydrate content, isoelectric point, fluorescence emission, molecular weight and sedimentation coefficient. The protein was found to be a glycoprotein with approximately 20% carbohydrate, primarily fucose and sialic acid. Over 50% of the amino acids are nonpolar, accounting for the hydrophobic nature of the protein. The isoelectric point was found to lie between pH's 6.0 and 7.0. The wave length maximum of fluorescence emission of bound ligand was 470 nm for excitation at 340 nm. The molecular weight was

found to be 106,000 by sedimentation equilibrium and 146,000 by sodium dodecyl sulfate polyacrylamide gel electrophoresis; the discrepancies between the two values can be attributed to the large carbohydrate content of the protein which makes molecular weight determination by SDS PAGE unreliable. With a sedimentation coefficient of 5.40 S, the protein has a frictional ratio of 1.59 which indicates an axial ratio of approximately 11:1 for an anhydrous protein or, more probably, a lesser axial ratio depending on the extent of hydration.

SIGNIFICANCE: The studies on the interactions of plasminogen, fibrinogen, plasmin inhibitor and plasma Factor XIII are relevant to the very significant roles that these proteins play in blood clotting and subsequent fibrinolysis. It is our objective to correlate the physical chemistry of these interactions with the physiological role which these proteins play. The studies on ricin are relevant to an understanding of its structure and to its function as a toxin. Ricin appears to be formed as a single polypeptide chain which then undergoes cleavage followed by association of the A and B chains and then oxidation of two sulfhydryls to form the disulfide bond; this study indicates how that association occurs. Bioassay studies on the toxicity of reduced ricin demonstrate a very good correlation between the concentration dependence of the toxicity and the concentration dependence of the formation of reduced ricin complex polymer, indicating that for reduced ricin, unlike the native form, polymerization is necessary for toxicity. The study on the monkey interphotoreceptor retinoid-binding protein is relevant to an understanding of the role that this glycoprotein might play in extracellular matrix reactions in the interphotoreceptor space.

PROPOSED COURSE: Studies on the interactions of the proteins involved in blood clotting and subsequent fibrinolysis will be pursued extensively, as this appears to be a very fruitful area of endeavor.

A manuscript describing the ricin studies is presently in preparation. We plan to continue work in this area with studies on the association of ricin chains which have been coupled to cell-specific antibodies, with studies on other related A and B chain type toxins and with studies on the correlation between primary structure and the association of A chains from one toxin and B chains from another.

A manuscript describing the monkey interphotoreceptor retinoid binding protein has been submitted for publication. It is anticipated that other studies on related proteins will be inititated.

Studies on other A-B type associations are planned; studies on human chorionic gonadotropin appear particularly promising.

PUBLICATIONS

Lewis, M.S., Carmassi, F. and Chung, S.I.: Cooperative Association of Plasminogen with Fibrinogen. Biochemistry (1984) 23:3874-3879.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10185-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

John W. Boretos, Physical Scientist, BEIB, DRS

Others:

John Doppman, M.D.	Radiologist	CR, CC, NIH
Edward Oldfield, M.D.	Neurosurgeon	NINCDS, NIH
Robert L. Dedrick, Ph.D.	Chem. Engr.	BEIB, DRS, NIH

COOPERATING UNITS *(if any)*
CR, CC, NIH; NS, NINCDS, NIH,
BEIB, DRS, NIH

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	0.7	0.7

CHECK APPROPRIATE BOX(ES)

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

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

The purpose of the project is to develop a catheter system for interventional radiology which is capable of traversing small and branching blood vessels (i.e. 1.5 to 4.0 mm) to reach the proximity of tumors and to administer chemotherapeutic drugs to these tumors in a controlled manner. A major obstacle to effective treatment is thought to be mal-distribution of the drug at the site due to inadequate mixing with the blood. A multi-lumen micro-catheter, which is propelled and directed through these small blood vessels via jets of fluid emanating from its distal end, has shown promise for negotiating heretofore difficult to reach areas. The catheter is controlled by an adjustable pressurized manifold operated by a joy stick. Provisions for on-line switching from a radiopaque contrast solution to the drug is provided at the manifold. The contrast agent serves as the propelling fluid as well as providing visual identification of the movement and location of the catheter. While fluid turbulence generated at lower pressure by the drug emanating at retrograde angles from the tip of the catheter adds significantly to the mixing of the drug within the blood stream. Animal tests have substantiated the feasibility of the catheter system for clinical use. A transparent model of the vascular network simulating blood flow of a pulsatile nature was designed to evaluate the efficiency of the jet flow at various delivering rates. Surfaces of the catheters have been modified with bonded hydrogels to reduce drag through conventional sheaths and further acts to minimize clotting on the surfaces. A radiopaque marker at the tip of the catheter can be readily located by fluoroscopy.

OBJECTIVES: The objective of this project is to develop a catheter system for interventional radiology which is capable of traversing small and branching blood vessels (i.e., 1.5 to 4.0 mm) to reach the proximity of cancerous tumors and to administer chemo-therapeutic drugs to these tumors in a controlled manner.

METHODS EMPLOYED: A multi-lumen micro-catheter which is propelled and directed through small blood vessels via jets of fluid emanating from its distal end has shown promise for negotiating heretofore difficult to reach areas. The catheter is controlled by an adjustable pressurized manifold operated by a joy stick. Radiopaque contrast solution is used to propel and visualize the movement of the catheter by being pumped selectively through the lumens of the catheter. As radiopaque fluid jets out of the catheter ports, thrust is transmitted to the tip of the catheter. Right, left, and forward motions can be controlled in this manner. The chemo-therapeutic drug is brought on-line by valvular switching at the manifold to replace the contrast agent. The jet action of the fluid emanating at a retrograde angle from the tip of the catheter generates fluid turbulence which adds significantly to the mixing of the drug within the blood stream. A transparent model of the vascular network simulating pulsatile blood flow is being used to evaluate the efficiency of the jet catheter system and its behavior in administering chemotheraputic drugs.

