NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

ANNUAL REPORTS

DIVISION OF INTRAMURAL RESEARCH

OCTOBER 1, 1986 TO SEPTEMBER 30, 1987

PREFACE

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is responsible for research on a wide spectrum of diseases affecting virtually every family in the Nation. These diseases are among the most common, chronic, disabling and costly facing us; they afflict millions of Americans of all ages, backgrounds and economic circumstances and constitute a tremendous drain in terms of human suffering and economic costs.

NIDDK's research programs, taken together encompass the various disciplines of internal medicine, less the cardiovascular system, allergy, and infectious diseases. Certain common scientific and biomedical denominators can be found in this broad array of diseases. Many of them overlap, with a common thread of molecular biology, cellular biology, endocrinology, metabolism, immunology, and nutrition running throughout. These shared fundamental mechanisms result in a unique symbiotic and synergistic effect on the Institute's research programs. New knowledge generated in one group of diseases clarifies and contributes to progress against the others. Thus, the Institute represents a unique entity: externally it serves multiple interests, but internally its programs are intertwined and benefit from this close relationship.

It is the goal of all our studies, intramural or extramural, to create new knowledge which would ultimately permit us to control, treat or prevent the diseases within our purview. Such new knowledge must begin at the cellular and subcellular level, in basic research studies, and be extended, where promising, to clinical investigations. We are justly proud of the distinguished record of achievement of the Institute's Division of Intramural Research in this long and arduous process and want to acknowledge warmly the skill and dedication of NIDDK's own.

This report is intended to chronicle the research advances of the past year, record ongoing intramural studies and indicate opportunities and plans for the future. We hope that it will convey to the reader a spirit of challenge and achievement and induce him to take an appreciative glance behind and a hopeful look ahead.

Phillip Gorden, M.D.
Director
National Institute of
Diabetes and Digestive and Kidney Diseases

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Annual Report of the

Mathematical Research Branch

National Institute of Diabetes and Digestive and Kidney Diseases

Current research projects of the Mathematical Research Branch reflect a broad range of interests in the development and application of theoretical models as well as quantitative methodologies to biological systems.

This research involves several different collaborations within the Branch and with other research groups, both at the NIH and elsewhere. This report describes recent work in the areas of molecular biology, synaptic neurobiology, electrical oscillations in nerve and secretory cells, auditory physiology, cell energetics, renal physiology, and microcirculation and facilitated transport.

Molecular Biology

We are refining previously developed algorithms for sequence comparisons. Our previous methods rapidly scanned the two dimensional matrix identifying subsequence alignments allowing mismatches but no gaps for an initial similarity score, and then performing a complete optimization in a band encompassing the most similar segment. The new methods perform an optimization of the subsequence alignments found in the initial scan to obtain an initial score which allows for insertions and deletions with virtually no increase in computation time. This algorithm has been implemented in nucleic acid and protein search programs. The programs allow the user to specify any scoring matrix. A tool for the evaluation of statistical significance of similarities has also been enhanced to include the new comparison method and a "local shuffle" in the Monte Carlo analysis, which will help distinguish biologically significant similarities. (Lipman and W. Pearson, U. Va. School of Med.)

We have extended the above approach for the analysis of local similarities between longer amino acid or DNA sequences. All subsequence similarities above a user specified threshold are located. A local optimization is carried out in a band centered around each subsequence as follows: starting at one end of the subsequence the optimization continues until all possible alignment scores go to zero. The position of maximum score becomes the starting point for an optimization which proceeds in the opposite direction until all scores go to zero. A traceback is made starting at the new maximum score position. This gives a high resolution view of the subsequence and its flanks. The sensitivity and selectivity of this method is easily controlled by the user and the output may be as a standard alignment or in graphic matrix form. (Lipman and W. Pearson, U. Va. School of Med.)

The construction of multiple alignments of proteins can often reveal important aspects of the evolutionary and functional relationships in a protein family that will not be seen using pairwise alignments. We have developed a method for the simultaneous alignment of up to 5 amino acid sequences. The computational complexity of the task is reduced by first finding short regions of maximal multiple similarity using a modification of a recently developed method, and aligning the remaining sequence with a modification of the standard optimization. (Lipman and Polner)

We have made an algorithmic breakthrough in the problem of multiple sequence alignment. We prove that knowledge of the measure of an arbitrarily chosen alignment can be used in combination with information from the pairwise alignments to considerably restrict the size of the region of the N-dimensional lattice which must be considered in the optimization process. The reduction implies fewer computations and less memory space needed to carry out the dynamic programming process. Our observations also suggest new variants of the multiple alignment problem. (Lipman and Carrillo-Calvet)

We are working on a practical tool for the multiple alignment of amino acid sequences based on an implementation of the algorithm mentioned above. The program is being written in the C programming language it will run on a wide variety of computers. It includes biologically realistic scoring rules and gap functions and allows the user to force specific alignment points if desired. The tool should allow the multiple alignment of up to 10 amino acid sequences. (Lipman and L. Fitzpatrick, National Library of Medicine)

Efforts to inform and educate molecular biologists about the new computational tools for sequence analysis have continued. We have experimented with a new format for teaching the underlying concepts and practical use of tools for the analysis of sequence similarity. A heavily oversubscribed one day course was given in cooperation with the National Library of Medicine. For each problem area, a short lecture was given, followed by "hands on" sessions working out specially chosen biological problems with state of the art software. There were no more than three scientists per microcomputer. The initial response has been very positive and a repeat course will be given for those who were passed over in the first course. Future courses are being planned to deal with other applications of computers in molecular biology, perhaps bringing in extramural scientists as teachers. (Lipman)

In related work, consultations have been conducted with the National Library of Medicine in their efforts to get more involved in the computational problems in molecular biology. In particular, we are working on improving the integration of the GENBANK DNA sequence database, the PIR amino acid sequence database, and the Brookhaven Protein Structure Database. The goal is to give experimentalists easy access to these databases and pro-

vide links between corresponding data in the databases. Tools will be provided for flexible access and appropriate analyses of these vital (and rapidly growing) sources of information. (Lipman)

Synaptic Neurobiology

Estimating the electrotonic structure of neurons. The usual formulae for determining the electrotonic structure of a neuron (Rall, 1969) assume that the neuron can be approximated by an equivalent cylinder with uniform membrane properties. However many neurons cannot be adequately approximated by an equivalent cylinder and their membrane properties may not be uniform, possibly due to a shunt produced by electrode penetration. Using models which include these complications (e.g., a large shunt at the soma, or a cell represented as two cylinders with unequal lengths) we have found significant discrepancies from naive application of equivalent cylinder formulae. Smaller discrepancies are seen with tapering structures. (Holmes and Rall)

In order to provide a method to estimate the electrotonic structure of a cell which cannot be approximated as an equivalent cylinder, we have endeavored to set up and solve a constrained inverse problem. That is, given a set of experimental parameter estimates (such as $_0$, $_1$, C_0, C_1) and an appropriate approximate simplified geometry, find (using suitable constraints) values for a set of unknown parameters (such as $_m$, L, and reflecting the electrotonic structure of the cell. We have done this for specific choices of knowns and unknowns and geometries, and we plan to generalize the procedure to take advantage of higher certainty of different sets of experimental parameters in different situations. (Holmes and Rall)

Workshop on Reassessment of Dendritic Neuron Models. Based upon Dr. Rall's proposal, The Neuroscience Institute (at Rockefeller University in NYC) is sponsoring a small workshop (August 6-12, 1987). This workshop will include Dr. Stephen Redman (Australian National Univ.), Dr. Julian Jack (Oxford Univ.), Dr. Idan Segev (Hebrew Univ. of Jerusalem), as well as Drs. Burke, Holmes and Rall, from NIH. The participants hope to analyze, discuss, and reach a consensus on several implications of trying to fit more complex theoretical models to the much more comprehensive anatomical and electrophysiological data that has recently become available, especially for spinal motoneurons. Some of the issues are described in the preceding paragraphs. By exploring several problems very thoroughly, we hope to agree on some useful guidelines for others who wish to apply dendritic neuron models to their experimental data. (Rall et al.

Work has continued on a biophysical theory to describe neuronal integrative properties which involve large numbers of excitable and/or passive dendritic spines. As outlined in previous reports, we have formulated a new cable theory in which the distribution of spines is treated by a continuum rather than dis-

crete approach. To explore continuum spine models with multiple dendritic branches, and with Hodgkin-Huxley-like membrane properties, we have developed new computational tools. We have investigated threshold properties, e.g., minimum number of synaptically-activated spines, or minimal density of spines, for the initiation and spread of activity. We have compared the effectiveness for synaptic amplification of placing excitable channels either in the spine head or on the dendritic shaft. We have also demonstrated that appropriate clustering of spines can enhance synaptic amplification and spread of activity. Finally, we have confirmed the results of Rall and Segev, 1986, namely that many spines on some branches may fire without spines on all branches firing. (Baer and Rinzel)

Long-term potentiation (LTP). In certain cells of the hippocampus repetitive high frequency afferent stimulation may result in a large increase in the size of the excitatory postsynaptic potentials (EPSPs) and this increase may last for a long time. This increase has been called long-term potentiation and is believed to be a possible mechanism for memory. Evidence suggests that LTP may occur at synapses on spines at which glutamate may activate NMDA and non-NMDA channels. NMDA channels mediate a Ca^{++} conductance which is blocked in a voltage-dependent manner by Mg^{++}. This conductance is negligible at rest due to Mg^{++} block, but increases with increasing depolarization as the block is released. We have modeled the conductance through NMDA channels using various functions designed to mimic published chord conductance plots. We are currently modeling LTP in a dentate granule cell using actual morphology obtained through anatomical studies. The model shows that large increases in Ca^{++} influx may occur depending on the frequency and number of synapses activated. (Holmes and W. B. Levy, Univ. Virginia)

Non-uniform distribution of ionic conductances. We are refocusing our previous studies on the effects of non-uniform Rm to emphasize the effects of non-uniform distributions of different ionic conductances on the effectiveness of synaptic inputs and on the electrophysiological properties in cortical pyramidal neurons. A cortical pyramidal neuron with dendritic spines is used in the modeling studies. The dendritic morphlogy for this neuron was obtained from anatomical studies. Non-uniform distributions of different ionic conductances result in a non-uniform resting membrane potential which has consequences for the effectiveness of synaptic inputs. (Holmes amd C. D. Woody, UCLA)

Electrical Oscillations in Nerve and Secretory Cells

Many theoretical models for nerve membrane behavior exhibit repetitive firing in response to a steady current whose intensity is above a threshold value I_1. (Mathematically, this threshold corresponds to a Hopf bifurcation.) However, if the current is applied as a slow ramp then the critical current level I_j for repetitive firing exceeds I_1, and moreover, it depends on the initial current I_0. We have obtained analytic results which

describe this memory effect and which estimate I_j, in the limit of a very slow ramp and in a noise-free environment. Numerical results show that I_j depends on the ramp speed, R. As R decreases, I_j first increases, but then for smaller R, I_j exhibits a decreasing trend rather than approaching our analytic prediction monotonically. We have obtained new insight into the mechanism for this decrease; we find that it is due to the fluctuations of roundoff error in the numerical calculation. Thus, for a very slow ramp, random fluctuations accummulate and diminish the memory effect. Our results suggest that both deterministic and stochastic approaches will be important for comparing theoretical and experimental results in systems where slow passage through a Hopf bifurcation is the underlying mechanism for the onset of oscillations. In this study, our results were illustrated in the context of an idealized nerve membrane model, the FitzHugh-Nagumo model. (Rinzel, Baer, and T. Erneux, Northwestern University)

The delay or memory effect has been studied for the Hodgkin-Huxley equations to obtain new insights into the phenomenon of nerve accommodation. Previous calculations of Jakobsson and Guttman(1981) had shown for very slow ramp speeds that threshold decreases as the ramp speed decreases. This was found surprising and it was suggested that the HH model, and squid axon in low calcium, exhibit "reverse accommodation", however a mechanism for the phenomenon was not proposed. Our results, as described above, show that "reverse accommodation" is not a pecularity of the Hodgkin-Huxley model, or of nerve membrane, but rather a feature of all excitable systems which exhibit Hopf bifurcations and that it reflects the influence of persistant random fluctuations. (Rinzel and Baer)

Over the past several years we have applied the techniques of singular perturbation and bifurcation theory to investigate a number of theoretical models for bursting oscillations which arise in the context of cellular electrical activity as well as in physical and chemical systems. Several different mathematical mechanisms for bursting have been identified. Our formal analysis of these complex nonlinear oscillations exploits the time scale differences between the fast and slow processes. In a series of intralaboratory lectures/discussions, this approach was reviewed and several fundamental questions were posed which relate to the mathematical justification for our formal and numerical techniques and, in particular, to the method of averaging. In this technique, the details of fast processes for action potential dynamics are not represented explicitly. Rather, one includes the average affect of an action potential on the slow processes, e.g. the net change in intracellular $[Ca^{++}]$ per action potential, and then a reduced model for only the "averaged" slow processes is studied. An idealized model of parabolic bursting (which includes action potential phase but not amplitude) has been formulated to serve as a test problem for extending further our formal averaging technique. (Rinzel, Baer, Sherman, and Carrillo-Calvet)

The Chay-Keizer model accounts for several aspects of the electrical bursting activity of pancreatic B-cells. However, it assumes a homogeneous and perfectly-synchronized islet; it does not include mechanisms for coupling. To study the phenomenon of synchronization, and to explain the fact that isolated B-cells do not show bursting, but rather chaotic spiking behavior, we have begun a study of coupling mechanisms. We have made significant extensions to the Chay-Keizer theory in order to model an isolated cell. Our reformulation replaces the Chay-Keizer modified Hodgkin-Huxley kinetics, and incorporates recent data from the whole cell voltage clamp experiments by Rorsman and Trube. Also, our model incorporates the fact that the calcium activated potassium (K-Ca) channel, which is thought by many to switch the cell between the active and silent phases, is large and only rarely open. Therefore, random single channel events can have a strong perturbing effect on the cell, producing irregular spiking. By treating the K-Ca channel as stochastic, our simulated single cell behavior looks strikingly like the experimental records. When many cells are tightly coupled electrically (with zero resistance gap junctions) then they, in effect, share the conductance of the K-Ca channels and organized bursting results as the number of cells in the cluster is increased. This biophysical hypothesis of channel-sharing was first formulated by Atwater and colleagues. (Sherman, Rinzel, and J. Keizer, University of Calif., Davis)

Auditory Physiology

The processing of complex sounds is examined theoretically and in model simulations at all stages of the auditory system. At the periphery, detailed cochlear models have been developed to account for the mechanics of basilar membrane motion and the biophysics of haircell function. Over the last year we have completed a study of the effects of haircell nonlinearities in the responses to multiple tones. We have also initiated a new study into the micromechanics of outer haircell-basilar membrane interactions. We have concluded a detailed study of the parameter sensitivities of a detailed model of the basilar membrane. (Shamma, Chadwick, Morrish, and Rinzel)

The models for cochlear processing are now used to generate regularly the cochlear responses to various speech sounds. Neural networks have been developed over the last year to accomplish the following tasks: (A) Generate accurate estimates of the spectral parameters of the sound using both temporal and spatial cues in the cochlear response patterns. (B) Binaural network to account for many attributes of spatial hearing using relatively simple topologies and no neural delays. (Shamma)

Adaptive algorithms mimicking supervised and unsupervised learning, memory and pattern recognition in central neural networks were developed and implemented. They were used to organize and classify American English vowels and to describe the acoustic features of fricative sounds. (Shamma)

Cell Energetics

A diffusion model of ATP and its byproducts has been developed for a cell system with ATP production and consumption. The model consists of a spherical cell with mitochondria in its interior and Na - K pumps distributed at its periphery. Species concentrations are obtained by solving a system of reaction diffusion equations in spherical coordinates. Chemical reactions include oxidative phosphorylation at the mitochondria and dephosphorylation in cytosol and at the plasma membrane dependent on equilibrium and non-equilibrium kinetics. Concentration profiles obtained for an MDCK cell, an established cell line from the dog kidney, have shown a significant concentration gradient for ADP but no limitation on the availability of ATP at the plasma membrane for physiologic rates of transport, even when the diffusivity of all species is reduced by two orders of magnitude in the plasmalemma. We have shown that in MDCK cells a diffusion to distance ratio for active sites greater than 0.1 cm/sec imposes no restriction on the availability of ATP. Ratios less than that pose a significant limitation. (Mejia and Lynch)

Renal Physiology

Theoretical and experimental work on a model for acid-base balance in the mammalian kidney is proceeding. Last year we developed a cylindrical model of a perfused renal tubule in a bath that computed the concentration of total CO2, net acid, total ammonia and a third buffer. We have now implemented a canonical tube model to be used in a model of the whole kidney. It is one-space-dimensional and includes differential equations in space and time for solute, flow and charge conservation as well as equations of motion and Henderson-Hasselbalch equations for chemical buffers. A fourth order difference scheme in space with Hermite interpolation and explicit integration in time has been used. This scheme is ammenable to partitioning of the tube equations from the bath or interstitium and embedding into a continuation algorithm, CONKUB, for solution as a function of model parameters. A comparison of solutions obtained from the cylindrical and axial models has shown that solutions of the latter tend to be bounded by wall and centerline values of the former. However, under conditions of active transport, for example, substantial radial gradients are observed. Transport parameters needed for realistic models will be measured in in-vitro laboratory experiments in the coming years. (Mejia and Knepper)

In the theory of acid-base balance, controversies concerning mechanisms of pH regulation are hinged in part on a lack of agreement about what is acid-base balance. We have continued the process of developing a rigorous description of pH balance that is based on physical principles. The hydrogen ion concentration of a control volume is determined by proton balance, where a control volume is any geometrically closed space with definable

inputs and outputs (e.g. a beaker, a cell, a kidney, etc.). Any general theory of proton balance should apply to any such control volume. Hence, the hydrogen ion concentration of a control volume must satisfy mass, volume and charge conservation equations, including electro-neutrality. The canonical tube model described previously is an example of an application of these basic principles. We plan to apply it to other control volumes. (Knepper and Mejia)

Microcirculation and Facilitated Transport

Myoglobin can facilitate the transport of oxygen in tissues. Oxygen can proceed along two channels, as free or combined oxygen. Moreover, there are membranes in the tissue which are impermeable to the carrier. Thus, the diffusion coefficient is discontinuous and this leads to an interface condition in the diffusion-reaction equations that describe the process.