MAJOR FINDINGS: The development of a multi-lumen micro-catheter which is propelled and directed through small blood vessels via jets of fluid emanating from its distal end shows promise for negotiating heretofore difficult to reach areas of the vascular network. The jet propulsion action can be regulated to serve as a means of administering chemotherapeutic drugs. Because of its angular retrograde flow, mixing of the drug within the blood stream is significantly enhanced over that of an open ended catheter of a single orifice. A radiopaque maker at the tip of the catheter allows constant fluoroscopic visualization to verify location during drug administration.

SIGNIFICANCE: Further development of the catheter system to increase fluid volumes under lower induced pressures along with characterization of the efficiency of intravascular drug administration. Clinical experience is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10186-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
John W. Boretos, Physical Scientist, BEIB, DRS

Others:
Milton Brightman, Ph.D.   Lab Chief   LNNS, NINCDS
Peter Bungay, Ph.D.   Chemical Engineer   CES, BEIB, DRS
Robert L. Dedrick, Ph.D. Chemical Engineer   CES, BEIB, DRS

COOPERATING UNITS *(if any)*

LNNS, NINCDS,;CES, BEIB, DRS, NIH.

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 0.3	OTHER: 0.7

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

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

The purpose of this project is to develop a capsule to serve as an aid to in situ implantation of multiple tissue fragments into the fourth ventricle of rats for the study of neuronal growth and ultimately, to reconstruction of severed neurological circuits. The capsule shall further serve as a mechanical constraint to maintain the tissue fragments in registration and in close juxtaposition while at the same time providing for angiogenesis from the overlying cerebellum. Various polymeric capsules of one millimeter diameter were developed having a range of dissolution characteristics based on methyl cellulose (demethyl ether) and methyl cellulose crosslinked with polyfunctional resins combined with crosslinked and non-crosslinked collagen. The capsules exhibited sufficient initial rigidity for ease of loading of the tissue fragments and insertion into the fourth ventricle, yet immediately became soft in cerebral fluid. Only materials with a proven history of biocompatability were used. Dissolution times could be varied from 30 minutes to 8 hours in increments of 30 minutes depending upon the degree of crosslinking selected. The presence of the collagen added a plasticizing effect to the polymer system and provided a pseudo-fibrous network once the polymer dissolved. This network remains for extended periods and may be yeneficial in supporting and confining the tissue fragments beyond the initial stages of growth. Preliminary studies showed the capsules to be tolerated by the animals and some evidence that conditions in the fourth ventricle are suitable for neuronal growth.

OBJECTIVES: The objective was to design a temporary capsule to serve as an aid in the in situ implantation of multiple tissue fragments into the fourth ventricle of rats in an effort to encourage neuronal growth and ultimate reconstruction of severed neurological circuits.

METHODS EMPLOYED: Various polymeric capsule systems of 1 mm diameter having a range of dissolution characteristics based on the dimethyl ether of methyl cellulose, crosslinked methyl cellulose and crosslinked and non-crosslilnked collagen were developed. The capsules were cast from solution by multiple dippings. Only materials with a proven history of biocompatability were used.

MAJOR FINDINGS: By combining methyl cellulose with cross-linked methyl cellulose in the presence of collagen, a capsule system was developed whose rate of dissolution in CSF could be predetermined. A range from 30 seconds to 8 hours was made available since the critical "incubation" time is as yet unknown. The presence of the collagen acted as a plasiticizer and further provided a psuedo-fibrous network which may help confine the tissue fragments once the bulk of the capsule dissolves. Initial studies showed the system to be well tolerated by the animals and some evidence of suitable condition for neuronal growth was observed.

SIGNIFICANCE: A physiologically compatible capsule having transient structure will aid in the study of the regeneration of neurons and revascularization of transplanted neural tissue and the tissues, substances and conditions which promote these processes. The knowledge gained may be applied clinically to patients with severed nerves.

PROPOSED COURSE: Further development of the composition of the capsule by incorporating substances that would promote neural regrowth. Characterization of the mass transfer conditions in the fourth ventricle of the rat brain after implantation of the capsule and tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10187-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mixing During Intra-Arterial Infusion via Catheters

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

R.J. Lutz   DRS, BEIB
J. Boretos   DRS, BEIB
E. Oldfield   NINCDS IR SN
J. Doppman   CC DR
D. Miller   CC DR
R. Pflueger   DRS, BEIB
C. Thompson   DRS, BEIB

COOPERATING UNITS (if any)
NINCDS
CC

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	.75	.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.)

Intra-arterial chemotherapy offers an alternative to systemic (iv) chemotherapy for localized neoplasms. Infusion of chemotherapeutic agents directly into the arterial supply of the neoplasm allows, under the proper circumstances, higher local tissue concentrations without a corresponding increase in systemic toxicity. Intra-arterial infusion rates are commonly in the range of 2 to 5 ml/min through a #5 or #7 French catheter. Results of such infusion have been varied and frequently suggest the possibility of preferential drug perfusion into local sub-regions of target tissue at position distal to the point of infusion. Often, some regions show areas of no drug effect, while other areas exhibit acute toxic responses. The suggestion has been put forward that fluid streaming from the catheter tip during infusion and, thereby, the absence of adequate drug mixing in the efferent blood supply to each arterial branch of the target tissue is one possible cause for the deliterious effects of this treatment modality. The aims of this project were to assess the degree of mixing of solutions infused from catheters into artery-like vessels, and to gain some insight into the fluid-mechanical factors that influence the mixing process. By means of model experiments, we have demonstrated that streaming can occur when infusion rates are too slow for the respective arterial size or arterial flow rates, and that drug solutions streaming at low infusion rates can advance preferentially into specific distal arterial branches. Mixing can be enhanced in numerous ways. e.g. by employing greater infusion rates, or by redesigning the catheter tips to use "jet" infusion of drug solution transverse to the perfusing blood flow.