To solve this class of problems, we have developed a new approach which involves the analysis of a boundary layer representation at each interface. Between adjacent membranes, the substrates are assumed to be in near chemical equilibrium. Matching conditions convert the problem into a system of nonlinear algebraic equations. This approach is computationally more efficient and more stable than our previous methods. Moreover, it allows us to write partly analytic expressions for the solution of the system, from which we obtain valuable physiological insights.

For example, we have identified analytically the following features. In a neighborhood of each membrane there are transfers between the flow of free and the flow of combined oxygen. There is a formal relation between these transfers which involves the shape of the oxygen-carrier dissociation curve. In order to effect this transfer through the oxygen-carrier chemical kinetics, a discontinuity on the combined oxygen concentration appears at the membranes.

The expressions also allow for an analytic discussion of the dependence of the facilitated transport on the parameters of the process. We find: (1) In the literature the value of the "off" coefficient of the oxygen-carrier chemical kinetics is emphasized. It was found here however that, for the steady state, the loading and unloading processes depend also on the "on" coefficient. (2) From the simple concept of two additive channels it was expected that less facilitated transport will occur when the free oxygen channel is enhanced. It was shown however that an increment on the diffusion coefficient of free oxygen in fact increases the amount of facilitated transport. (3) When the transport path is relatively short and no chemical equilibrium can be established inside the path it was assumed that the facilitated transport will be smaller than otherwise. It has now been shown that in some cases the opposite can occur; the condition of chemical equilibrium can effect an "excessive" loading or unloading.

Finally, we have extended our model for the transport-reaction process to include discrete spatial regions where consumption of oxygen takes place. These regions correspond to mitochondria in the transport path. Inside the mitochondria, no carrier (myoglobin) exists, and oxygen is consumed according to Michaelis-Menten kinetics. The boundary of a mitochondrion is impermeable to the carrier. The model involves two diffusion-reaction systems (one for the cytosol and the other for the mitochondria) and interface conditions at the cytosol-mitochondrion boundaries. (Gonzalez-Fernandez)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 13,001-14 MRB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical formulations and analysis relevant to experimental neurophysiology.

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

PI:	W. Rall	Senior Research Physicist	MRB, NIDDK
Others:	W. R. Holmes	NRSA Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*
Lab. of Neural Control, NINCDS
Dept. of Zoology, Univ. of California, Berkeley
Dept. of Anatomy, Yale University
Dept. of Neuroscience, The Hebrew Univ. of Jerusalem

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.0	.3

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

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

RESEARCH AREA. Basic neuroscience involving structure/function relations for such neuronal structures as synapses, dendritic branching, and dendritic spines (as well as neuron populations with cortical symmetry), and for such functions as synaptic transmission, amplification and dendro-dendritic interactions in the context of spatio-temporal input patterns, logical processing of input, and neural plasticity, as in conditioning and learning.

RATIONALE. To combine experimental data from neuroanatomy and from electrophysiology with biophysical models of nerve membrane (passive, synaptic and excitable) into a comprehensive theory which can lead to new insights and to testable theoretical predictions (which can, in turn, be used to design better experiments), it was necessary to create, explore and test mathematical and computational models (of increasing complexity).

METHODOLOGY. Our methods include both analytical solutions and computational solutions of boundary value problems (involving partial differential equations) in the tradition of classical physics. They include also formulation and solution of problems in terms of systems of ordinary differential equations; when this is done explicitly for a compartmental model of a neuron, it is possible to accomodate a remarkable variety of dendritic branching and non-uniform distributions of membrane properties and synaptic inputs.

RESULTS. Earlier results are summarized in Chapter 3 of "The Handbook of Physiology: The Nervous System, Vol. 1", Kandel, Brookhart, & Mountcastle, eds.; American Physiological Society (1977). More recent results are described in Chapter 22 of "Synaptic Function"; Edelman, Gall & Cowan, eds.; Wiley (1987).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13,002-15 MRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mathematical description of substrate transport in capillary-tissue structures.

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

PI: J. M. Gonzalez-Fernandez Research Mathematician MRB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.03	1.0	.03

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of this work is to develop mathematical models of the blood flow and transcapillary exchanges in capillary networks. An effort is being made to incorporate in the models the histological structure of capillary networks as well as different flow patterns from available experimental information. In this model the extraction of substrates with different chemical kinetics at the tissue site will be described. It is expected that this could be used in experimental situations where the extraction of different substrates are measured simultaneously, thus helping to infer the flow pattern features of the microcirculation. In particular a model of the diffusion-consumption of oxygen in striated muscle containing myoglobin (facilitated diffusion) is being developed and pertinent numerical results examined.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 13,004-13 MRB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical description of cellular neuroelectric signal transmission.

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

PI:	J. Rinzel	Chief, MRB	MRB, NIDDK
Others:	S. M. Baer	Staff Fellow	MRB, NIDDK
	A. S. Sherman	NRC Fellow	MRB, NIDDK
	H. Carrillo-Calvet	Visiting Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*
Lab. of Cell Biology & Genetics, NIDDK
Dept. of Chemistry, Univ. of California, Davis
Dept. of Eng. Science & Appl. Math., Northwestern Univ.

LAB/BRANCH
Mathematical Research Branch

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	3.0	.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.)*

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of neuroelectric signaling for individual neurons. Among the topics of current interest are: (i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) propagation of action potentials along axons; (iii) stimulus-response and threshold properties for repetitive-firing of action potentials; (iv) complex bursting patterns of membrane potential oscillations which arise through endogenous membrane properties and/or interneuronal coupling.

Because qualitatively related mathematical or biophysical problems may arise in other contexts, e.g. chemical and biochemical oscillations, or e.g. excitation-secretion coupling, this project may consider models from such applications.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods drawn from both classical and modern applied mathematics. These methods may include finite difference or finite element numerical integration, bifurcation theory, perturbation techniques, and nonlinear dynamical systems theory. One goal of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet physiologically reasonable, equations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13,014-06 MRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Probabilistic Analyses of Nucleic Acid Sequences.

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

PI:	D. J. Lipman	Research Scientist	MRB, NIDDK
Others:	G. P. Polner	Visiting Fellow	MRB, NIDDK
	H. Carrillo-Calvet	Visiting Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)* Physical Sciences Lab., DCRT
Biophysics Lab., BRR, Food & Drug Admin.
Dept. Biochemistry, U. Virginia School of Medicine

LAB/BRANCH

Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.94	1.9	.04

CHECK APPROPRIATE BOX(ES)

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

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

This project has focussed on the analysis of amino acid and nucleic acid sequence data as it pertains to molecular biology and molecular evolution. Continuing areas of interest include:

The development of computational tools for molecular biologists. We developed a complete set of refined sequence comparison tools for molecular biologists which will soon be widely distributed. We have made an algorithmic breakthrough in the problem of multiple sequence alignment and are developing a practical implementation of the method.

We have continued our work on the relationship of amino acid sequence to protein structure and function. In one area we have new results on the relationship of amino acid hydrophobicity and solvent accessibility. Another ongoing project is the analysis of patterns of sequence conservation and its relationship to structure and function.

We are developing new and more effective formats for educating experimental molecular biologists on the use of computational tools. A recent workshop combining lectures and "hands on" problem solving was held in conjunction with the National Library of Medicine.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13,015-06 MRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Probabilistic modeling of biological information systems.

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

PI: W. J. Wilbur Guest Worker MRB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 13,017-04 MRB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Sound processing in the auditory system.

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

PI:	S. A. Shamma	Guest Worker	MRB, NIDDK
Others:	J. Rinzel	Chief, MRB	MRB, NIDDK
	R. Chadwick	Biomedical Engineer	BEI, DRS
	K. A. Morrish	Staff Fellow	MRB, NIDDK

COOPERATING UNITS *(if any)*

Biomedical Engineering & Instrumentation Br., DRS

LAB/BRANCH

Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION

NIDD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	.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 project involves research on the processing of speech and complex sounds at three levels of the auditory system:

1. Peripheral stages - theoretical models of cochlear function to explain the results of various physiological and psychophysical experiments.
2. Neural network models to process and extract important parameters of speech and other sounds for both monaural and binaural hearing.
3. Learning algorithms mimicking adaptive central auditory neural networks to perform storage and recognition tasks.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 13,018-03 MRB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI: K. A. Morrish Staff Fellow MRB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 13,019-03 MRB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical study of excitability properties in coupled nerve membrane patches.

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

PI: S. M. Baer Staff Fellow MRB, NIDDK

COOPERATING UNITS *(if any)*
Dept. Engineering Sciences & Appl. Math., Northwestern Univ.
Dept. of Biological Sciences, Florida State U., Tallahassee

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.60	1.58	.02

CHECK APPROPRIATE BOX(ES)

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

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

Biophysical theory regards the electrophysiological interaction between passive and active patches of nerve membrane as functionally significant. Important examples include dendritic spines with both passive and excitable spine head membrane, myelination, and the interaction between dendrite and axonal membrane.

The aim of this project is to explore, using mathematical modeling, analysis, and numerical computation the functional implications of these interactions.

Last year we expanded our research efforts to include a new class of excitability problems involving a slowly-varying control parameter. We are pleased to report that this study revealed some new and valuable insights into the biophysical phenomena of accommodation.

Areas of research initiated this year include the evaluation and study of the dynamics of an alternative mathematical model of the Hodgkin-Huxley squid data and applications of cable theory to assist experimenters in understanding the function of the first synapses in the visual system.

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

ANNUAL REPORT OF THE LABORATORY OF CELLULAR AND DEVELOPMENTAL BIOLOGY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

This laboratory conducts research in a wide variety of areas which are united by a common theme of relationships to development and differentiation. Most of the research also has a methodologic common thread in using the techniques of modern molecular biology. In a spectrum of activities that range from atomic level structural studies to investigations of mammalian development, each working group has productive interactions with several other groups, making the laboratory more productive and innovative than the sum of its constituents.

As I have done in the past, I will discuss the work of the laboratory by topical areas rather than by individual Sections. This thematic analysis of projects and progress serves to emphasize the cohesive yet eclectic approach that we take to study of basic questions in the biology of cells.

Transitions

Dr. John Kerestezy, Chief of the Laboratory of Nutrition and Endocrinology (our progenitor) from 1964 to 1969, died at the age of 81 in April 1987. Kerestezy attracted B.T. Kaufman to the laboratory and they began a series of important studies on folates and folate metabolizing enzymes together. He was largely responsible for development of the Pilot Plant which has evolved over many years to its current role in biotechnology.

Dr. Sidney Chernick, a Scientist Director in the USPHS, retired at the end of June. Sid had been at NIH since 1953. He was a valued advisor to many at NIH with an encyclopedic knowledge of the endocrine literature. His collaborations with R.O. Scow during their three and a half decades as coworkers were innovative and productive. His presence in the laboratory will be missed.

Macromolecular Structure

One of the research areas that has progressed most rapidly in LCDB over the past five years has been study of the structure and function of the extracellular ribonuclease of B. amyloliquefaciens, barnase, and its intracellular protein inhibitor, barstar. The primary and crystal structure of barnase have been determined by us and our collaborators, respectively. The two proteins form a 1:1 complex and both have simple two-state thermal transitions, making them an ideal system for study of protein folding mechanisms and protein-protein interactions.

In previous reports, we have documented the cloning of genes for both barnase and barstar and described the overproduction of structurally intact but inactive barnase bearing a site directed mutation of the active site residue His-102. The gene for barstar have now been sequenced. The derived protein composition agrees with that previously determined for the

protein except for the presence of two cysteinyl residues, undetected in the amino acid analysis. Plasmids bearing both enzyme and inhibitor genes express active barnase without being lethal to the host, as are plasmids containing the barnase gene alone. Coupling either the barnase or the barstar genes to composite control regions containing highly efficient promoters and sequences which signal protein export and processing has allowed production of large amounts of protein, over 300 mg/L of E. coli culture. The genetic constructions lead to secretion of the protein into the periplasmic space and medium, making purification very easy. As discussed in previous reports, the study of barstar structure and barnase-barstar interactions has been nearly impossible due to the limited amounts of the inhibitor that could be obtained from B. amyloliquefaciens. Cloning and expression of the barstar gene have led to a 10^5-fold increase in the amount of protein obtainable from a bacterial culture, obviating the previous difficulties in such studies.

Now that we have the ability to produce large amounts of both proteins and mutants thereof, what is the course of the project? There is clearly enough work for a large group of scientists and equally clearly interest in this system and related, homologous nucleases from both pro- and eukaryotic sources. We have begun studies on the folding of barnase, showing that mutations of the three prolyl residues to other amino acids alter the thermal stability of the protein without altering the nature or kinetics of the folding transition. A collaboration with four European laboratories has been established to study folding mechanisms and crystal structures of mutant forms of the nuclease. Physical studies of the inhibitor, its derivatives, and interactions of inhibitor and enzyme will be the focus of efforts in LCDB. The barnase/barstar complex structure is of particular interest since it is one of the few enzyme/inhibitor complexes known in which the enzyme is not a protease and the inhibitor a pseudosubstrate.

The second system whose structure has been under intensive investigation in the laboratory for a number of years is chromatin, at the levels of core particle, chromatosome, and 30nm fiber. Several years ago we described the precise association, in vitro, of a sea urchin 5S rRNA gene and chicken erythrocyte histone to form a positioned core particle. Derivatives of this DNA segment have been constructed, amplified and purified as a 146 bp fragment in milligram quantities. This DNA has been associated with histones and crystallized in collaborative studies with T. Richmond and A. Klug of the MRC, Cambridge. Richmond has now obtained x-ray diffraction patterns of these crystals; the pleasing result is that the crystals containing the defined sequence DNA diffract to less than 5 angstroms on the least-resolved axis. This leads to a greater than two-fold augmentation of the crystallographic information available for these crystals vis a vis previously analyzed crystals of core particles which contained random sequence DNA. At the level of resolution afforded by these crystals, it should be possible to accurately follow the path taken by DNA as it winds about the histone octomer, determine the helical periodicity of DNA and the nature of the irregular bends previously detected for the nucleic acid, identify the individual histone molecules and define their interactions with nucleosomal DNA.

We have made tandemly repeated polymeric DNA fragments based on the positioning 5S sequence to use as model systems for the study of higher order chromatin structure. Repeat lengths from 160 to 255 bp with repeat numbers from 3 to >50 have been constructed. Previously, we reported that core histones associated properly with such DNA fragments and provided evidence that some degree of higher order folding occurred with the histone octomer alone. We have continued efforts to further add to such complexes the H1/H5 histones which are known to be of importance in formation of the 30nm fiber of chromatin. Unfortunately, the goal has not yet been achieved. Given the importance of understanding this level of chromatin structure, the level which has been most often implicated as being pivotal in regulation of transcriptional activity of the eukaryotic genome, we will continue to pursue such studies.

Chromatin structure and transcriptional regulation

Other investigations in LCDB also relate to the structure of the core particle and, by extension, possibly to the role of core particle structure (as opposed to higher order structure) in transcription. DNA undergoes a change in twist as a function of temperature. Analysis of the degree to which DNA in a nucleosome is free to twist can be made by treating minichromosomes with topoisomerase at different temperatures and determining the degree of supercoiling of the nucleic acid after removal of proteins. Such an analysis of DNA associated with chicken histones by Morse and Cantor indicated that ca. 200 bp of DNA were constrained from twisting for each nucleosome present in a minichromosome. Recently, Huberman and his co-workers found that in an in vivo situation, yeast histone only constrained 30% of the DNA from twisting - this could result from other factors labilizing DNA, from a large fraction of the plasmid they studied being transcribed and therefore free to twist or from a fundamental difference in core particle structure for yeast vs. chicken histones.

We have examined these possibilities by study of the thermal untwisting of plasmid chromatin in yeast nuclei, nuclear extracts, and highly purified minichromosomes. Different length plasmids have been used, some containing non-transcribed sequences, as have been plasmids reconstituted with chicken histones. The summation of a number of experiments is quite clear - DNA associated with chicken histones is highly constrained from thermal untwisting, irrespective of the milieu in which it is incubated, and DNA associated with yeast histones is relatively free to untwist, irrespective of the fraction of that DNA which is transcriptionally active. While chicken histone nucleosomes constrain 200 bp of DNA, yeast histones constrain only about 50-100 bp of DNA per nucleosome from untwisting. We speculate that these differences mirror a fundamental difference in nucleosome structure for the two organisms; the possibility that this difference may be related to the fact that >50% of the yeast genome is transcribed (as opposed to ca. 3-5% of the chicken genome) is intriguing.

Another study has asked what the role of nucleosome structure in transcriptional regulation might be. A plasmid containing a frog 5S gene was assembled with chicken histones in vitro and then transcribed in a X. borealis extract which is TFIIIA dependent. Increasing amounts of histones

associated with the DNA led to decreases in the level of transcription, suggesting that nucleosomes interfered with either initiation or elongation or both. Using two restriction endonucleases, with recognition sites near the initiation site or well distal to it, we digested the reconstituted plasmid chromatin with one or the other enzyme, and then assessed transcriptional properties. The results show that a plasmid which has a nucleosome near the initiation site is not a substrate for transcription while chromatin which has a nucleosome away from the initiation site, but still on the gene, is capable of being transcribed. From this we suggest that transcriptional initiation is not possible on histone-associated DNA sequences and that transcriptional elongation can proceed along DNA associated with histone octomers.

For a full description of the structure of a transcriptionally regulated gene when it is active and when it is repressed, one would like to study the complex of trans-acting factors with chromatin which has been assembled in vivo. While others have made considerable progress in identification of trans-acting factors based on their interactions with naked DNA, we, coming from a chromatin background, would like to study such factors interacting with histone-complexed DNA, the substrate which the factors approach in the cell. Given this thought, we began several years ago the study of yeast plasmids which exist as chromatin. We detailed the structure of one, the TRP1ARS1 plasmid, and documented the compositional origins of several of its structural features. We then set out to devise methods for purification of the plasmid, as chromatin, and reported two years ago the successful culmination of these efforts.

More recently, we have modified the conventional purification method in a fashion which reduces the period of time necessary for isolation of the chromatin from 4-5 days to a single day. Briefly, we inserted into a nucleosome-free region of the plasmid a 77 bp segment of pUC19 containing the E. coli lac operator DNA sequences. After preparation of spheroplasts and release of the plasmid chromatin from nuclei, we add a fusion protein consisting of galactosidase and the lac repressor. Repressor binds to the chromatin containing the operator sequences. Addition of anti-galactosidase immobilized on beads allows selective removal of the chromatin complex from the lysate. After washing, an inducer of the lac operon, IPTG, is added to release the minichromosome from the insoluble bead complex. The material is 50-90% pure and suitable for many types of analysis without further purification.

By several criteria, including linking number change from naked DNA, histone content and micrococcal nuclease digestion pattern, the chromatins purified by the two methods are essentially identical. Having the ability to isolate yeast plasmid chromatin, we have spent a good bit of time in the past year inserting regulated DNA sequences into such plasmids in order to allow our studies of structure of active and repressed genes to proceed. We have constructed plasmids which include, in addition to the TRP1ARS1 vector DNA and the E. coli lac operator, the PHO5 gene, the HSP26 gene, the yeast 5S rRNA gene, and regulatory sequences abutting the GAL1/10, STE3 and STE6 genes.

An important consideration when studying multicopy (>100/cell) plasmids is whether regulation of the amplified gene is similar to that of a single genomic copy. For the HSP26 gene, we have evidence that this is true. Determination of steady-state levels of mRNA and decay rates for HPS26 message lead to the conclusion that at least half, if not all, of the amplified plasmid genes are regulated by heat or other stress in a fashion like that of the wild type, single copy genomic gene. Consistent with this, recent data indicate that the yeast heatshock regulatory factor identified by others is present in the plasmid chromatin preparation; mobility shift gel electrophoresis analyses using a synthetic oligonucleotide containing the cis-acting regulatory sequences were positive in assays with proteins present in the plasmid chromatin. While realizing the necessity for similar controlling experiments for each gene we study, the current results reinforce our hope that this approach to chromatin structure/function relationships will be a fruitful one.

Development and Differentiation

Yeast gene regulation, sporulation and mating type switching are examples of differentiation or development; we also study these processes in a series of more complex species, including Dictyostelium, sea urchin and mouse.

We have previously detailed studies of gene regulation during Dictyostelium development. Critical findings were (1) identification of a repetitive, simple repeat DNA element, $[A\text{-}A\text{-}C]_n$, which is on the coding strand of a number of nearly-coordinately regulated genes, (ii) demonstration that a protein encoded by one of the genes containing the sequence had partial homology to the ras oncogene family and contained a probable GTP-binding domain, and (iii) demonstration of several modes of gene regulation by cyclic AMP, one involving intracellular nucleotide and likely functioning through the protein kinase mechanism and a second involving extracellular cAMP acting as a paracrine hormone and possibly transducing its signal through the phospholipase C/PIP_2/diacyl glycerol/calcium path.

Our interests in the past two years have turned to definition of the molecular mechanisms for signal transduction to gene regulation by cAMP during Dictyostelium development. Pharmacological experiments provided the clues to lead to the postulated mechanisms listed above. We now are seeking the genes which synthesize the proteins involved in the transduction processes. This pincer approach starts from both the signal, at the periphery of the cell, and the end result, transcription in the cell nucleus.

The ras-like, GTP binding protein is a likely candidate for a transducing molecule in the scheme, based on information concerning the role of guanine nucleotide binding proteins in vertebrates. In collaboration with P. Devreotes at Johns Hopkins University, we have measured the level of translatable mRNA for the cAMP receptor during development. Based on that information, we constructed a cDNA library from RNA isolated slightly prior to the time of maximal receptor activity. Using an antibody to the receptor and this cDNA expression library, we have isolated two clones with largely overlapping restriction enzyme maps that we feel likely to include

the gene for the cAMP receptor. If true, having the genes for two proteins likely to be involved in signal transduction in Dictyostelium and having the ability to transform the organism, developed by others in the past two years, we will be in a position to rigorously analyze the mechanism of gene regulation by cyclic nucleotides during development of this simple eukaryotic organism.

Somewhat more complex in body form than Dictyostelium is the sea urchin. We have previously reported studies of the chromatin structure of urchins during the switch in histone types that occurs during early embryogenesis; the chromatin structure of the early histone genes, a family of genes which are developmentally regulated; and the cloning of a urchin collagen gene, the only collagen gene thus far identified in an early deuterostome.

Due to uncertainties about the relationship between the collagen gene we cloned and the well typed vertebrate collagens, we have synthesized an undecapeptide encoded by the exon of the collagen gene; we specifically picked a region of the gene which contained a discontinuity in the $[Gly\text{-}X\text{-}Y]_n$ motif in hopes that the epitope would be more likely to be exposed at a disruption of the triple helical theme. Antibody to the peptide was produced in rabbits. The antisera labeled a peptide of M_r 208000 in Western blots of total pluteus proteins separated by SDS gel electrophoresis. The peptide was selectively sensitive to digestion by collagenase, confirming its identity as a collagen. Embryo dissociation and tissue fractionation suggested that the peptide was associated with the endoskeleton and its adherent primary mesenchyme cells. In situ labeling of whole mount pluteus embryos by immunofluorescence confirmed the localization of the epitope to the periphery of the endoskeleton; further it demonstrated the absence of the peptide in basal lamina.

Since collagen is a secreted protein, we assessed the site of synthesis of the mRNA by in situ hybridization, in collaboration with L. and R. Angerer. Expression of the message is 80% in primary and 20% in secondary mesenchyme. The timing of synthesis is as previously reported, commencing at blastula and persisting at roughly the same levels through the mature pluteus larva. There are features of the size, structure, and sites of synthesis of this collagen gene that are characteristic of both fibrillar and nonfibrillar collagen genes (proteins) of vertebrates. It remains to be determined whether early deuterostomes have collagens that are equivalent to vertebrate types or have a primitive, multifunctional form of this important protein.

This is the first mesenchyme specific gene defined in sea urchins. Of interest will be characterization of the control of expression of the gene - particularly since mRNA is present in both primary and secondary mesenchyme while the protein product is confined to areas around primary mesenchyme only. Since micromeres (the progenitors of primary and some secondary mesenchyme) can be isolated from the 16-cell embryo and will differentiate in culture, these questions are approachable.

Collagen is known to be important in morphogenesis in sea urchins; we are also studying other proteins involved in cell-cell interactions. Workers in D. McClay's laboratory identified (with monoclonal antibodies) sets of cell surface proteins which were expressed in specific cells. While in that laboratory, one of our group determined that the epitope recognized by one of the monoclonals was carbohydrate, used the antibody to isolate the modified protein, removed the carbohydrate and generated a polyclonal antibody to the protein itself. Using this, we have now isolated the gene for the protein from a cDNA expression library. Characterization of the gene is now underway. This protein is of particular interest in terms of morphogenesis since it appears to be a cell adhesion molecule by in vitro assays.

Generalized or specific factors involved in sea urchin embryogenesis and tissue specification are not known. In previous studies by a current LCDB member and others, it was shown that insulin, insulin-like growth factors, and anti-insulin antibodies altered features of early embryogenesis in chicken. This suggested that peptides related to these vertebrate hormones might play a role in growth of the young embryo. We have now extended such studies to the developing embryo of the sea urchin.

Addition of insulin at concentrations of 10-10000 ng/ml to cultures of urchin embryos just after fertilization leads to differential effects on the amount of mRNA present for three specific genes, Spec1, early histone and collagen, at blastula stage. Message content for the first two genes increases at 10 and 100 ng/ml and decreases at higher concentrations. In contrast, collagen message increases in concentration as insulin is increased over the entire range of doses studied. Insulin binding to membranes of urchin embryos increases about ten-fold from the egg to pluteus larva stage; the level of binding is at least an order of magnitude less than that observed for, e.g., mammalian liver membranes. Immunofluorescence microscopy of pluteus larvae demonstrates a highly specific localization of epitopes which react with an antibody to vertebrate insulin - only the apical section of the embryo, particularly the midgut, contains a reactive protein. If insulin-like molecules are functional in echinoderms, which lack a pancreas, the midgut seems a reasonable location for cells which secrete them.

A still larger organism we study during embryogenesis and early development is the mouse. We have previously documented studies of the synthesis, modification and localization of the three major proteins of the zona pellucida, an extracellular glycocalyx which surrounds the maturing oocyte, fertilized egg and pre-implantation embryo. We have cloned a nearly full length cDNA for ZP3, the zona protein which functions as a species specific sperm receptor, defined the timing and cellular localization of its transcription, and determined its chromosomal localization.

This year we have extended these studies in several significant areas. We have used the cDNA to isolate genomic murine clones spanning nearly 20 kb which encode the ZP3 gene. The clones contain several thousand base pairs 5' to the gene, giving us confidence that controlling sequences important for regulation of this oocyte-specific gene can be identified. We are

currently constructing deletions through the 5' flanking region and coupling these to a reporter gene, luciferase, for microinjection into growing oocytes to perform this analysis. A second approach to identification of regulatory regions has been to clone the human ZP3 gene from a genomic library. Comparison of the flanking sequences of the murine and human genes may reveal conserved features important in regulation of the vertebrate zona genes. At this point, analysis of the murine gene has revealed the presence of at least six exons and the occurrence of a four times tandemly repeated, ca. 60 bp sequence about 1 kb 5' to the transcription start site. This sequence is partially homologous to the Alu family and brain ID sequences. It will be highly interesting to determine if similar sequences flank other oocyte specific genes. In that context, we have recently identified a clone from our ovarian cDNA expression library which seems to contain the coding sequences for ZP2, the major zona protein. The clone was isolated by a similar protocol to that used for the ZP3 clone, primary screening with a polyclonal anti-zona antiserum followed by secondary screening with a ZP2 specific monoclonal antibody. Identification of that clone as the gene coding for this protein remains to be confirmed by sequence analyses of both protein and DNA.

Endocrine Studies

Another group in LCDB also studies mouse early development. However, in this case, the interest arises from a longstanding involvement in lipid transport and particularly the role of lipoprotein lipase (LPL) in same. A recessive mutation known as cld (combined lipase deficiency) in mice leads to deficiencies in activity of both hepatic lipase and LPL in the homozygous state. Affected animals die with massive hyperlipidemia within days after they begin to feed. We have previously reported immunologic studies indicating that the amount of LPL protein in affected animals exceeded that in normal or heterozygous littermates. Since LPL is a glycoprotein, it was thought possible that defects in glycosylation might account for the lack of activity and secretion of the enzyme.

LPL acts at the luminal surface of capillaries; tissue cells other than endotehlium are believed to synthesize the enzyme. We have examined the distribution of LPL in two tissues of normal and cld/cld mice using indirect immunocytochemistry. In heart of normal mice, LPL is present in small blood vessels, near the endothelium. In contrast, lesioned mice lack LPL in blood vessels but contain large amounts of immunoreactive protein in myocytes, supporting the contention that the mutant mice are unable to secrete the enzyme from the cells which synthesize it. In liver of both normal and cld/cld newborn mice, LPL is found within hepatocytes. Double antibody studies suggest that the protein is not in lysosomes. The significance of the observation of others (supported by this study) of the transient presence of LPL in livers of newborn rodents is unknown.

In an effort to provide a more malleable substrate to facilitate the study of the mechanisms of the cld mutation and its effects on lipid metabolism, we began several years ago to establish a tissue culture system for cells from the mutant mice. Initial results were far from encouraging. Recently, we have found that treatment of confluent cultures of cells from

brown fat of cld/cld or normal mice with triiodothyronine, insulin and octanoic acid leads to conversion of >70% of the cells to adipocytes, hopefully solving the problem. Support for this hope derives from observations which indicate that the cultured cells mirror the state of adipocytes in the whole animal. Thus, adipocytes from normal animals release LPL to the medium; those from mutant mice do not. Immunocytochemical studies demonstrate that cells from cld/cld mice contain large amounts of LPL protein while those of normal mice contain only traces at the periphery of the cell. In exact analogy to the studies of heart detailed above, these results show that the mutant mice synthesize a nonsecretable form of LPL while normal mice synthesize and secrete active LPL. This established the validity of the tissue culture system for future studies of this abnormality in lipid metabolism.

Another approach to abnormalities of lipid metabolism has also utilized cytochemical localization of cellular chemicals. Fibroblasts exposed to low density lipoproteins in culture accumulate cholesterol. Using filipin (a fluorescent probe that complexes to cholesterol), antisera to membranes with high cholesterol content, and an antibody to lysosomal membrane proteins, we have shown colocalization of epitopes reactive with all three probes in cultured human fibroblasts incubated with LDL. This indicates that the accumulated cholesterol is present in the lysosomes of the cultured cells. We will now carry out similar studies with variations of physiological and pathological conditions that are known to affect cholesterol metabolism.