OBJECTIVES: To assess the degree of mixing produced by infusion of solutions through various types of catheters into a flowing stream inside arterial-like vessels.

To gain insight into the fundamental fluid-mechanic events which influence the mixing during catheter infusion.

To determine methods for improving mixing during intra-arterial infusions.

METHODS EMPLOYED: Flow visualization techniques are employed to qualitatively determining mixing properties during infusion through various types of catheters. Several flow systems are used. The most fundamental consists simply of several sizes of straight, glass tubing (5mm and 9.5mm ID) to simulate the artery. The fluid consists of either water or glycerine/water mixtures to assess the influence of fluid density and fluid viscosity. For a well-controlled geometry and stable catheter, placement, straight sections of hypodermic tubing are used as catheters for infusion. A range of fluid flow rates are used in the study, both steady and pulsatile, and a range of infusion rates are provided by constant infusion pumps. The infusion solution consists of a water-soluble dye in either water or glycerine/water mixtures to study bouyancy effects. The infusion events are recorded on video tape for visual analysis.

A more complex flow system is constructed which is a plastic cast of a carotid arterial geometry, containing an internal carotid artery and the major distal branching vessels. This model is connected to a mock circulatory loop that provides pulsatile flow comparable to internal carotid blood flow. The model system is being used to evaluate several types of clinical catheters.

MAJOR FINDINGS: Infusion rates that are too slow for the respective arterial diameter or for the arterial flow rates do exhibit definite streaming patterns. Depending on the position of the catheter inside the artery, infused solutions could preferentially flow into one distal artery branch and not another, thereby resulting in uneven regional distribution of drug to the tissue of interest. The greater the artery flow rate, the greater must be the infusion rate to promote mixing. Catheters that utilize small, lateral "jet" holes appear to enhance mixing at lower infusion rates than straight catheters. The enhanced mixing is promoted by impingement of the lateral jet onto the artery walls. During pulsatile flow, streaming from the catheter is promoted by the plug like nature of arterial flow during systole, and the predominant mixing events occur during the slow flow diastolic phase of the pulse. The magnitude of the net forward flow during diastole can affect the degree of mixing.

SIGNIFICANCE: Inadequate mixing of drugs infused via intra-arterial catheters can result in variable drug exposure to distal tissue regions. Excessive drug exposure, particularly to sensitive normal tissue such as the eye, can result in unwanted toxic effects. Inadequate drug exposure can result in reduced therapeutic effect at a particular tumor site. With inadequate mixing, the exposure at all sites would be random and unpredictable.

PROPOSED COURSE: To continue to study the fluid-mechanical factors that influence mixing from catheters of various geometries and in vessels of various shapes and sizes using flow visualization and using pulsatile flows.

To develop a methodology of quantitating the degree mixing, e.g. using a series of photocell devices to determine dye concentrations from fluid flow samples from numerous branch vessels that are distal to the point of infusion.

Possibly, to conduct a mixing experiment using whole blood in the carotid model while infusing BCNU and cis-platin.

To conduct in vivo studies of mixing using radio opaque dyes at various infusion rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10188-01 BEI

PERIOD COVERED
February 1, 1984 to September 30, 1984

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

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

P.F. Morrison   Physical Scientist   BEIB, DRS
R.L. Dedrick   Chief, ChE Section   BEIB, DRS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, NINCDS, NIH (E.H. Oldfield)

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

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

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

Concentration profiles of cis-platin in the cortex and cerebellum of the rat were analyzed with the intent of determining transport and reaction rate parameters. These parameters are needed to prospectively model various tumor treatment modalities for expected efficacy.

A reaction-diffusion model was formulated for cis-platin infusion from point and near-point sources. Both infinite and finite spherical transport regions were modeled, the latter to demonstrate that drug flow into the cerebral spinal fluid is of secondary importance. It was shown that the experimental concentration profiles corresponded to steady state conditions, and could be modeled by simpler steady-state mathematics.

Convection due to diluent flow from the infusion cannula was added to the reaction-diffusion model. Steady-state analysis showed that convection substantially modified the concentration profiles only over the first millimeter from the cannula tip.

More realistic geometry was introduced modelling the cerebellum as a prolate ellipsoid. Average total Pt concentrations for sections across this ellipsoid were fit to corresponding experimental tissue section data. The resulting fit, constrained by an independent measure of infusate recovery, provided estimates for cis-platin capillary permeability (9.03×10^{-7} cm/sec) and reaction rate (.005/min). The tissue diffusion constant was estimated as 1.9×10^{-6}/sec, similar to creatinine due to the small hydrodynamic radius of cis-platin. The permeability is within the range predicted from octanol-saline partition coefficient/permeability correlations. The reaction rate compares with values reported for other tissues.

ASSOCIATE INVESTIGATORS

E.H. Oldfield   Neurosurgeon   SN, NINCDS

OBJECTIVES: To determine the transport parameters and theoretical framework that describe the movement of drugs through brain tissue, thus providing a basis for the prospective evaluation of various treatment modalities.

METHODS EMPLOYED: Reaction-convection-diffusion equations were formulated for several boundary and source conditions. Initial work was focussed on the antitumor agent, cis-platin. The tissue was represented physically as a nearly homogeneous medium with capillaries and reaction sites evenly distributed throughout the tissue mass. Movement of cis-platin was considered to be dominated by its transport through the extracellular fluid; equilibration with the intracellular fluid occurred on a somewhat slower time scale. Free drug was lost from the tissue either by reaction or by its passage across capillary walls with subsequent loss to other body tissues. Analytical solutions were obtained wherever possible, finite difference methods being employed when analytical solutions were not obtainable.

MAJOR FINDINGS: Time-dependent solutions have shown that steady state cis-platin concentration profiles are reached in about three hours. Thus simpler steady state solutions may be applied to continuous infusion times of thirty hours or longer. Finite diffusion range calculations have shown that little significant transport of drug occurs across the brain-cerebral spinal fluid boundary. Likewise, the non-point-source nature of the infusion cannulus was shown to decrease the concentration profile by only 5% relative to the point source result. Convection effects were found to be substantial over the first millimeter from the cannula tip but were insignificant beyond this (where most of the experimental data were concentrated).

Experimental data, giving the average Pt concentrations for sagittal tissue sections cut across the cerebellum, were shown to be represented by a spherical diffusion-reaction solution integrated over sections cut through an equivalent prolate spheroid. For cannulus tip to section distances between 0.5 mm and the minor axis distance, theory predicted the linear distance-log concentration relation observed experimentally.

Transport parameters were determined for cis-platin: capillary permeability was 9.03×10^{-7} cm/sec; reaction rate was .005/min; diffusion constant was 1.9×10^{-6} cm^2/sec.

SIGNIFICANCE: Availability of transport parameters now allows one to estimate the range of effective cis-platin concentrations within brain tissue when the tissue is dosed by flow across various brain surfaces.

PROPOSED COURSE: The modelling will be extended to include transport descriptions of tumor as well as normal tissue. Various treatment modalities (e.g. microinfusion and balloon implants) will be evaluated for expected efficacy. In addition, the modelling will be extended to other drugs such as the transport of dopamine in the treatment of Parkinsons disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10189-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

P.F. Morrison   Physical Scientist   BEIB, DRS
Cheryl Daniels   Chemical Engineer   BEIB, DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

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

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

Probabilistic analayses were performed to assess the effects of time variant exposure to carcinogen on cancer risk estimates.

Equations were formulated from several statistical models of cancer that allow one to determine the effects on risk estimates of (1) a time-varying toxic agent concentration and of (2) a migrating exposed population. The incidence models examined included the one-hit, multihit, and Weibull models. Present results show that the same risk estimates are obtained from the one-hit and multihit models when either the time-averaged dose or the fully time-dependent dose is used. The Weibull model requires that time-dependence be carried through the risk estimation procedure or else order of magnitude errors may occur (such as for the 30 year arsenic risk estimate when exposure actually occurs only over a third of this time). Migration was described by a first order partial differential flow model and was coupled to the incidence models via a residence time distribution formalism. Failure to include such a description of population migration results in both multihit and Weibull models overpredicting estimated risk, e.g. a Weibull model applied to arsenite toxicity will overpredict the incidence of skin cancer by ten-fold if an out-migration rate of 12% per year is ignored.

In addition, the multistage model for an arbitrary number of stages has been solved for constant concentration exposure. In turn, this multistage model along with multihit and Weibull models, was used to recast the form of cancer incidence expected at the cellular level to that expected at the organ level, the correct form to use when fitting to animal dose-response data.

OBJECTIVES: To find improved procedures for estimating the incidence of cancer induced in a human population exposed to carcinogens. First efforts include determination of the magnitude of the effect of time-variant exposure, and of the effects of scaling events expected at the cellular level to the organ level.

METHODS EMPLOYED: Various hit-type models of carcinogenesis were cast into stochastic differential equation form with transition coefficients taken as linear functions of carcinogen concentration. In addition, the Weibull formalism was cast into an incidence form dependent upon instantaneous carcinogen concentration. Cancer prevalences (for a non-migrating exposed population) were then estimated from these expressions by integrating them over several time-dependent concentration patterns. They were then compared to the prevalences obtained by solving corresponding time-averaged concentrations, a first order approach often used in environmental health studies.

In addition, the effect on risk estimates was assessed for various levels of migration in an exposed population (exposed at constant concentration). First order partial differential equations were formulated to describe the population dynamics. and a residence time distribution model was used to couple the resulting time-dependent population density expression with the carcinogonesis models.

The effects of scaling from the cellular to the organ level were assessed by a straightforward substitution of hit model results into a binomial distribution dependent organ response.

MAJOR FINDINGS: The same risk estimates (prevalences) are obtained from the one-hit and multihit models whether or not time-averaged concentrations are used.   The particular Weibull model used does require that full time-dependence be retained during the estimation of prevalence, or else order of magnitude errors may occur. Failure to include population migration causes both the multihit and Weibull models to overpredict risk. For aqueous arsensite exposure, the skin cancer risk may be overestimated ten-fold if a 12% out-migration rate is ignored.

Cell to organ scaling results for the multistage model require that the model be solved for the high dose range. High dose incidence and prevalence results (distinct from the Crump model) have been obtained as well as parameter partial derivatives useful for likelihood calculations.

SIGNIFICNACE: Criteria are now available to determine when time-variant exposure must be taken into account, thus allowing less biased risk estimates to be made.

PROPOSED COURSE: The magnitude of the cell to organ scaling effect will be determined for various hit models and hit-levels, and improved risk estimation equations will be derived. A long term goal for some tumors is to interpret and requantify the transition probabilities of the hit models in terms of the underlying molecular biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10190-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

R.S. Chadwick, Biomedical Engineer, BEIB
D. McGuire,   Mathematician,   BEIB
D. Goldstein, Senior Investigator, EHL, NHLBI
H. Kaiser,   Chief   EHL, NHLBI

COOPERATING UNITS *(if eny)*
NHLBI, Endocrine - Hypertension Laboratory

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.35	.25	.1

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

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

This is a combined theoretical and experimental study designed to assess the importance of vessel tortuosity as a determinant of peripheral vascular resistance. An apparatus was designed and built to measure the relative hydrodynamic conductance of a machined tortuous tube. A range of physiological Reynolds numbers was obtained with aqueous solutions of glycerol. Theory relates the findings for a single tube to the input resistance of a vascular tree.

OBJECTIVES: To assess the effect of tortuosity on the resistance of a vascular network, and to determine the relative importance of tortuosity at different branching levels of the vascular tree.