Insulin is of high importance in lipid metabolism; the mechanism of action of insulin has been of interest to members of LCDB for a number of years. For insulin to act, it must first be transferred across capillary endothelium from blood to target cell receptors. We have studied the concentration dependence and kinetics of insulin effects on tissues using perfused adipose tissue, isolated adipose tissue, and adipocytes. The concentration studies show that half-maximal responses occur at 200-500, 30, and 8 microunits/ml for the three systems, respectively. Further, the time course of the response to insulin was markedly longer in the perfused, whole animal system than in isolated tissues. These data support the idea that insulin is transferred across capillary endothelium and extravascular spaces by a receptor-mediated process, as recently suggested by others.

The role of insulin in regulating lipolysis in adipocytes is studied by another group in LCDB. We reported in the past the development of methodology that allows isolation of rat adipocytes in a quiescent state, one which mirrors the in vivo situation. Using these adipocytes and altering the content of cAMP in cells by a variety of pharmacologic agents, we studied the relationship of cAMP content (assayed by measurement of the degree of activation of the cAMP-dependent protein kinase) and lipolysis. At low levels of cAMP, inhibition of lipolysis by insulin paralleled reduction in cAMP concentrations. At higher levels of cAMP, however, insulin inhibited lipolysis in a cAMP-independent fashion. Several other phenomena in adipocytes have recently been found to also have such a bimodal dependence on insulin.

Our previous findings were most simply interpreted by postulating an insulin dependent phosphatase which acted on hormone sensitive lipase, leading to the cAMP-independent inhibition of lipolysis at high levels of activation of A-kinase. Due to the small amount of the lipase in adipocytes, we initially examined the phosphorylation state of other, more abundant cellular proteins which can be detected easily by gel electrophoresis and autoradiography. One prominent peptide of M_r 65000 is phosphorylated and its phosphorylation is reduced by insulin in a manner that is not related to cAMP-dependent kinase activity. The concentration dependence of dephosphorylation of this protein is exactly parallel to the dose-response curve for inhibition of lipolysis previously noted, strongly supporting our hypothesis.

To extend and confirm such studies, we have developed methods for purification of the hormone sensitive lipase from rat adipocytes. Nearly 70% recovery of the enzyme from quiescent cells is possible by HPLC and gel electrophoresis. The purified enzyme will be used to generate antibody for cloning the lipase gene from an adipocyte cDNA expression library and for studies of the sequences which are phosphorylated. The lipase is phosphorylated at three sites in lipolytically quiescent cells; these phosphorylations must be by enzymes other than A-kinase, since this protein is not active in these adipocytes. In corroboration of the studies described above, one of these sites is not phosphorylated in lipase isolated from cells treated with insulin. Addition of exogenous kinase to the lipase preparation leads to phosphorylation of a fourth site. In addition to this phosphorylation, increases in cAMP have recently been shown to lead to a translocation of the hormone sensitive lipase from the cytosolic fraction to the lipid phase of adipocytes.

Another interrelationship between kinase activities and cAMP production has been studied in the laboratory. In isolated membranes, protein kinase-C was shown to stimulate the activity of adenylate cyclase. Studies of the substrate dependence of this reaction have demonstrated that a transferable ATP gamma phosphate was required for this activation. In contrast to expectation, the G-proteins of the cyclase system are not the substrates for this phosphorylation; we speculate that the catalytic subunit of cyclase may be the C-kinase target.

The subcellular distribution of the G-proteins, both inhibitory (G_i) and stimulatory (G_s) has been studied in the last year. Proteins were specifically labeled by radioactive tagging using pertussis or diptheria toxin catalyzed reactions. As expected, G_s subunits were exclusively found in plasma membranes when cells were fractionated. In contrast, G_i subunits were distributed in both plasma membranes and low density microsomal membranes. Addition of insulin to adipocytes leads to a translocation of a large portion of these regulatory, transducing subunits to the plasma membrane. This disposition of G_i proteins and its rearrangement under insulin stimulation parallels that which we and our collaborators have previously described for the glucose transporter. Coordinate regulation of these two molecules intimately involved in the regulation of adipocyte metabolism by insulin is likely to be an important feature in the hormonal control of lipid metabolism and may signal general features of the control of cellular metabolism by other peptide hormones.

Giving attention to the other major pancreatic hormone, a group in LCDB studies the development of glucagon responsiveness. MDCK cells, an established dog kidney cell line, lose the ability to respond to glucagon stimulation when they are transformed by Harvey sarcoma virus. We showed in the past that a variety of pharmacologic agents, many of which increase intracellular cAMP, led to restoration of glucagon responsiveness in these transformed cells. Furthermore, 8-Br-cAMP also led to the differentiated, hormone reponsive state.

Particularly effective in inducing glucagon sensitivity was culture for several days in the presence of prostaglandin E_2. Induction was inhibited by serum, the phorbol ester TPA and epidermal growth factor (EGF). Inhibition by EGF was shown to occur distal to the cAMP stimulated step in the differentiation pathway.

We have recently studied EGF receptors during the PGE_2 induced differentiation of MDCK cells. EGF binding increases rapidly after addition of the prostaglandin, peaking in 18 hours. Binding then decreases, dropping to about 20% of the maximal value by 48 hours. Induction and subsequent desensitization of EGF receptors is also seen when cells are cultured with 8-Br-cAMP. Paradoxically, we find that EGF binding to isolated plasma membranes is decreased when membranes are treated with the catalytic subunit of protein kinase A.

Protein chemistry

In addition to the studies of enzymes and proteins cited above, two other groups in the laboratory have primary interests in two physiologically important proteins, dihydrofolate reductase (DHFR) and apolipoprotein B (apoB). As noted in the introduction to this report, DHFR and folates have been a subject of investigation in LNE and LCDB for nearly three decades. The enzyme is of particular interest since it seems to be the primary target of methotrexate (MTX), a clinically important agent in the treatment of cancer, arthritis and autoimmune disorders. We have previously reported methods for the affinity purification of this enzyme using MTX coupled to a solid support, purification of the enzyme from a number of vertebrate hepatic tissues, and characterization of the kinetics and alterations in activity brought about by chemical or solvent modifications. In the past year, our focus has been on the role of glutamyl derivatization in inhibition of the enzyme by MTX and 10-DAM. Folates exist in the cell as polyglutamates. We have previously investigated the effect on activity of the extent of substitution of this substrate. We now find that different levels of glutamyl substitution of MTX also alter the effectiveness of the inhibitor. Most striking effects were observed for the sheep enzyme where increase from one to six glutamyl residues decreased the K_i by over three-fold. Less marked effects, some biphasic, were found with the enzymes from cow and chicken liver. Glutamyl derivitization of 10^{-6} M led to lesser, noncoordinate effects on inhibitory efficacy.

ApoB is the major structural protein of VLDL and LDL particles. It is essential for synthesis, release and catabolism of these particles, the

major transport vehicles for cholesterol and triacylglycerol. The protein is synthesized in three isoforms by both small intestine and liver. We reported previously the development of methods which allow measurement of production rates of the proteins by the two organs and alterations in the ratios of isoforms synthesized by physiological variables, including fasting and feeding. Glucose infusion increases and starvation decreases the ratio of synthesis of the smaller to the larger isoform.

Biotechnology

Two areas of research in LCDB are closer to the application of modern molecular biological expertise to the clinic than the experiments reviewed above. Both have the potential to deal with major health problems.

Noted above were the studies of ZP3, the putative murine sperm receptor. We reported previously that antibodies to ZP2 or ZP3 could passively immunize mice against pregnancy; presumably the antibodies coated the oocyte and formed a physical barrier to sperm penetration. The contraceptive action was reversible; as antibody titers diminished, new cohorts of oocytes which lacked zona proteins at the time of immunization could grow, be shed, and be fertilized. We began attempts to apply this knowledge to production of an active contraceptive vaccine two years ago.

We synthesized peptides dictated by the coding sequence of the ZP3 cDNA clone as one approach and isolated the galactosidase-ZP3 fusion protein from the lambda gtll-bearing E. coli strain as another. Immunization of mice with the latter protein led to induction of an antibody response to both galactosidase and ZP3; the titers for galactosidase were much higher than those for the zona protein. Given our previous observations that antibody produced in rat could passively immunize mice, we have now immunized rats with the fusion protein and anticipate that titers of anti-ZP3 will be sufficiently high that a test of the contraceptive effect will be worthwhile. As previously noted, should this strategy prove successful, its results can be rapidly extended to a variety of domestic animals since the mouse ZP3 cDNA clone cross-hybridizes with genomic Southerns of a wide spectrum of mammals.

The Biotechnology Unit of LCDB is engaged in large scale fermentation, tissue processing, and protein purification in support of various groups within NIH. A total of 180 large scale preparations were carried out in the past year. A number of eukaryotic and prokaryotic microorganisms were grown and processed in volumes varying from 10 to 1200 liters. Additionally, several large scale purifications were done by the staff of the Biotechnology Unit and mammalian tissue culture cells were provided to several research groups in volumes up to 50 liters. While this service facility, unique on the NIH campus, provides materials and expertise for a variety of scientists, this group also performs research and development functions in several areas. In the past year, the Pilot Plant has been physically modified to be a biosafety level 3 large scale (BL3-LS) facility. With these modifications and development of a detailed protocol, the Unit has carried out a number of fermentations of B. pertussis for preparation of nearly a gram of pertussis toxin to be toxoided and used for clinical trials as a more efficacious and safer vaccine for prevention of whooping cough.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 15004-12 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Hormone Responsiveness During Cellular Differentiation

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

P.I.:	Michael C. Lin	Research Chemist	LCDB	NIDDK
Others:	Yvonne Wu	Senior Staff Fellow	LCDB	NIDDK
	Beatrix White	IRTA Fellow	LCDB	NIDDK

COOPERATING UNITS *(if any)*

Eugenio Santos, LMM, NIAID

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.6	0.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The goal of our research effort is to understand the regulation of hormone responsiveness during cellular differentiation. Acquisition of hormone sensitivity often accompanies differentiation, therefore, by understanding its regulation, we will better understand the process of differentiation. In order to allow better control of experimental conditions, a model system using cultured cells was established. We found that in a dog kidney cell line, MDCK cells, glucagon responsiveness was selectively lost after transformation by Harvey murine sarcoma virus. This loss of hormone sensitivity can be restored to the transformed cells by culturing the cells in the presence of prostaglandin E_2. The induction by PGE_2 seems to be mediated by cyclic AMP. We also found that this induction process is inhibited by a serum factor, epidermal growth factor and a phorbol ester (TPA). Using these differentiation inhibitors, we are attempting to define the nature of this cyclic AMP-dependent process. It is apparent that the inhibitory effect of EGF resides downstream beyond the activation of cyclic AMP-dependent protein kinase. In the presence of PGE_2 during induction, EGF receptors undergo a biphasic regulation. EGF binding increases initially and reaches a peak in 18 hr, then followed by a decrease to less than 20% of the maximal binding in 48 hr. The induction and the subsequent desensitization of EGF receptors suggest a cyclic AMP-dependent modulation of EGF effect. Using a semi-purified preparation of EGF receptors, we are currently studying their phosphorylation by both EGF- and cyclic AMP-dependent processes. In addition, we also found that the ras viral protein, p21, production is decreased when cells are induced to differentiate. Since a role in signal transduction has been suggested for p21, we are also examining the potential interaction between the viral protein and receptors for growth factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15005-12 LCDB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	Constantine Londos	Research Chemist	LCDB, NIDDK
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	John J. Egan	Guest Worker	LCDB, NIDDK
	Soraya Naghshineh	Staff Fellow	LCDB, NIDDK
	Andrew S. Greenberg	Clinical Staff Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

I. A. Simpson, S. W. Cushman, MCNEB, NIDDK, K. P. Huang, ERRB, NICHHD, S. I. Taylor, DB, NIDDK

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.5	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.)*

With isolated adipocytes from rat epididymal rat pads as a model system, we have examined various aspects of hormonal control of metabolic processes. A) Phosphorylation of the purified hormone-sensitive lipase by exogenous protein kinases has been compared with phosphorylation of the lipase by endogenous kinases in cells subjected to various hormones. The enyzme contains several phosphorylation sites, one of which is dephosphorylated by insulin in intact cells in a manner independent of changes in cAMP-dependent protein kinase (A-kinase) activity. Moreover, the phosphorylation of this site does not require elevation of A-kinase. B) Since our previous findings suggest that insulin activates a phosphatase which acts on the hormone-sensitive lipase, we have examined the effects of insulin on the phosphorylation state of other, more abundant cellular proteins, which are more easily detected by SDS-PAGE and autoradiography. Insulin reduces the phosphorylation of one prominent A-kinase substrate (65 KD), and this effect is not explained by a reduction in cellular A-kinase activity. Thus, the above findings indicate that insulin stimulates the dephosphorylation of target sites for A-kinase and other kinases. C) Adenylate cyclase linked receptors (R) and GTP regulatory components (G), both stimulatory (R_sG_s) and inhibitory (R_iG_i) are thought to reside exclusively in plasma membranes. However, we find one component of the inhibitory circuit, $G_i\alpha$, in both cell surface and low density microsomal membranes. Insulin stimulates the translocation of a substantial fraction of this GTP-binding protein to the plasma membrane. D) Previously, we found purified protein kinase C stimulates adenylate cyclase in isolated membranes. A transferable ATP γ-phosphate is required, indicating that phosphorylation of a component of the cyclase system occurs. However, the GTP-binding complexes are not C-kinase substrates, indicating that another component, perhaps the cyclase catalytic unit, is the C-kinase target.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 15100-17 LCDB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Protein Nucleic Acid Interactions: Chromatin Structure and Function

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

P.I.:	R.T. Simpson	Chief	LCDB	NIDDK
Others:	S. Chambers	Senior Staff Fellow	LCDB	NIDDK
	A. Dean	Research Chemist	LCDB	NIDDK
	F. dePablo	Visiting Scientist	LCDB	NIDDK
	A. Dranginis	Staff Fellow	LCDB	NIDDK
	R. Morse	NRSA Fellow	LCDB	NIDDK
	D. Pederson	Senior Staff Fellow	LCDB	NIDDK
	J. Brubaker	Biological Lab Tech	LCDB	NIDDK
	T-C. Wu	Chemist	LCDB	NIDDK

LAB/BRANCH: Laboratory of Cellular and Developmental Biology

SECTION: Developmental Biochemistry Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.0	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

As an approach to understanding the composition and structure of transcriptionally regulated genes, we have devised methods for purification of yeast episomal chromatin. One of these, using conventional biochemical methods, has been described previously. We have improved this method by inserting the E. coli lac operator into the plasmid DNA and then using repressor-operator affinity and antibodies to allow partial purification (>50%) in a single day. We have now inserted several transcriptionally regulated genes into the plasmid and will purify chromatin and determine its structure when the genes are either active or repressed. One of these genes, HSP26, appears to be regulated in identical fashion in the multicopy plasmid environment and as a single copy in the yeast genome.

The role of chromatin structure in transcriptional regulation has been studied in model systems. An investigation of transcription of the 5S rRNA gene in Xenopus oocyte extracts suggests that initiation is blocked by histones but elongation is not. Studies of thermal untwisting of DNA show that DNA is constrained on the surface of nucleosomes containing chicken histones and relatively unconstrained when associated with yeast histones.

Messenger RNA for a collagen gene is expressed only in cells of the mesenchyme lineage during sea urchin embryogenesis. The protein coded by the gene was localized immunologically to the primary mesenchyme cells which elaborate the larval endoskeleton. Other studies of urchin embryogenesis have documented the presence of insulin-like molecules which may function as factors involved in general or specific aspects of development and have led to cloning of the gene for a putative cell adhesion molecule which is expressed in a temporally and spatially specific fashion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15102-27 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Study of a Ribonuclease and its Inhibitor from Bacillus amyloliquefaciens

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

P.I.: Robert W. Hartley	Research Physicist	LCDB	NIDDK
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COOPERATING UNITS *(if any)*

G.G. Dodson, Dept. of Chem., University of York
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LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

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

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

Two proteins, barnase, the extracellular ribonuclease of Bacillus amyloliquefaciens, and barstar, its intracellular inhibitor, are used as a model system for the study of protein folding a protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes.

Recombinant DNA techniques are being applied to the project with three major aims: 1) to facilitate production, 2) to examine the structural and control sequences of the genes and 3) to tailor specifically designed modifications in the sequences to test theories of protein folding.

The lethal effect of the cloned wild-type barnase gene in either E. coli or B. subtilis can be repressed by expression of the cloned barstar gene placed on the same plasmid. Secretion vectors for both proteins have been devised.

Both genes have been sequenced, providing confirmation of the barnase sequence (110 residues) and a derived sequence (89 residues) for barstar which agrees well with previous amino-acid analysis.