METHODS EMPLOYED: An experimental apparatus has been designed and built that determines the functional relationship between the relative hydrodynamic conductance and Reynolds number for a tortuous tube made by a numerically controlled milling machine. A range of physiological Reynolds numbers is obtained by controlling the viscosity of aqueous solutions of glycerol. Theoretical aspects involve understanding the conductance of a single tortuous tube, as well as relating the single tube findings to a vascular network.

MAJOR FINDINGS: Preliminary measurements suggest that large decreases in conductance occur for Re > 10, indicating that there are large inertial loses. Hence vessels which normally have neglible pressure drop can have significant pressure drops in the tortuous state. Even at neglible Reynolds number. the new theory suggests that significant decreases in conductance can result from extreme tortuosity.

SIGNIFICANCE: These findings suggest that the tendency for increased vessel tortuosity with age can be a factor in elevating vascular resistance.

PROPOSED COURSE: Theoretical work at non-neglible Reynolds number as well as flow visualization and laser doppler velocity profile measurements on the model for a range of Reynolds numbers are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10191-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Continuous Optical Monitoring for Bacterial Cultures

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

G.M. Maxwell, Staff Fellow, BEIB, DRS
Ernst Freese, Chief, LMB, NINCDS

COOPERATING UNITS *(if any)*
LMB, NINCDS

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD 20205

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

CHECK APPROPRIATE BOX(ES)

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

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

Several routine measures in molecular biology involve monitoring and/or controlling the population density of bacterial colonies grown in liquid medium by withdrawing samples and measuring turbidity. The purpose of this project is to develop a system to continuously monitor cell population by measuring associated changes in optical properties of the culture.

Current work is aimed at determining the feasibility of such techniques for short term study (8 hours). Current and future effort will be directed at determining and improving the long term stability (several days) of the transducer, improving the noise characteristics of the system, and developing the capability for simultaneous monitoring of multiple channels (32).

OBJECTIVES: To determine the limits of continuous monitoring of bacterial in a liquid culture media and if feasible, to develop a system capable of monitoring many cultures simultaneously.

METHODS EMPLOYED: An initial analysis was performed to estimate the dynamic range required for a common type of experiment; following which a prototype system was constructed and tested. Subsequent testing and modification are currently underway to determine the necessary parameters and design constraints. The probe uses two red LED light sources and photodiode detectors mounted in an immersable bracket. Both scattered and transmitted light are measured and these signals are then compared against independent spectrometer measurements of the medium's special density.

PROPOSED COURSE: Two major questions remain to be answered concerning the system's feasibility. The first involves determining if bacteria will attach to the source and detector in the culture medium and if so, how long it will take for this process to limit the systems' sensitivity. The second problem is to determine an appropriate technique for calibrating the system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10192-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Temperature Monitor for EM Induced Hyperthermia

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

G.M. Maxwell, BEIB, DRS
R. Levin, BEIB, DRS

COOPERATING UNITS (if any)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, MD   20205

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

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

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

The purpose of this project was to develop the associated electronics for multiple EM compatible commercially available temperature probes used to monitor the temperature in a hyperthermic phantom. A prototype system was constructed and, following testing and modification, a 16 channel system was constructed. Final testing and calibration are currently being performed.

OBJECTIVE: To develop a sixteen channel temperature probe system for use in phantom studies of EM induced hyperthermia.

METHODS: A non-turbing commercially available thermistor probe was tested. Subsequent design and modification of a current source and signal conditioning electronic were performed to obtain an acceptable level of stability (19% day) and resolution .05°C. The complete system has been constructed.

SIGNIFICANCE: Induced hyperthermia through application of an external EM field is an extremely complex undertaking. Before clinical tests of such a device can be performed, extended phantom studies are necessary to adequately quantify the effects of the applied field. Accurate and non-perturbing temperature measurements are a necessary adjunct to this endeavor.

PROPOSED COURSE: Final testing and evaluation of the complete system will be performed in the near future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10193-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Toposcopic Catheter for Clinical Gastroenterological Use

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

D.R. Shook, Biomedical Engineer. BEIB,DRS

COOPERATING UNITS *(if any)*
Naval Hospital Bethesda, Naval Medical Command, National Capital Region. USN
(E.L. Cattau, T.J. Spurling); Esophogeal, Gastric and Colonic Diseases,
Digestive Diseases and Nutrition Division, NIADDKD (K.J. Vener).

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.3	PROFESSIONAL: 0.3	OTHER:

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

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

The unique characteristics of the toposcopic catheter have suggested its
application in the gastrointestinal system. The operation of the catheter
promises to facilitate the catherterization of the pancreatobiliary ducts
through the Papilla of Vater. An initial series of patients has undergone
routine endoscopic retrograde cholanopancreatography (ERCP) using the
topocatheter for contrast infusion. An extensively modified topocatheter is
passed through an appropriately positioned endoscope. The catheter tip is
placed within the ampulla, and the toposcopic element is everted. In basic
ERCP, contract medium is then injected for appropriate diagnostic procedures.
In addition, the topocatheter's ability to atraumatically negotiate small,
tortuous ducts, is being applied to catheterization of remote pancreatic ducts
for sampling of pure secretions, and of the cystic duct, for sampling and
possible infusions for dissolution of gall stones.

OBJECTIVES: Develop techniques for selective toposcopic catheterization of small, tortuous ducts of the gastrointestinal system and optimize apparatus for varied diagnostic and therapeutic applications.

MAJOR FINDINGS: Experimental work has progressed sufficiency to justify catheterization of patients during routine ERCP procedures. A patient series has commenced using a topocatheter modified for insertion and operation through a fiberoptic endoscope. The far reaches of the pancreatic duct have been safely catheterized and opacified at lower than conventional pressure.

SIGNIFICANCE: Catheterization of the far reaches of the pancreatic duct and branches coupled with sampling of pure secretions will reveal here-to-fore unavailable data on secretory activities as functions of digestion time and pathological state. Also of interest to clinical research is conveying fiberoptic probes for measuring chemical activity levels as near to the secretory source as possible. The topocatheter may greatly facilitate these applications without mechanical trauma and with much less risk of pancreatitis. The topocatheter has the potential to repeatably and atraumatically negotiate the cystic duct for sampling of fluids and possible infusion of chemotherapy for dissolution of gall stones.

PROPOSED COURSE: Optimize catheter system for diverse clinical applications. Substantiate the use of the toposcopic concept in unique GI applications. Develop related devices and explore additional use for the catheter.

Related Project Number

Z01-RS-10-01 BEIB (Vascular use)
Z01-RS-10-01 BEIB (Polymer Processing)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10194-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimized Polymer Processing for Advanced Catheter Development

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

D.R. Shook, Biomedical Engineer, BEIB, DRS
B. Bourin, Guest Researcher, BEIB, DRS

COOPERATING UNITS *(if any)*
Diagnostic Radiology, CC (J.L. Doppman); Esophogeal, Gastric and Colonic
Diseases, Digestive Diseases and Nutrition Division, NIADDKD (K.S. Vener)

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Mechanical Engineering Section,

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda. MD   20205

TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.5	OTHER: 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.)*

Advanced techniques of plasticating extrusion can produce materials of
optimized anisotropic properties and very fine dimensional characteristics.
Availability of such materials then supports development of unique polymeric
medical devices, such as the toposcopic catheter. Unique polymer resins are
first characterized specifically for extrusion characteristics and predictive
models of rheological behavior are constructed. Die designs can now be
implemented to take advantage of unique polymer character and to overcome the
most critical processing difficulties. The models way also be used to
eliminate certain polymers from consideration. Current efforts are to
optimize the topocatheter material for maximally safe operation and to allow
development of the topocatheter for arterial dilatation.

OBJECTIVES: To produce advanced and state-of-the-art extrusions to allow development of unique catheter and general medical devices for diagnostic and therapeutic applications.

MAJOR FINDINGS: The processing system has been assembled and optimized for fine wall tubing extrusion. Major problems with the pressure and temperature control systems have been corrected. The rheological model of the topocatheter polyurethane is being developed.

SIGNIFICANCE: The availability of advanced materials processing technology is often the critical element in development of unique polymeric medical devices. The toposcopic catheter is an excellent example of a very useful device whose development was substantially delayed by lack of high quality processing capabilities. The economics of the medical device industry often restrict development efforts to known low-risk technologies. The present processing capabilities will permit and encourage the development of unique device designs that will reveal clinical and basic research applications not heretofore envisioned.

PROPOSED COURSE: To optimize topocatheter materials for maximum safety; to broaden topocatheter applications to arterial dilatation by customizing material anisotropies; to implement design modifications to allow convenient and reliable steering of the topocatheter.

RELATED PROJECT NUMBER

Z01-RS-10015-09-BEIB
Z01-RS-10-01 BEIB (GI use)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10195-01 BEI

PERIOD COVERED
April 1984 to September 30, 1984

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

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

C. Wooten	Electronic Engr.	BEIB, DRS
W.S. Friauf	Ch., EEES	BEIB, DRS
R.L. Berger	Physicist	LTD, NHLBI
G. Hemphill	Electronic Tech.	BEIB, DRS

COOPERATING UNITS (if any)
CDC, Atlanta

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	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.)

New rare earth chelate fluorescent probes, with a decay time constant much longer than the background decay time-constant of typical organic materials offer the potential of a great improvement in sensitivity by appropriate timing of the response. However, to rival radioimmunoassay methods the required sensitivity improvement is so great that overload recovery of the fluorescence detector is a major problem. Extensive past work on this problem is largely inapplicable to the very low speed and level requirements of this situation. Consequently initial effort is being applied to determining the optimum fluorescence detection device and ancilliary signal overload limiting circuitry. Problems related to the fluorescent probe at extremely low sample concentrations will be studied in collaboration with CDC, which is interested in this approach as another tool for AIDS research. Finally an evaluationn of the details of excitation, optical filtering, and digital signal processing will be carried out and optimized.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10196-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

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

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

C. Wooten	Electronic Engineer,	BEIB, DRS
P. Smith	Visiting Scientist,	BEIB, DRS
R. Nakamura	Investigator	LPD, NIMH
R. Phillips	Investigator	DCBR, NIMH

COOPERATING UNITS *(if any)*
NIMH

LAB/BRANCH
Biomedical Engineering and Instrumentation Branch

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
DRS, National Institutes of Health, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.7	PROFESSIONAL: 0.5	OTHER. 0.2

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

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

Many behavioral studies and EEG activity studies use visual stimuli to elicit a response and then use a reward system to encourage that response.

One particular system is used to measure monkey responses to transiently displayed pattern stimuli. An image is drawn on a CRT screen from computer memory, it is then shuttered by an electro-optic element for viewing times down to 5 ms and can therefore provide a repetition rate of 10 frames per second. A lens assembly including the electro-optic element is mounted in a housing suitable for projection of a video CRT image onto a viewing screen. Computer input to a control box provides a predetermined voltage to the electro-optic element which causes it to rotate the plane of polarization of the transmitted light by 90° and shutter it. In conjunction with this testing system, a water reward system was developed to provide the monkeys with a controlled dosage of water according to their response or non response to visual images.

Another visual stimulus system involves the use of a slide projector to project the images on a ground glass screen directly in front of a monkey who is tethered to a chair to pick up EEG recordings. A capacitive touch panel is provided for positive response to the image and a resistive touch panel is provided for the negative response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10197-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Experimental Parameters on Derived SSD's

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

C.R. Swyt Physical Scientist BEIB DRS
R.D. Leapman Special Expert BEIB DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging & Microspectroscopy - Office of the Chief

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

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

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

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

The derivation of a single scattering distribution (SSD) from a collected electron energy loss (EELS) plural scattering spectrum (PSS) may fail if the collected spectrum incorrectly represents the amount of plural scattering. Errors in elemental edge identification and quantitation for the thicker (greater than about 1000A in carbon for 100keV beam energy) biological specimen are then compounded.