Effects of several site-directed mutations of barnase on activity and thermal stability have been studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15200-27 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies on Folic Acid (Dihydrofolate Reductase) and Vitamin A

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

P.I.:	Bernard T. Kaufman	Research Chemist	LCDB	NIDDK
Other:	John Bieri	Scientist Emeritus	LCDB	NIDDK

COOPERATING UNITS *(if any)*

Dr. Roy L. Kisliuk, Tufts University, Boston, MA, Dr. J. Cecil Smith, Jr., USDA, Beltsville, MD, Dr. Carmin Allegra, NCI

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Nutritional Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Folate cofactors exist intracellularly as polyglutamate derivatives. The discovery that certain antifolate drugs are converted to similar polyglutamates has raised the possibility of a role for such derivates in chemotherapy.

Using dihydrofolate reductases (DHFR) isolated from various animal livers certain comparative aspects of DHFR inhibition by polyglutamyl derivates of the drugs methotrexate (MTX) and 10-deazaaminopterin (10-DAM) as compared with the potent inhibitory properties of the corresponding monoglutamates were investigated. The most striking effects were seen with sheep liver DHFR. Polyglutamylation of MTX causes stepwise increases in inhibition. Six Glu residues is 3-X more inhibitory than the monoglutamate. Increasing the Glu chain on 10-DAM decreases the inhibitory effects up to a chain length of 3. Further increases now result in increasing inhibition. The kinetic parameters of dihydropteroylpentaglutamate and dihydropteroylglutamate (dihydrofolate) appear to be identical in this study. However, when the pentaglutamate is used as substrate instead of dihydrofolate, the degree of inhibition is correspondingly decreased 2- to 5- fold for both the MTX and 10-DAM pentaglutamate derivatives.

The amount of carotenoids appearing in the plasma of normal men under standardized conditions is highly variable, whereas all subjects ingesting pure β-carotene gave maximum responses in about 30 hr, the magnitude of the responses varied at least 3-fold. Beta-carotene when fed as the pure compound was comparable to the same dose from cooked carrots. Study of the plasma response to different dietary carotenoid sources revealed that plasma carotenoid concentrations do not accurately reflect the dietary intake of certain pro-vitamins, ie., lutein, lycopene, etc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15302-17 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Biochemical Studies of Hepatic and Intestinal Function

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

P.I.:	Herbert G. Windmueller	Research Chemist	LCDB, NIDDK
Others:	Albert E. Spaeth	Chemist	LCDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Nutritional Biochemistry Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Chylomicrons and very low density lipoproteins (VLDL) transport triacylglycerol in the blood stream. They are composed of a core of mostly triacylglycerol surrounded by a lipid-protein monolayer. The structural protein of chylomicrons and VLDL is apolipoprotein B (apoB). ApoB is also found in low density lipoproteins (LDL), a metabolic derivative of chylomicrons and VLDL. Clinical findings indicate that LDL may be involved in the development of coronary arteriosclerosis.

We have used the rat to study the synthesis and metabolism of apoB. ApoB exists in the rat in three forms, B-100, B-95 and B-48. M_r of B-48 is around 240,000, about one-half that of B-100 and B-95. We showed earlier that rat liver synthesizes all forms of apoB, whereas the intestines synthesizes only B-48. In vivo studies showed that B-48 is cleared from blood much faster than the other forms. Other findings indicated that B-95 and B-100 are incorporated into different populations of hepatic VLDL. Thus, the metabolic fate of VLDL and possibly LDL, could be determined by the form of apoB present in the lipoprotein.

We have developed a method for measuring in vivo, in the rat, incorporation of [^{3}H]leucine into individual forms of apoB. The method is based on the ability of Triton WR-1339 to block clearance of all forms of apoB from the blood stream. Glucose infusion increased, and fasting decreased, the ratio of B-48 to the larger forms in VLDL without affecting the ratio of B-100 to B-95. Surgical stress and Triton injection, in contrast, lowered the ratio of B-100 to B-95 without affecting the ratio of B-48 to the larger forms. These findings suggest that synthesis of each form of apoB is regulated individually by hormonal/nutritional factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15400-13 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Hormones, Lipoprotein Lipase and Lipid Metabolism

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

P.I.:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
Others:	Sidney S. Chernick	Scientist Director	LCDB, NIDDK
	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
	Hiroshi Masuno	Visiting Fellow	LCDB, NIDDK
	Carmen Mateo	Visiting Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Thomas Olivecrona, Dept. of Physiol. Chem., Univ. of Umea, Sweden; Drs. W. Virgil Brown and Kazuhiro Oka, Div. of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine, New York, NY

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	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.)*

Mice born with combined lipase deficiency develop severe hyperlipemia and die within 3 days if allowed to suckle. This condition is caused by a recessive mutation (cld) in the T/t complex of chromosome 17, and is characterized by marked functional deficiencies of both lipoprotein and hepatic lipases. We reported earlier that brown adipose tissue, heart and diaphragm muscle of cld/cld mice synthesize lipoprotein lipase that is normal in size, but the enzyme is inactive and retained in the tissues. Since lipoprotein lipase is a glycoprotein and mutations in the T/t complex of chromosome 17 can affect glycosylation of proteins, it seemed possible that defective glycosylation could account for lack of activity, and possibly lack of secretion, of lipoprotein lipase in cld/cld mice.

We are now studying this possiblility in adipocytes cultured from brown adipose tissue. We found that cells cultured from both cld/cld and normal mice readily converted to adipocytes when grown in medium containing triiodothyronine, insulin and octanoic acid. However, only adipocytes from normal mice released active lipoprotein lipase to the medium. Preliminary studies, using fluorescent immunocytochemical techniques, showed that adipocytes from cld/cld mice contained intracellular lipoprotein lipase, whereas adipocytes from normal mice had lipase, in small amounts, only on cell surfaces. These findings show that cultured adipocytes from defective mice synthesize a non-secretable form of lipoprotein lipase, whereas those from normal mice synthesize and secrete active lipase. Thus, cultured adipocytes can be used to study in vitro the genetic and chemical nature of the lipoprotein lipase deficiency in cld/cld mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 15401-15 LCDB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Transport of Lipids, Hormones and Enzymes in Tissues, Cells and Membranes

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

P.I.:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
Others:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
	Sidney S. Chernick	Scientist Director	LCDB, NIDDK
	Lynne Amende	Senior Staff Fellow	LCDB, NIDDK
	Carmen Mateo	Visiting Fellow	LCDB, NIDDK

COOPERATING UNITS (if any)

LAB/BRANCH: Laboratory of Cellular and Developmental Biology

SECTION: Endocrinology Section

INSTITUTE AND LOCATION: NIH, NIDDK, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Insulin initiates its effects on metabolism by binding to specific receptors on the surface of cells. For insulin in blood to react with these receptors in vivo, the hormone must cross the capillary endothelium and extracellular space to reach the cells. Recent studies by others in cultured endothelial cells indicate that insulin may be transported across capillary endothelial cells by a receptor-mediated process. We report here a study of transport of insulin across capillary endothelium in perfused rat adipose tissue. Since we were unable to measure directly the concentration of insulin in the extravascular fluid, we measured transport of insulin across endothelium by the effect of intraarterially infused insulin on oxidation of [U-^{14}C]glucose to CO_2.

Glucose oxidation was constant in adipose tissue perfused with 0 or 50 microunits of insulin per ml. The rate of oxidation was doubled in 90 min at 100 microunits/ml, and maximal in 40 min at 200 microunits/ml and maximal in 20-30 min at 500 microunits/ml. The slow decline in oxidation rate when insulin infusion was stopped suggests that insulin was sequestered in the tissue. The half-maximal response to insulin occurred at a much higher insulin concentration in perfused tissue than in incubated adipocytes and incubated adipose tissue, and the time required for maximal response was longer in perfused adipose tissue. The data indicate that transfer of insulin from blood to parenchymal cells in perfused adipose tissue was restricted. The minimal amount of insulin needed for a response by adipocytes in perfused tissue was estimated to be less than 1% of that in blood. Our findings are consistent with the concept that insulin is transferred across capillary endothelium by a receptor-mediated process.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15404-03 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Ultrastructural Immunocytochemistry of Lipid Metabolism in Cells and Tissues

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

P.I.:	E. Joan Blanchette-Mackie	Research Biologist	LCDB, NIDDK
Others:	Robert O. Scow	Chief, Endocrinology Section	LCDB, NIDDK
	Nancy K. Dwyer	Biologist	LCDB, NIDDK
	Lynne Amende	Senior Staff Fellow	LCDB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Thomas Olivecrona, Dept. Physiol. Chem., Univ. of Umea, Sweden; Drs. Kazuhiro Oka and W. Virgil Brown, Div. of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine, New York, N.Y.

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Although lipoprotein lipase acts on chylomicrons and VLDL at the luminal surface of capillaries, the enzyme is synthesized in parenchymal, not endothelial, cells. Mice born with combined lipase deficiency (cld/cld) have mostly inactive lipoprotein lipase in heart and brown adipose tissue, and are unable to clear triacylglycerol from the blood. Heart of normal mice had lipoprotein lipase, visualized with fluorescent immunochemistry, associated with small blood vessels, whereas heart of cld/cld mice had lipoprotein lipase within myocytes, but not in blood vessels. Brown adipocytes cultured from normal mice had lipoprotein lipase only on cell surfaces, whereas adipocytes from cld/cld mice contained lipoprotein lipase within the cells. These findings suggest that secretion of lipoprotein lipase is impaired in myocytes and adipocytes of cld/cld mice.

Active lipoprotein lipase is present in liver of both normal and cld/cld mice. Fluorescent immunochemical studies in tissues from both groups of mice showed that the enzyme was located in hepatocytes.

Non-esterifed cholesterol has been localized in cultured human fibroblasts incubated with low density lipoproteins. Cholesterol was visualized with fluorescent antibodies to cholesterol and with filipin fluorescence. Lysosomes were also localized, with fluorescent antibodies to lysosomal membrane protein. Intracellular co-localization of the fluorescent probes demonstrates accumulation of cholesterol in lysosomes of fibroblasts in culture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15405-05 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Aggregation of Human Platelets Induced by Decompression: Mechanism and Prevention

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

P.I.: Makio Murayama Research Chemist LCDB NIDDK

COOPERATING UNITS *(if any)*

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

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 15500-27 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Large Scale Processing of Biological Material

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

P.I.:	Joseph Shiloach	Research Chemist	LCDB	NIDDK
Others:	Jeanne E. Kaufman	Biol. Lab. Tech.	LCDB	NIDDK
	Nahum Andorn	Visiting Fellow	LCDB	NIDDK
	Ilse Blumentals	Guest Researcher	LCDB	NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Pilot Plant operation encompasses several different types of activities:

1. Providing large amounts of bacteria, proteins, mammalian cells, etc. for the needs of various research programs.

2. Conducting process development work to assist various investigators with the development of new and improved production and recovery methods.

3. Performing independent research associated with optimizing growth of microorganisms and mammalian cells, and with the production of bioproducts.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15503-06 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Developmental Gene Expression

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

P.I.:	Alan R. Kimmel	Senior Staff Fellow	LCDB	NIDDK
Other:	Charles Saxe	Staff Fellow	LCDB	NIDDK
	Stephen Saxe	Staff Fellow	LCDB	NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In eukaryotes, extracellular molecules interact with cell surface receptors to alter the concentration of intracellular second messengers. These second messengers can ultimately promote cell proliferation and cytodifferentiation. We have now identified two intracellular molecules, cAMP and IP_3, which in Dictyostelium regulate the expression of specific gene families during development. It is suggested that these molecules affect the activity of the cAMP-dependent protein kinase, the Ca^{++}/calmodulin-dependent protein kinase and protein kinase C. Other components of the transmembrane signalling system have been partially characterized. GTP-binding, regulatory proteins mediate the action of some ligand activated receptors. We have begun a molecular genetic analysis of the signal transduction system in Dictyostelium. Genes have been isolated which encode a cell surface receptor and a GTP-binding protein. A single gene for the receptor is present in the genome. Interestingly, multiple mRNA forms are differentially expressed during development. The latter protein is encoded by two different genes. Data suggest that the protein is involved in intracellular communication and membrane associated functions.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 15506-04 LCDB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Gene Expression in Early Mammalian Development

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

P.I.:	Jurrien Dean	Senior Investigator	LCDB, NIDDK
Others:	Maurice Ringuette	Visiting Associate	LCDB, NIDDK
	Steven Chamow	Staff Fellow	LCDB, NIDDK
	Margaret Chamberlin	FAES Graduate Student	LCDB, NIDDK
	Anne Baur	Chemist, GS-11	LCDB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Developmental Biochemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.6	3.6	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Gene expression during oogenesis plays an important role in early mammalian development. The genes coding for the mouse zona pellucida are expressed during oogenesis where they are translated into three sulfated glycoproteins designated ZP1, ZP2 and ZP3. We have isolated cDNAs coding for ZP2 and ZP3 from an ovarian lambda gt11 expression library using monoclonal antibodies specific to ZP2 and ZP3. The identity of the ZP3 clone has been confirmed by a comparison of its nucleic acid sequence with the amino acid sequence of an internal ZP3 peptide. By Northern blot analysis and in situ hybridization we have shown that ZP3 is expressed uniquely in oocytes as a 1.5 kb poly(A)$^+$ mRNA. ZP3 transcripts are not detectable in resting oocytes (15 um) but become very abundant during oocyte growth and represent 0.1-0.2% of the poly(A)$^+$ RNA in 50 um diameter oocytes. There is a subsequent dramatic fall-off of ZP3 transcripts in the latter stages of oocyte growth (65 um) which closely parallels the decline in zona protein synthesis. ZP3 is a single copy gene and is located on mouse chromosome 6. There appears to be neither gene amplification nor gene rearrangement to account for the tissue specific expression of ZP3, although the ZP3 locus is hypomethylated in ovarian tissue (where it is expressed) compared to somatic tissue. We have isolated genomic clones containing both the mouse and human ZP3 genes and are in the processes of defining their intron and exon structures. We are particularly interested in investigating the 5' flanking regions which may modulate the tissue specific expression of the zona genes. We have previously reported that monoclonal antibodies to the ZP2 and ZP3 were effective, longterm but eventually reversible contraceptive agents. Based on our recent ability to clone the zona genes, we are now exploring the use of synthetic zona peptides and ZP/β-galactosidase fusion proteins as active immunogens for the development of a contraceptive vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 15507-10 LCDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Macromolecular Structure

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

P.I.:	Alasdair C. Steven	Visiting Scientist	LCDB	NIDDK
Other:	Adelia C. Bauer	Physiologist	LCDB	NIDDK
	Margaret E. Bisher	Microbiologist	LCDB	NIDDK
	Colin D. Ockleford	Visiting Scientist	LCDB	NIDDK
	David A.D. Parry	Guest Researcher	LCDB	NIDDK

COOPERATING UNITS *(if any)*

B. Trus, DCRT; M. Unser, BEIB; P. Ross, LMB,NIDDK, J. Cowell, FDA; J. Brown & W. Newcomb, U.Va; L. Black, U. Md.; J. Maizel & P. Steinert, NCI; R. Podolsky, NIAMS; Co. Steer, LMB, NIDDK; F. Studier, Brookhaven Nat'l Lab., LMB/BRANCH

FOREIGN: A. McDowall & J. Dubochet, EMBL, Heidelberg, R.R.G.

Laboratory of Cellular and Developmental Biology

SECTION

Section on Structural Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMISTRY AND METABOLISM

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The Laboratory conducts research in such apparently disparate areas as differentiation, morphogenesis, endocytosis, endocrinology, membrane transport, detoxication and protein behavior and does so very different methods that are being applied to solve the identified problems. Resolution is being attempted by approaches that stem from enzymology, carbohydrate chemistry, cell biology and molecular biology. Although seemingly diverse, there is a common element to each of the subjects summarized here that is appropriate to the Laboratories' designation: biochemical and metabolic approaches are being brought to bear on major problems encompassed by the Institute's charge. It is the close proximity of experienced investigators from diverse scientific disciplines, discussing their distinct approaches to a given problem with each other, that provide synergistic effects for the resolution of the questions under investigation.

A. Growth, Differentiation and Morphogenesis

Several groups are active in this broadly designated area by exploring different aspects of development of mammary tissue and its distinct protein products, as well as an investigation of the origin and development of the bud scar of yeast.

1. Hormone-dependent Development of Mammary Gland

The molecular and cytological events involved in the development of mammary gland are being explored. Although estrogen has long been recognized other investigators had reported that epidermal growth factor (EGF) inhibits the induction of casein synthesis by mouse mammary tissue in vitro. However, since the circulating level of EGF increases during lactation, and since functional EGF receptors are retained by the lactating cells, it seemed unlikely that EGF is an inhibitor of mammary differentiation in vitro. Current studies demonstrate, that EGF actually inhibits the induction of casein synthesis in vitro only when insulin, an essential hormone, is present in the culture medium at pharmacological concentrations.

EGF has been found to substitute effectively for prolactin (P) in the induction of α-lactalbumin activity (assayed as lactose synthetase) in rat mammary explants cultured in the presence of insulin and glucocorticoid. The time courses of induction with EGF or P are similar. Both EGF and P also promote similar elevations in the accumulated level of α-lactalbumin mRNA. However, although P produces a large increase in the accumulated level of immunoprecipitable α-lactalbumin (determined with polyclonal antibody), the induction of immunoprecitable α-lactalbumin by EGF is less than 10% of that which occurs with P. It appears, then that EGF, like P, can support α-lactalbumin gene expression and the formation of enzymatically active α-lactalbumin, but that P is required post-translationally for the production of

immuno-active α-lactalbumin. A less likely interpretation is that the α-lactalbumin activity induced by EGF corresponds to a protein distinct from the α-lactalbumin present in rat milk.

2. Tissue Specific and Hormonal Regulated Gene Expression

Here the approach is mainly from the discipline of molecular biology whereby the milk protein system is being used as a model for defining the cis-regulatory elements and trans-acting factors that determine the tissue specificity and hormone induced expression. Additionally, a study is under way of the mechanism of activation and repression imposed on the major immediate early gene of the human cytomegalovirus (HCMV) in different host cells.

The molecular basis of mammary specific gene expression is being studied through analysis of cis-acting regulatory elements in milk protein genes and their cognate trans-acting factors. Applying mobility shift assays, Exonuclease III and DNAaseI protection, it was shown that nuclear proteins from mammary epithelial cells form a multiple nucleoprotein complex with the whey acidic protein (WAP) gene promoter/upstream region. Whereas some of the DNA sequences were recognized by proteins present in a variety of different cell types, other sequences were recognized by proteins preferentially or exclusively present in mammary gland nuclear extract. Furthermore, a promoter fragment of the WAP gene, encompassing the sites of protein-DNA interaction was found to confer the expression of 'non - mammary' genes in lactating mammary glands of transgenomic animals. This indicated a physiological role of the protein binding sites.

In the second project an in vitro system is being established which mimics the in vivo activation of the human cytomegalovirus (HCMV). This might allow the study of molecular mechanisms of viral gene activation. Upon virus infection of the HCMV, immediate early gene 1 (IE1) is the first viral gene to be expanded. It appears that the IEI gene enhancer mediated transcriptional stimulation in vitro involves its recognition by specific trans - acting factors present in the nuclear extract. DNaseI protection analyses reveal at least 13 sites in the enhancer promotor region that are protected specifically by nuclear proteins. A correlation was made between protein binding to specific sequences and transcriptional stimulation in vitro.

3. Polysaccharides in Morphogenesis

The ongoing research attempts to provide an understanding of the molecular mechanism of morphogenesis. The topics currently under study are the formation of the primary septum of yeast, i.e., the bud scar, and the biosynthesis of a glucan, the latter a major structural component of the cell wall of yeast and other fungi.

By transformation of Saccharomyces cerevisiae cells harboring a disrupted gene for chitin synthetase 1 with a yeast DNA library, a strain was isolated that over-produces chitin synthetase 2 by a factor of 50. Studies are continuing to establish whether the plasmid contained in those cells bears the structural gene for chitin synthetase 2 and to determine the function of this enzyme in yeast.

Chitin synthetase 1 was purified by a new procedure involving a hybrid protein with β-galactosidase and antibodies against the enzyme have been elicited in rabbits.

The solubilized GTP-binding factor from S. cerevisiae (1→3) β-glucan synthetase has been partially purified.

B. Proteins and Enzymes

At the center of it all are the protein catalysts. In this laboratory, two groups are directly oriented toward protein chemistry and enzymology. A third group is approaching the problem of metabolism disease by analyzing the genes of a hereditary enzyme defect.

1. Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms.

Work is directed toward the relationship of protein sequence to conformation and enzyme activity. One laboratory is engaged in studies on protein structure and the mechanism by which a protein molecule, which is synthesized as a random coil, can fold into a specific secondary and tertiary structure, without any external help. The main subject of research is swine pepsinogen, a monomeric protein of molecular weight =39,630, which is stable at pH's between 6 and 8.5. Below pH 6 pepsinogen activates itself by proteolytic loss of its first 44 amino acids, to produce an enzymatically active protein, pepsin. Pepsin is stable only at pH's below 6. Both proteins are unfolded by exposure to high pH, temperature or concentrations of denaturants, such as urea. After such unfolding, pepsinogen can refold to its normal structure, when returned to native conditions, whereas pepsin cannot. Interest is in the mechanism of this refolding reaction and the influence of the change in sequence on the behavior of the two proteins. Using techniques such as ultra-violet, circular dichroic and fluoresence spectroscop, together with chemical modification and peptide chemistry, the structures of the native and unfolded species have been characterized. Using rapid kinetic techniques, such as stopped-flow and T-jump, intermediate, partly folded forms have been detected in the folding reaction, their structures partially determined and the nature of the chemical reactions which separate them from the native and unfolded forms investigated.

One group is investigating the enzymes of detoxication, three dozen or so enzymes that are distributed ubiquitously among higher animals with the apparent function of detoxifying xenobiotics, i.e., foreign compounds. As a result of such efforts it is now becoming clear that these enzymes generally have two properties in common: 1) they function in a manner that is designed to convert xenobiotics into readily excretable and pharmacologically inert compounds. 2) The enzymes themselves are characterized by a very broad substrate specificity with particular avidity for lipophilic ligands.

Under investigation are the enzymes concerned with the detoxication of amines. Isoenzymes have been prepared in homogeneous form from rabbit liver, amine N-methyl transferases A and B, each of which catalyzes, with very similar specificity, the transfer of methyl groups from S-adenosyl-L-methionine to a large number of amines. The amine acceptors include primary, secondary and tertiary amines of very different carbon skeleton that include aliphatic, aromatic and heterocyclic amines. It will be noted that the product of methylation of azaheterocycles is frequently a quaternary ion. A second type of conjugation reaction with amines is that in which amines serve as receptors for a sulfuryl group from 3'-phosphoadenosine 5'-phosphosulfate. The result is the formation of a sulfamate, a reaction that occurs with a variety of both primary and secondary amines and is catalyzed by amine N-sulfotransferase isolated from guinea pig liver. One substrate is cyclohexylamine which undergoes sulfuryl transfer to form sulfamate,the sugar substitute cyclamate.

3. The Genetic Lesions of Tay-Sachs Disease

Tay-Sach disease is a group of disorders caused by mutations in the α-chain polypeptide of the A form of β-hexosaminidase, a lysosomal enzyme composed of two chains (α,β). Such lesions result in a spectrum of disease states ranging from severe to mild. Although the disorder is in general rare, both French Canadians living in Eastern Quebec as well as Ashkenazi Jews, have a 10-fold higher gene frequency then the general population for a severe form of the disorder known as "classic" Tay-Sachs disease.

From previous work in this laboratory, certain French Canadians were found to lack a 7.6 kilobase fragment of the α-chain gene including the promoter region, exon 1 and part of intron 1, whereas the gene from Ashkenazi patients appeared grossly intact. During the past year, the exact deletion borders in the α-chain gene of a French Canadian patient were identified by sequence analysis of the deletion junction in the mutant, and in corresponding regions of the normal gene. This analysis also demonstrated the presence of similarly oriented Alu sequences at the 5', 3' deletion boundaries suggesting that the deletion may have arisen during homologous recombination from unequal crossing over between Alu sequences. In addition, we have isolated genomic clones from a λ library constructed with DNA from an Ashkenazi Jewish patient with classic Tay-Sachs disease, that span almost the entire 40 kilobase α-chain locus. Twelve of the exons have been sequenced as well as 24 of the 28 splice junction regions.

No deviations from the corresponding regions of the normal gene have been found as yet.

C. Biochemistry, Function and Regulation of Membranes

Under the heading of membranes are a broad range of projects that range from nuclear membranes, through exocytosis and membrane transport, to the endocrinology of thyroid disease.

1. The Role of the Nuclear Envelope in Intracellular Protein Sorting

The proper compartmentalization of proteins destined for the cell nucleus is likely to play a role in the regulation of cell growth and development. Synthetic peptides containing the amino acid sequence responsible for the nuclear localization of the SV40 Large T antigen and a modified sequence present in a cytoplasmic variant have been used to study protein import into the nucleus. These synthetic peptides have been used to generate polyclonal and monoclonal antibodies which bind specifically to the nuclear localization sequence. Such short peptides, when chemically coupled to the large fluorescent protein B-phycoerythrin, specifically target the protein conjugate to the nucleus. Transport of such conjugates across the nuclear envelope was demonstrated after micro-injection into cultured cells or in an *in vitro* import assay using rat liver nuclei. Transport is time, temperature and energy dependent; only conjugates containing the localization sequence are properly transported. The nuclear pore complex transverses the nuclear envelope and may mediate uptake into the nucleus. We have shown that the outer nuclear membrane is an important site of membrane glycoprotein synthesis. We have also demonstrated that proteins bearing cytoplasmically oriented,O-linked GlcNAc are components of the nuclear pore complex. The nuclear pore glycoproteins can be selectively labelled using the lectin wheat germ agglutinin. This lectin reversibly blocks import into the nucleus. Monoclonal antibodies have been raised against these nuclear pore glycoproteins and O-linked GlcNAc was found to be part of the immunodeterminant. These findings raise the exciting possibility that cytoplasmic glycosylation may be involved in the assembly or function of the nuclear pore.

2. Mechanisms Regulating Iron Metabolism in Human Erythroleukemic Cells

The regulatory mechanisms which determine the level, locus and affinity of the hepatic receptor for asialoglycoproteins in health and disease states are the subject of study.

Asialoglycoproteins serve as ligands of the hepatic asialoglycoprotein receptor (ASGP-R) and induce down-modulation of the functional expression of the receptor without altering immunological integrity. The molecular basis for the phenomenon was investigated in a well-differentiated human hepatoma cell line (Hep G2). This ligand-induced modulation was found to be related to a decrease in protein-bound sialic acid. The low concentration of cell protein-bound sialic acid appears to be the result of the exogenous asialoglycoprotein, i.e. the ligands themselves, serving as substrates for sialotransferases. This results in a competition with the cell's own glycoproteins for the limited amount of sialic acid synthesis. This

glycoproteins for the limited amount of sialic acid synthesis. This mechanism, in which the concentration of intracellular asialoprotein appears to act as modulator, may regulate the amount of asialoprotein that enters the cells.

3. Cell Regulation by the Action of Pharmacodynamic and Autoimmune Agents on the Cell Membranes

The thrust of work in this section is toward an understanding of the mechanisms by which hormonal and pharmacological agents regulate cell activity and the means by which these mechanisms are subverted by pathologic agents to express themselves in metabolic and digestive diseases. The specific theme centers around the understanding of thyroid physiology, development, and regulation with respect to normal body function as well as pathologic states.

Structure-function relationships in the mechanisms by which glycoprotein hormones (thyrotropin), autoantibodies, certain bacterial toxins (cholera and pertussis, for example), the anti-viral protective agent, interferon, α_1-adrenergic agents, insulin, and insulin-like growth factors (I and II) interact with and transmit their message through the cell membrane to affect thyroid or fibroblast function and pathology are being defined. Studies using monoclonal antibodies and the idiotype antiidiotype theory have continued to explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general, and the auto immunity of Graves' disease, Hashimoto's disease, and diabetes in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of different hormones and signal transduction mechanisms in thyroglobulin biosynthesis, in thyroglobulin biodegradation to T_3 and T_4, and in the transport of T_3, T_4, monoiodotyrosine, diiodotyrosine, and other amino acids from the lysosome. The role of phosphate and carbohydrate moieties in thyroglobulin structure and post-translational processing is being studied. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell growth and development. Studies have been initiated to clone the TSH receptor and define its structure and regulatory control at a gene level.

4. Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

The work relates to understanding the biochemical events associated with the normal function of the thyroid and to such pathological conditions of the thyroid as Graves' disease.

Regulation of FRTL-5 cells, a continuous strain of rat thyroid cells, involves both cyclic AMP and calcium as second messengers. TSH uses both pathways, while alpha 1-adrenergic agents utilize only a calcium signal. Efflux of iodide into the follicular lumen of the thyroid and iodination of thyroglobulin, essential steps in thyroid hormone formation, are regulated by TSH and adrenergic agonists (such as norepinephrine) through calcium mobilization. The transducing mechanism for generation of this second messenger is the phosphodiesterase-mediated hydrolysis of membrane phosphoinositides, in particular phospatidylinositol 4.5 bisphosphate. The products of this cleavage are inositol trisphosphate, which releases calcium from intracellular storage sites, and diacyglycerol which is important in phospholipase C activation and cell growth. Stimulated metabolism of membrane phosphoinositides is also associated with release of arachidonic acid, metabolites of which are implicated in iodide efflux and growth. Thiocyanate, a goitrogen for humans, competes for the transport and metabolism of iodine by the thyroid. Thiocyanate reacts with tyrosyl residues of thyroglobulin in the region where thyroid hormone activity is segregated, with release of a thiocyanate containing peptide. Thyroglobulin isolated from iodine deficient goiters, and animals with spontaneous goiter, suggest that thyroglobulin related proteins are involved in hormone release and the pathogenesis of iodine deficient goiter. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of thyrotropin and pharamacologic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17001-21 LBM

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanism Regulating Iron Metabolism in Human Erythroleukemia Cells

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

PI:	G. Ashwell	Institute Scholar	LBM, NIDDK
Others:	R. Koenig	Visiting Fellow	LBM, NIDDK
	C. Steers	Expert	LBM, NIDDK
	P. Weiss	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3	.5

CHECK APPROPRIATE BOX(ES)

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

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

Asialoglycoproteins serve as ligands of the hepatic asialoglycoprotein receptor (ASGP-R) and induce down-modulation of the functional expression of the receptor without altering immunological integrity. The molecular basis for the phenomenon was investigated in well-differentiated human hepatoma cell lines (Hep G2). This ligand-induced modulation was found to be related to a decrease in protein-bound sialic acid. The low concentration of cell protein-bound sialic acid appears to be the result of the exogenous asialoglycoprotein, i.e. the ligands themselves, serving as substrates for sialotransferases. This results in a competition with the cell's own glycoproteins for the limited amount of sialic acid synthesis. This mechanism, in which the concentration of intracellular asialoprotein appears to act as modulator, may regulate the amount of asialoprotein that enters the cells.

GPO 814-018

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17002-17 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Enzymatic Basis of Detoxication

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

PI:	William B. Jakoby, Chief Laboratory of Biochemistry and Metabolism		LBM, NIDDK
Others:	S. Ansher	Sr. Staff Fellow	LBM, NIDDK
	S. Ramaswamy	Visiting Associate	LBM, NIDDK

COOPERATING UNITS *(if any)*

Peter Crooks University of Kentucky, Department of Medicinal Chemistry, Lexington, Kentucky

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Under investigation are the enzymes concerned with the detoxication of amines.

Two Isoenzymes have been prepared in homegeneous form from rabbit liver, amine N-methyl transferases A and B, each of which catalyzes, with very similar specificity, the transfer of methyl groups from S-adenosyl-L-methionine to a large number of amines. The amine acceptors include primary, secondary and tertiary amines of very different carbon skeleton that include aliphatic, aromatic and heterocyclic amines. It will be noted that the product of methylation of azaheterocycles is frequently a quaternary ion.

A second type of conjugation reaction with amines is that in which amines serve as receptors for a sulfuryl group from 3'-phosphoadenosine 5'-phosphosulfate. The result is the formation of a sulfamate, a reaction that occurs with a variety of both primary and secondary amines and is catalyzed by amine N-sulfotransferase isolated from guinea pig liver. One substrate is cyclohexylamine which undergoes sulfuryl transfer to form sulfamate, the sugar substitute cyclamate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17003-20 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Polysaccharides in Morphogenesis

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

PI:	E. Cabib	Senior Research Chemist	LBM, NIDDK
Others:	S. DasGupta	Visiting Fellow	LBM, NIDDK
	A. Sburlati	Visiting Fellow	LBM, NIDDK
	S. Silverman	Senior Research Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

By transformation of Saccharomyces cerevisiae cells harboring a disrupted gene for chitin synthetase 1 with a yeast DNA library, a strain was isolated that over-produces chitin synthetase 2 by a factor of 50. Studies are continuing to establish whether the plasmid contained in those cells bears the structural gene for chitin synthetase 2 and to determine the function of this enzyme in yeast.