In order to determine the experimental condition which is the source of the particular error in the collected spectrum, software was written to generate plural scattering spectra from a given single scattering distribution. These spectra weregenerated with modifications to simulate the effects of various experimental conditions that cause the amount of plural scattering to be incorrectly estimated in a collected spectrum.

The derived incorrect SSD's were catalogued with the experimental condition that is the source of the particular distribution. This catalogue will help the investigator to identify and correct the experimental conditions which have modified the collected plural scattering spectra. This should yield greater accuracy in edge identification and quantitation.

OBJECTIVES: The objective of this work was to correlate incorrect derived single scattering distributions with the experimental condition that is the source of a particular form of incorrect distribution.

METHODS EMPLOYED: A FORTRAN program was written which generated plural scattering distributions for any specimen thickness to mean free path ratio from a single scattering distribution by the appropriate spectral convolutions using real fast Fourier transforms. Spectra simulating various experimental conditions which yield incorrect collected plural scattering distributions were generated and the single scattering distributions derived.

MAJOR FINDINGS: The experimental conditions which most often lead to failure of the single scattering derivation are 1) detector saturation at the zero loss peak, 2) thickness variations in the analytical volume, and 3) contamination build up or specimen drift.

SIGNIFICANCE: The cataloged incorrect single scattering distributions can be compared to that derived from a collected spectrum. The experimental condition affecting the data acquisition can be identified and corrected. This will permit accurate element identification and quantitation from electron energy loss spectra.

PUBLICATIONS

C.R. Swyt, R.D. Leapman, "Removal of Plural Scattering from EELS: Practical Considerations", Microbeam Analysis -1984, (in press).

	PROJECT NUMBER
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	Z01 RS 10198-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Concurrent Dynamic Focussing of Two WDS Spectrometers

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: C.R. Swyt Physical Scientist BEIB DRS
Other: C.E. Fiori Physical Scientist BEIB DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD   20205

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

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

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

The Cameca MBX Microprobe has been interfaced to the DEC PDP 11/60 computer controlled Hitachi H700H TEM-STEM elemental imaging system. Elemental x-ray image acquisition is now possible on the microprobe with either the energy dispersive (EDS) or wavelength dispersive (WDS) detectors. In many cases the WDS detectors are preferred to the more efficient EDS detector because of their superior resolving power and high peak to background ratio. Also for many speicmens an image magnification of 100x or 200x is most informative. At these magnifications the large area (1mm at 100x) which is digitally scanned results in WDS spectrometer defocussing relative to the source of the x-rays and thus artifactual variation in pixel intensity across the elemental images.

In order to overcome the defocussing problem, software to dynamically focus both spectrometers in synchronism with the scanned electron beam was written. The 11/60 computer controls two Canberra Industries Axis Positioners using their Model 6726 Telecomputer Interface as the executive link.   The spectrometer crystals are driven to the focussed positions for each beam location by controlling two positioner stepping motors.   The focussed positioner settings are calculated from calibration files of beam coordinates at image limits and corresponding focused spectrometer settings obtained with standard specimens for each element of interest.

OBJECTIVES: To obtain low magnification elemental x-ray images with the Cameca MBX wavelength dispersive spectrometers, free of intensity variations due to spectrometer defocussing.

METHODS EMPLOYED: A FORTRAN program to run simultaneously with image acquisition software on the DEC PDP 11/60 computer was written to adjust WDS spectrometer crystal positions to maintain focus as the electron beam of the Cameca MBX Microprobe is digitally stepped across a specimen. A Canberra Industries 6726 Executive module provides full duplex serial communication between the 11/60 computer and 6648 Axis positioners which control the position, speed and direction of two stepping motors to position the crystals.

MAJOR FINDINGS: The ability to dynamically focus both spectrometers has been of particular importance in imaging Ca and A in the collaborative work on neurofibrillary tangles (NFT) being done with scientists in LCNSS of NINCDS. The low magnification images of the distribution of these elements in the pyramidal cell region of the hippocampus of the brains of subjects known to have suffered from amyotrophic lateral sclerosis and parkinsonism-dementia on Guam have provided, in a few images, the large amounts of data necessary to verify the hypothsis that these elements are involved in NFT formation.

SIGNIFICANCE: Low magnification x-ray images free of intensity variations due to spectrometer defocussing will enable researchers to obtain quantitative x-ray data on the distribution and concentrations of elements of interest from up to 512x512 analysis points distributed uniformly over up to 1mmx1mm areas of specimen from one computer controlled acquisition. Because the WDS spectrometers can be dynamically focussed simultaneously, images for two elements can be acquired and compared without problems of image registration or modification of specimen chemistry between the images.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RS 10199-01 BEI

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Derivation of Single Scattering Distributions from EELS Spectra

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*
C.R. Swyt Physical Scientist BEIB DRS
R.D. Leapman Special Expert BEIB DRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biomedical Engineering and Instrumentation

SECTION
Electron Beam Imaging and Microspectroscopy

INSTITUTE AND LOCATION
DRS, National Institutes of Health, MD 20205

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

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

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

Plural scattering effects in an electron energy loss spectra (EELS) acquired from biological sections of thickness greater than about 1.0 mean free path (600 A at 100keV beam energy for carbon) may lead to errors in elemental edge identification and quantitation.