Chitin synthetase 1 was purified by a new procedure involving a hybrid protein with β-galactosidase and antibodies against the enzyme have been elicited in rabbits.

The solubilized GTP-binding factor from S. cerevisiae (1→3) β-glucan synthetase has been partially purified.

GPO 914-018

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17004-19 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

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

PI: Peter McPhie Research Chemist LBM, NIDDK

COOPERATING UNITS *(if any)*

Irwin Chaiken, MCNEB, NIDDK; Preson Hensley, Dept. of Biochemistry, Georgetown University; Russell Howard, LPD, NIAID; Phillip Lazarovici, BB, NICHD

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This laboratory is engaged in studies on protein structure and the mechanism by which a protein molecule, which is synthesized as a random coil, can fold into a specific secondary and tertiary structure, without any external help. The main subject of research is swine pepsinogen, a monomeric protein of molecular weight = 39,630, which is stable at pH's between 6 and 8.5. Below pH 6 pepsinogen activates itself by proteolytic loss of it's first 44 amino acids, to produce an enzymatically active protein, pepsin. Pepsin is stable only at pH's below 6. Both proteins are unfolded by exposure to high pH, temperature or concentrations of denaturants, such as urea. After such unfolding, pepsinogen can refold to its normal structure, when returned to native conditions, whereas pepsin cannot. I am interested in the mechanism of this refolding reaction and on the influence of the change in sequence on the behaviour of the two proteins. Using techniques such as ultra-violet, circular dichroic and fluorescence spectroscopies, together with chemical modification and peptide chemistry, the structures of the native and unfolded species have been characterized. Using rapid kinetic techniques, such as stopped-flow and T-jump, intermediate, partly folded forms have been detected in the folding reaction, their structures have been partially determined and the nature of the chemical reactions which separate them from the native and unfolded forms investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17008-04 LBM

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of the Nuclear Envelope in Intracellular Protein Sorting

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

PI:	J.A. Hanover	Senior Investigator	LBM, NIDDK
Others:	M. D'Onofrio	Visiting Fellow	LBM, NIDDK
	M.K. Park	Visiting Fellow	LBM, NIDDK
	B. Wolff	Guest Worker	LBM, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The proper compartmentalization of proteins destined for the cell nucleus is likely to play a role in the regulation of cell growth and development. Synthetic peptides containing the amino acid sequence responsible for the nuclear localization of the SV40 Large T antigen and a modified sequence present in a cytoplasmic variant have been used to study protein import into the nucleus. These synthetic peptides have been used to generate polyclonal and monoclonal antibodies which bind specifically to the nuclear localization sequence. Such short peptides, when chemically coupled to the large fluorescent protein B-phycoerythrin, specifically target the protein conjugate to the nucleus. Transport of such conjugates across the nuclear envelope was demonstrated after micro-injection into cultured cells or in an in vitro import assay using rat liver nuclei. Transport is time, temperature and energy dependent; only conjugates containing the localization sequence are properly transported. The nuclear pore complex transverses the nuclear envelope and may mediate uptake into the nucleus. We have shown that the outer nuclear membrane is an important site of membrane glycoprotein synthesis. We have also demonstrated that proteins bearing cytoplasmically oriented, O-linked GlcNAc are components of the nuclear pore complex. The nuclear pore glycoproteins can be selectively labelled using the lectin wheat germ agglutinin. This lectin reversibly blocks import into the nucleus. Monoclonal antibodies have been raised against these nuclear pore glycoproteins and O-linked GlcNAc was found to be part of the immunodeterminant. These findings raise the exciting possibility that cytoplasmic glycosylation may be involved in the assembly or functioning of the nuclear pore.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 17009-02 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Tissue Specific and Hormone Regulated Gene Expression

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

PI:	L. Hennighausen	Senior Research Chemist	LBM, NIDDK
Others:	P. Ghazal	Visiting Fellow	LBM, NIDDK
	H. Lubon	Visiting Fellow	LBM, NIDDK
	C. Pittius	Special Volunteer	LBM, NIDDK

COOPERATING UNITS *(if any)*

University of Erlangen, West Germany (B. Fleckenstein); Integrated Genetics Framingham, MA (K. Gordon); Ludwig Institute, Bern, Switzerland (B. Groner); and Laboratory of Molecular Genetics, NICHD (H. Westphal)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The molecular basis of mammary specific gene expression is being studied through analysis of cis-acting regulatory elements in milk protein genes and their cognate trans-acting factors. Applying mobility shift assays, Exonuclease III and DNase I protection, it was shown that nuclear proteins from mammary epithelial cells form a multiple nucleoprotein complex with the whey acidic protein (WAP) gene promoter/upstream region. Whereas some of the DNA sequences were recognized by proteins present in a variety of different cell types, other sequences were recognized by proteins preferentially or exclusively present in mammary gland nuclear extract. Furthermore, a promoter fragment of the WAP gene, encompassing the sites of protein-DNA interaction was found to confer the expression of 'non - mammary' genes in lactating mammary glands of transgenomic animals. This suggested a physiological role of the protein binding sites.

In the second project an *in vitro* system is being established which mimics the *in vivo* activation of the human cytomegalovirus (HCMV). This might allow the study of molecular mechanisms of viral gene activation. Upon virus infection the HCMV, immediate early gene 1 (IE1) is the first viral gene to be expressed. It appears that the IEI gene enhancer mediated transcriptional stimulation *in vitro* involves its recognition by specific nuclear *trans*-acting factors. DNase I protection analyses revealed at least 13 sites in the enhancer/promotor region that interact specifically with nuclear proteins. A correlation was made between protein binding to specific DNA sequences in the enhancer/promoter region and transcriptional stimulation *in vitro*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 17024-04 LBM

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Genetic Lesions of Tay-Sachs Disease

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

PI:	R. Myerowitz	Senior Staff Fellow	LBM, NIDDK
	S. Lontkowski	Howard Hughes Medical student	LBM, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

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

This project transferred from GBB. The former project number was Z01 DK 52013-03GBB.

Tay-Sach disease is a group of disorders caused by mutations in the α-chain polypeptide of the A form of β-hexosaminidase, a lysosomal enzyme composed of two chains (α,β). Such lesions result in a spectrum of disease states ranging from severe to mild. Although the disorder is in general rare, both French Canadians living in Eastern Quebec as well as Ashkenazi Jews, have a 10-fold higher gene frequency then the general population for a severe form of the disorder known as "classic" Tay-Sachs disease.

We previously found that French Canadian patients lacked a 7.6 kilobase fragment of the α-chain gene including the promotor region, exon 1 and part of intron 1, whereas the gene from Ashkenazi patients appeared grossly intact. During the past year we have identified the exact deletion borders in the α-chain gene of a French Canadian patient by sequence analysis of the deletion junction in the mutant and corresponding regions of the normal gene. This analysis also demonstrated the presence of similarly oriented Alu sequences at the 5', 3' deletion boundaries suggesting that the deletion may have arisen during homologous recombination from unequal crossing over between Alu sequences. In addition, we have isolated genomic clones from a λ library constructed with DNA from an Ashkenazi Jewish patient with classic Tay-Sachs disease, that span almost the entire 40 kilobase α-chain locus. Twelve of the exons have been sequenced as well as 24 of the 28 splice juction regions. No deviations from the corresponding regions of the normal gene have been found as yet.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18000-22 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Hormone Dependent Development of Mammary Gland

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

PI: Y.J. Topper Chief LBM, NIDDK
Section on Developmental Biology

Others: L. Sankaran Expert LBM, NIDDK

COOPERATING UNITS *(if any)*

Division of Cancer Biology and Diagnosis, NCI (P. Qasba)

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	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.)*

Other investigators have reported that epidermal growth factor (EGF) inhibits the induction of casein synthesis by mouse mammary tissue in vitro. However, since the circulating level of EGF increases during lactation, and since functional EGF receptors are retained by the lactating cells, it seemed unlikely to us that EGF is an inhibitor of mammary differentiation in vitro. The current studies demonstrate, in fact, that EGF inhibits the induction of casein synthesis in vitro only when insulin, an essential hormone, is present in the culture medium at pharmacological concentrations.

We reported previously that EGF can substitute effectively for prolactin (P) in the induction of α-lactalbumin activity (assayed as lactose synthetase) in rat mammary explants cultured in the presence of insulin and glucocorticoid. The time courses of induction with EGF or P are similar. Both EGF and P also promote similar elevations in the accumulated level of α-lactalbumin mRNA. However, although P produces a large increase in the accumulated level of immunoprecipitable α-lactalbumin (determined with polyclonal antibody), the induction of immunoprecitable α-lactalbumin by EGF is less than 10% of that which occurs with P. It appears, then that EGF, like P, can support α-lactalbumin gene expression and the formation of enzymatically active α-lactalbumin, but that P is required post-translationally for the production of immuno-active α-lactalbumin. A less likely interpretation is that the α-lactalbumin activity induced by EGF corresponds to a protein distinct from the α-lactalbumin present in rat milk.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18002-14 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies on the Pathogenesis of Sialic Acid Storage Disease

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

PI:	Frank Tietze	Research Chemist	LBM, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project was transferred to the Laboratory of Molecular and Cellular Biology. The new project # is Z01 DK 57503-14 LMCB.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 18007-08 LBM

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

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

PI:	Evelyn F. Grollman	Medical Officer (Research)	LBM, NIDDK
Others:	Sonia de Qualteli Doi	Visiting Fellow	LBM, NIDDK

COOPERATING UNITS *(if any)*

Richard J. Montali: National Zoological Park; Alfred Halpren, Sao Paulo Medical School; Sidney Shifrin, Chemist, NCI, DCBD; N.J. Philp, University of Pennsylvania School of Medicine; Donatella Tormbaccin, USUHS

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Cell Regulation

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Regulation of FRTL-5 cells, a continuous strain of rat thyroid cells, involves both cyclicAMP and calcium as second messengers. TSH uses both pathways, while alpha 1-adrenergic agents utilize only a calcium signal. Efflux of iodide into the follicular lumen of the thyroid and iodination of thyroglobulin, essential steps in thyroid hormone formation, are regulated by TSH and adrenergic agonists (such as norepinephrine) through calcium mobilization. The transducing mechanism for generation of this second messenger is the phosphodiesterase-mediated hydrolysis of membrane phosphoinositides, in particular phospatidylinositol 4.5 bisphosphate. The products of this cleavage are inositol trisphosphate, which releases calcium from intracellular storage sites, and diacyglycerol which is important in phospholipase C activation and cell growth. Stimulated metabolism of membrane phosphoinositides is also associated with release of arachidonic acid, metabolities of which are implicated in iodide efflux and growth. Thiocyanate, a goitrogen for humans, competes for the transport and metabolism of iodine by the thyroid. Thiocyanate reacts with tyrosyl residues of thyroglobulin in the region where thyroid hormone activity is segregated, with release of a thiocyanate containing peptide. Thyroglobulin isolated from iodine deficient goiters, and an animal with spontaneous goiter, suggest that thyroglobulin related proteins are involved in hormone release and the pathogenesis of iodine deficient goiter. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of thyrotropin and pharamacologic agents.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 18008-21 LBM

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Regulation by Pharmacodynamic and Autoimmune Agents Acting on Cell Membranes

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

PI:	Leonard D. Kohn, M.D.	Medical Director, USPHS, and Chief, Section on Cell Regulation	
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LAB/BRANCH M. Sheppard (Guy's Hosp., London)
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Section on Cell Regulation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.0	6	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Structure-function relationships in the mechanisms by which glycoprotein hormones (thyrotropin), autoantibodies, certain bacterial toxins (cholera and pertussis, for example), the anti-viral protective agent, interferon, α_1-adrenergic agents, insulin, and insulin-like growth factors (I and II) interact with and transmit their message through the cell membrane to affect thyroid or fibroblast function and pathology are being defined. Studies using monoclonal antibodies and the idiotype antiidiotype theory have continued to explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general, and the auto immunity of Graves' disease, Hashimoto's disease, and diabetes in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of different hormones and signal transduction mechanisms in thyroglobulin biosynthesis, in thyroglobulin biodegradation to T_3 and T_4, and in the transport of T_3, T_4, monoiodotyrosine, diiodotyrosine, and other amino acids from the lysosome. The role of phosphate and carbohydrate moieties in thyroglobulin structure and post-translational processing is being studied. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell growth and development. Studies have been initiated to clone the TSH receptor and define its structure and regulatory control at a gene level.

1986-7

ANNUAL REPORT OF THE LABORATORY OF CHEMISTRY

NATIONAL INSTITUTE OF DIABETES, DIGESTIVE AND KIDNEY DISEASES

SECTION ON BIOCHEMICAL MECHANISMS

TRH ANALOGS

The simple tripeptide, L-pyroglutamyl-L-histidyl-L-proline amide (TRH), exerts marked cardiovascular, behavioral and analeptic effects, through activation of the sympathoadrenomedullary system. These effects appear to be unrelated to its action on the hypothalamo-pituitary axis to release thyrotropin and prolactin. Involvement of TRH in many nonendocrine functions of brain is also suggested by its distribution and the presence of high affinity binding sites outside the hypothalamus and pituitary. TRH has shown promise in the treatment of various forms of shock, as an analeptic, antidepressant and in promoting the regeneration of injured spinal cord. Practical clinical utility of the peptide is limited, however, by this very multiplicity of biological activities, as well as by its very low biological half life. The presence of degrading enzymes in blood serum, a difficulty in crossing the blood-brain barrier because of its polar structure, and the unavailability of facilitated or receptor-mediated transport - all serve to limit severely the survival of exogenous TRH and its delivery to the brain. On the other hand, the multiplicity of significant (or even vital) physiological activities of TRH argues strongly for the search for synthetic analogues which can not only overcome these limitations of stability and penetration but also achieve separation of the various activities.

The synthetic analogues used in our previous and current studies have all involved modification (or replacement) of the imidazole ring of histidine; these analogues have produced dramatic dissociation of some activities, suggesting that the different physiological functions of TRH may be mediated through different receptors or subtypes thereof. In contrast to TRH, 4-F-Im-TRH and 2-CF_3-Im-TRH do not bind to pituitary GH_4 cells in vitro nor stimulate prolactin release from them; such results would immediately suggest the analogues to be nonfunctional. On the other hand, systemic injection or direct microinjection into the rat brain of either analogue not only results in increased cardiovascular (CVS) effects (heart rate, blood pressure) comparable to those found with TRH, but also in release of prolactin at 2-3 times the level observed with TRH. In the whole animal, therefore, prolactin release can be controlled from receptor sites outside the pituitary. Enhanced CVS activity is also evident in 4-CF_3-Im-TRH and 4-NO_2-Im-TRH, and it would seem that the receptor for CVS activity is essentially indifferent to the position, size or nature of the imidazole ring substituent. The fallacy of this conclusion is demonstrated by the greatly reduced CVS activity of 4-I-Im-TRH and the total inactivity of 2,4-I_2-Im-TRH. Furthermore, replacement of histidine by an aliphatic amino acid (e.g., norvaline) also results in the loss of CVS activity. The spectrum of CVS and other activities are summarized in Table I.

TABLE 1

Physiological Activities of TRH Analogues [a]

Compound [b]	CVS Activity	Prolactin Release	TSH Release [c]	CNS Activity [c]
TRH	+++	+	+++	+++
4-F-TRH	++	++ [d]		
4-CF_3-TRH	+++	++		
2-CF_3-TRH	+++	+++		
4-I-TRH	0(+)	+ [e]	0	
2,4-I_2-TRH	0	+	0	
4-NO_2-TRH	+++	0	0	
Nva^2-TRH	0	+	0	+++ [f]

[a] By intra-arterial administratiion in conscious rats, unless otherwise indicated.
[b] Substitutions are all on imidazole ring of histidine.
[c] Reported in the literature.
[d] Both CVS and prolactin release are observed following central administration.
[e] Active at higher dose (30 μmol/kg).
[f] Ten times more potent than TRH in analeptic activity test.

It is evident from the Table that the structural requirements for CVS activity differ markedly from those for prolactin release and that, for the latter, an imidazole ring may not be necessary at all. Equally striking is the evidence that structure-activity factors for the release of thyrotropin-stimulating hormone (TSH) do not parallel those for prolactin release. Although data is still being assembled, it is already apparent that CNS activity will show its own unique structure dependence.