A large FORTRAN software package has been written which allows the investigator to derive the single scattering distribution (SSD) from a collected plural scattering spectrum (PSS). Formulations for the SSD were developed for spectra with small dynamic range and low energy loss edges and for spectra requiring amplifier gain change but with edges that can be background subtracted. Algorithms were then developed for each case. The required deconvolutions are performed using a real fast Fourier transform (FFT). The fourier coefficients (FC) of the SSD may be extracted from the FC of the total PSS using a logarithmic formulation or from the FC of the background subtracted core edge distributions divided by the FC's of the low loss distribution.

OBJECTIVES: The objective of this work was to develop algorithms and software to extract single scattering distributions from electron energy loss spectra from thicker specimens.

METHODS EMPLOYED: Two algorithms for extracting the SSD from a PSD were developed. The first gives the FC's of the SSD in terms of a logarithm of the Fourier coefficients of the complete PSD deconvoluted by the zero loss contribution. This approach is useful for spectra with edges at low energy losses which cannot be background subtracted. The other algorithm developed is applicable to spectra with large dynamic range and edges that can be background subtracted. The background subtracted core edge distribution is deconvoluted by the low loss distribution to give the SSD. The programs are written in Dec FORTRAN-77 using real fast Fourier transforms. Each approach has been tested on spectra acquired on the Hitachi H700H TEM-Stem in our laboratory and spectra submitted for analysis by researchers in other laboratories.

MAJOR FINDINGS: The algorithms developed are successful for specimens up to several mean free paths in thickness.

SIGNIFICANCE: Frequently, the thickness OF the only available specimen is greater than a small fraction of the mean free path and plural scattering will occur resulting in complicated spectra with intensities redistributed compared to the single scattering distribution. The identification of the elements in the analytical volume may be difficult and quantitation impossible. This program allows the investigator to remove the plural scattering from the spectrum so that specimens up to several mean free paths in thickness can be analysed. Though written for the computer interfaced Hitachi H700H TEM-STEM system, the software is completely transportable and has been requested by a number of investigators at both university and commerical laboratories including Westinghouse Hanford, Tracor Northern and the University of Alberta.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE	PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT	Z01 RS 10200-01 BEI

PERIOD COVERED

October 1, 1983 to September 30, 1984

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

Cell Culture Incubator

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

C.P. Mudd Biomedical Engineer ACES, BEIB, DRS
H.W. Tipton, Mech. Engr. Tech, ACES, BEIB, DRS
J. Robbins, Research CC

COOPERATING UNITS *(if any)*

CC

LAB/BRANCH

Biomedical Engineering and Instrumentation

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

DRS, National Institutes of Health, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

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

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

This project is set up to proceed in two phases. In phase 1, a standard incubator will be modified to allow uniform, low level irradiation of cell cultures while maintaining control of temperature, humidity and CO_2. The cell holders will be mounted on a carousel and rotated to ensure uniform exposure. The top of the incubator will be removed and a lexan top used to allow the passage of radiation.

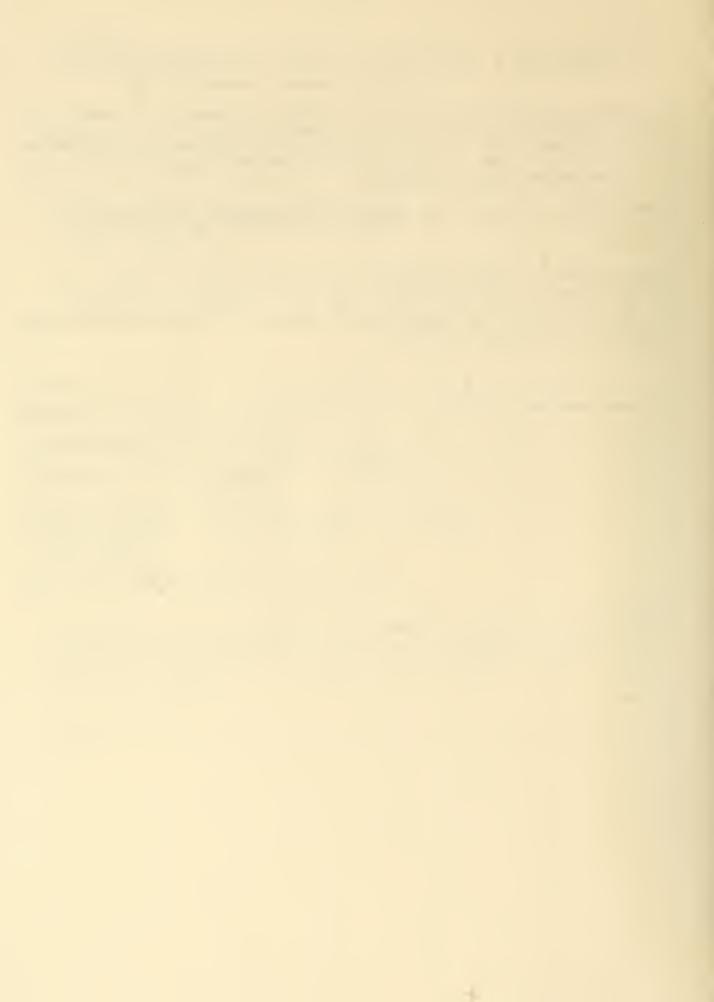Based upon the results in phase I, a larger version will be constructed for phase 2.

OBJECTIVES: To provide an environment with controlled temperature, humidity, and CO_2 in which cell cultures can be irradiated uniformly for periods up to 8 hours. The temperature must be maintained at 37°C ±1°C, the CO_2 at 1%, and humidity at 95 to 100%. The entire unit must be mounted on a cart for easy storage during non-irradiation periods.

SIGNIFICANCE: This study is an attempt to determine the relationship between irradiation levels and the ratio of malignant cell mortality to normal cell viability.

PROPOSED COURSE: A standard incubator will be used in the first phase of this project to provide the temperature, humidity and CO_2 control. The unit will be modified to hold and rotate the cell cultures and provide a radiation-transparent top. Based upon the results of this smaller version, a larger unit will be constructed later.