It is now quite clear that at least four of the biological activities of TRH involve uniquely different receptors and that, after decades of effort in various laboratories, the separation of these activities has at last been achieved. Thus, 4-NO_2-TRH, highly selective for CVS activity, may be useful in the treatment of various forms of shock without a concommitant enhancement of thyroid activity or of prolactin release. On the other hand, 2,4-I_2-Im-TRH or Nva^2-TRH may be useful as diagnostic tools for the assessment of pituitary function without the risk of increased blood pressure and tachycardia induced by TRH. The iodinated analogue is particularly useful since it can be

prepared readily with radioactive iodine. Furthermore, each of these selective agonists should provide a useful research tool for the study of the role and mechanism of TRH involvement in the respective function.

Strong conclusions about structure-activity correlation are not yet possible. We have theorized that the imidazole ring of histidine is necessary for the CVS activity of TRH but is not essential for prolactin-releasing activity. The unexpected loss of CVS activity in 2,4-I_2-Im-TRH may be due to steric hindrance to binding at the TRH receptor. In addition to size, ring substituents vary in electronegativity, polarity, hydrophobicity and ability to participate in intra- and/or intermolecular hydrogen bonding. One or more of these variables may stabilize the physiologically relevant conformations of TRH, interfere with binding or promote binding to a specific receptor. In addition, a given substituent may stabilize either the π or τ tautomer of imidazole in histidine.

A number of other new imidazole-modified analogues of TRH have already been prepared and others are in progress. With data on the pharmacology and neurobiology of all these analogues, we hope to identify the structural requirements and limitations for each type of activity, as well as the role of imidazole pK, aromaticity and hydrophobicity. In order to determine whether both ring nitrogens are necessary for activity and whether imidazole tautomers can be differentiated, we are currently preparing analogues of TRH with other heterocyclic rings in place of imidazole. Receptor-specific analogues will also be prepared with increased resistance to enzymic degradation and more lipophilic prodrugs are planned to accelerate penetration to the brain.

ANTIMALARIALS

Our development, in 1971, of a photochemical route to ring-fluorinated aromatics and heteroaromatics has led to the synthesis of a wide variety of fluoro analogues of imidazole-based metabolites. Many of these compounds have shown interesting properties as agonists or antagonists and have proved useful as research tools and as possible chemotherapeutic agents. A striking difference has been found between 2-fluoro-L-histidine (2-FHIS) and the 4-fluoro isomer. While the former compound is readily incorporated into new protein in place of histidine (both in bacteria and mammals), the 4-fluoro isomer is not incorporated at all. Furthermore, 2-FHIS shows antibacterial, antiviral, antileukemic and antimalarial properties; again, the 4-fluoro isomer shows none of these activites.

We have become particularly interested in the antimalarial properties of 2-FHIS, since the compound is uniquely and selectively active against Plasmodium falciparum, that parasite which is notoriously resistant to chemotherapy. The organism has the unusual property of inducing production, within an invaded erythrocyte, of a protein containing as much as 70% histidine. The protein is found in "knobs" which are seen on the erythrocyte surface; these knobs are responsible for a very strong adherence of the infected erythrocytes to capillary endothelium, thereby sequestering parasitized cells which would normally be destroyed during passage through the spleen.

In cultures of infected erythrocytes, low concentrations of 2-FHIS not only inhibit cytoadherence but prevent maturation of the parasite and the appearance of knobs entirely. The assumption that these antiparasitic properties are due to the incorporation of 2-FHIS into the histidine-rich protein is probably unwarranted, since the treated parasite shows a general decrease in protein synthesis and rather low incorporation of ^{3}H-2-FHIS. As one of several hypotheses for the mechanism of action, we propose that 2-FHIS interferes with histidine as a promoter of the transport of some other amino acids into the cell. This hypothesis is supported by our earlier findings that 2-FHIS inhibits protein synthesis in cell and organ cultures but not in cell-free systems. Studies are in progress on the effect of 2-FHIS on facilitated amino acid transport.

Laboratory-scale production of these histidine analogues is extremely time-consuming, involves multiple low-yield steps, and is limited to small batch operation. Our recent efforts to find alternative, and more economical routes have been successful - at least for 2-IHIS. Readily available 2,4-diiodo-L-histidine can be converted into mixtures of 2-IHIS, 4-IHIS and HIS by photoreduction, catalytic hydrogenation or reduction with titanium trichloride. The last method is especially promising, providing yields of 2-IHIS up to 20% in this one-step process. More recently, we have found that 2,4-diiodo-L-histidine can be reduced selectively with hot 3N HCl to 2-iodo-L-histidine, without formation of any of the 4-iodo isomer. While both the 2-fluoro and 2-iodo analogues show high antimalarial activity in vitro, tests with monkeys show the 2-fluoro compounds to be too toxic and the 2-iodo compound to be inactive. It is possible that mammals possess a metabolic system for deiodination of the iodo analogue. Deiodination of 4-iodohistidine in rats had been observed previously. In the initial in vitro screening, 2-azidohistidine was also found to have some activity against P. falciparum. In order to attempt any structure-activity correlation, much more data is needed - particularly on 2-substituted histidines. We are now developing new synthetic methods to obtain such compounds, based on (1) cyclization of dibenzoylaminoethylenes with acyl halides and (2) imidazole ring substitution with photochemically generated radicals. Further clues to the design of effective antimalarials may be achieved from knowledge of the mechanisms of action of these histidine analogues. To this end, a synthesis of ^{14}C-2-fluorohistidine has been developed. We have also demonstrated that H-4 in 2-iodohistidine can be exchanged with isotopic hydrogen under alkaline conditions.

CHEMISTRY OF IMIDAZOLES AND BIOIMIDAZOLES

In various sections of this report, we describe significant and valuable applications of histidine analogues in biochemical and pharmacological studies. Such studies could have been performed many years ago, but for the fact that these analogues had not been available through classical or obvious synthetic routes. Even methods suitable for simple imidazoles may not be applicable to complex bioimidazoles, because of the additional functional groups and chirality. Thus, nonclassical methods (e.g., photochemical radical substitution, one-electron reduction, etc.) were developed to fit these gaps.

Even more novel methods are always being sought to provide analogues still inaccessible. We have now developed procedures for the conversion of amino-histidines into azido and nitrohistidine, of amino to chloro, bromo and iodo, of trifluoromethyl into methyl, cyano, carboxy, carbomethoxy, etc. Recently, we synthesized 2- and 4-(pentafluoroethyl)-histidines by photochemical radical substitution. These compounds are converted by base into the corresponding (trifluoroacetyl)-histidines, which have such reactive carbonyl groups that they may serve as affinity labels for histidine-binding sites. The trifluoro-acetylimidazoles can be reduced to the secondary alcohols, also obtainable by direct condensation of imidazoles with trifluoroacetaldehyde. In turn, the secondary alcohols can be oxidized to the trifluoroacetyl ketones. Upon treatment with methanolic base, (trifluoromethyl)-histidine can be converted into (trimethoxymethyl)-histidine and pentafluoroethyl into the corresponding ketal. These ortho functionalities are also of interest as potential covalent affinity labels.

Ring-trifluoromethylated imidazoles show the unique property of losing hydrogen fluoride above pH 8 to form metastable difluorodiazafulvenes, which then react with any available nucleophile to form new covalent bonds. Such intermediates, derived from trifluoromethylhistamine or histidine, may be able to serve as covalent affinity labels for specific binding sites, both in vitro and in vivo. It would be desirable, therefore, to have available a series of trifluoromethyl analogues with a range of reactivities, and to be able to correlate reactivity with some substituent parameter. Our discovery of a simple photochemical method for the trifluoromethylation of imidazoles has made available a large series of analogues for study. We have now found that the reactivities of some members of the group can be correlated with the special electronic effects of certain substituents (capable of hyperconjugation or back-bonding). Computer analysis of reactivity data for a series of trifluoromethylimidazoles has provided a linear free energy relationship in which log k_r correlates with both inductive and resonance components of the respective substituents. According to computer-based predictions, the fluoro group would provide the ideal combination of acidity and reactivity under physiological conditions. We have, therefore, developed procedures for sequential photochemical introduction of fluorine and trifluoromethyl into imidazoles and have verified the predicted reactivities. We are now involved in the preparation of peptide hormones containing these substituents. Photochemical introduction of the trifluoromethyl group has been found practical for more complex imidazoles and studies are under way for the synthesis of the trifluoromethyl analogue of the anti-ulcer drug, cimetidine.

HYPOXIC CELL SENSITIZERS

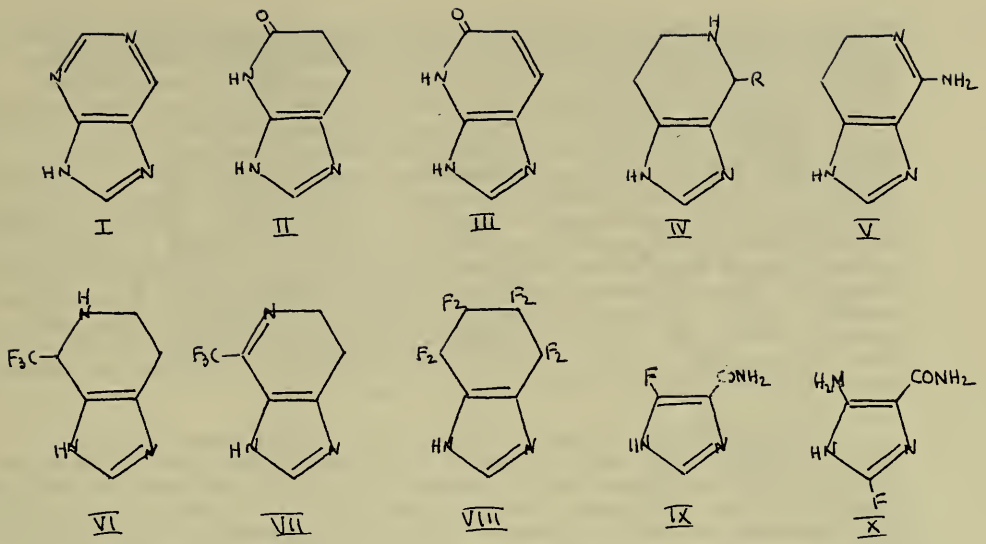
The valuable properties of nitroimidazoles as radiation sensitizers and as selective cytotoxic agents for cancer treatment have stimulated considerable research into mechanisms of action and metabolic fate of the drugs. We have proposed three theories for the mechanism of action: (1) Thiols are known to add to the 4,5-double bond of nitroimidazoles and, thus, such compounds may intefere with normal cellular functions by binding cysteine, glutathione, SH enzymes, etc. (2) Nitroimidazoles may be reduced in vivo, to hydroxylamino-

imidazoles which can function as supernucleophiles in cleaving the phosphate ester bonds of polynucleotides; unfortunately, synthetic hydroxylaminoimidazoles have been found so unstable that their potential as nucleophiles cannot be investigated. As an alternatiave, we are devising synthetic methods for hydrazinoimidazoles; these compounds should be significantly more stable than hydroxylaminoimidazoles and, yet, should possess the same nucleophilic power inherent in hydroxylamine functions. Our primary interest is in 2-hydrazinoimidazoles: efforts to prepare these compounds by displacement of fluorine in 2-fluoroimidazoles have been unsuccessful; we are now trying reduction of 2-diazonium imidazoles with borohydride-metal combination. (3) Reduction of the nitro group by nonnucleophilic agents leads to nitro radicals; we believe these heterocyclic radicals capable of alkylating cell constituents and interfering with metabolism. To this end, we are now studying the anaerobic reduction of nitroimidazoles with one-electron transfer agents (e.g., titanous chloride).

Misonidazole is an alkylated 2-nitroimidazole which has been found quite effective in sensitizing cancer cells to radiation and in reducing the radiation dose needed to effect significant cell destruction. Unfortunately, the compound has to be used at such high levels as to produce serious side effects and may not be released by FDA. We have postulated that the introduction of nitro groups into more natural imidazoles (histamine, histidine, etc.) may produce the desired alien molecule. Indeed, several such compounds have shown in vitro activity comparable to that of misonidazole. Evaluation of the clinical effectivness in animals of this series of compounds is in progress.

IMIDAZOLE ANTIVIRALS

The notable success of virazole and deazapurine systems as antivirals has stimulated research into further modifications of the purine (I) ring system, especially those involving replacement of ring nitrogen with carbon. Analogues synthesized to date have required laborious multistep processes and have given only low yields. We have devised a number of simple syntheses which produce deazapurine analogues in good yield and with few steps. Reduction of 4-nitrohistidine ester or of 4-nitroimidazolepropionic ester leads to II. Reduction of 4-nitrourocanic ester gives the stable 4-aminourocanic ester, but subsequent irradiation converts the trans olefin to cis and the product cyclizes to III. Condensation of histamine with aldehydes gives series IV and cyclization of 4-(trifluoromethyl)-histamine with ammonia gives V. Series VI is obtained by condensation of histamine with trifluoroacetaldehyde, VII by cyclization of 4-(trifluoroethyl)-histamine with base, and VIII by photochemical reaction of imidazole with 1,4-diiodo-perfluorobutane. Compounds which we have previously found to have significant antiviral activity (IX and X) are also being modified somewhat. Systems III-VII can be dehydrogenated to the fully aromatic systems with selenium dioxide. These compounds, with or without ribose attachment, will be evaluated for antiviral activity, particularly against AIDS.



CHEMISTRY, BIOCHEMISTRY AND PHARMACOLOGY OF BIOINDOLE ANALOGS

Tryptophan is an essential amino acid, serving as the precursor of the neurotransmitter, serotonin, and of the hormone, melatonin, in addition to its roles in enzymes and in receptor proteins. Tryptophan is metabolized in mammals by a pyrroloxygenase in the liver, where it can serve as a precursor of nicotinamide (Vitamin B) in some animals. In other tissues, tryptophan and related indoles are metabolized by a distinct oxygenase, the activity of which is dramatically increased (up to 100-fold) upon administration of bacterial lipopolysaccharides or interferon. The role of this oxygenase in the response of the organism to infection is unknown, however. We anticipated that certain 2-substituted tryptophans might serve as selective "suicide substrates" for these oxygenases. Analogs of tryptophan with electronegative substituents at C-2 had not been previously prepared. We have obtained 2-chloro and 2-bromo-L-tryptophan by radical halogenation, 2-trifluoromethyl-L-tryptophan by photochemical substitution, and 2-nitro-L-tryptophan as a minor product of direct nitration. Both the trifluoromethyl and nitro groups can be converted readily into other functions; some of these derivatives are of potential value as affinity and photoaffinity labels, as antibacterial agents and as photosensitizers in radiation therapy. 5-Azido-L-tryptophan has already been found effective as a photoaffinity label for tryptophan synthase.

The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine

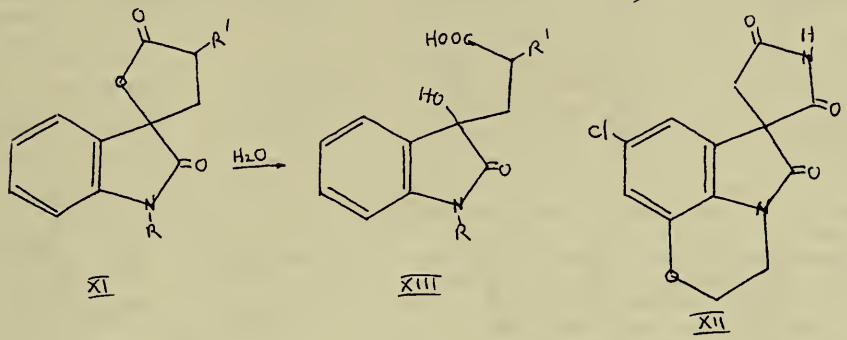
tautomer. An enzyme carboxyl groups should also promote indolenine formation, suggesting the indolenine to be the true substrate for certain tryptophan enzymes. The first conclusive support for this concept is found in the demonstration that 2,3-dihydro-L-tryptophan and oxindolyl-L-alanine, analogs of the indolenine tautomer of tryptophan (tetrahedral carbon at C-3), are potent competitive inhibitors of tryptophan synthase and tryptophanase. Furthermore, the two enzymes show opposing specificity for the C-3 diastereoisomers of 2,3-dihydro-L-tryptophan suggesting that these enzymes catalyze their reactions via enantiomeric indolenine intermediates. The chiral center at C-3 in oxindolyl-L-alanine racemizes too readily to permit a study of opposing enzyme specificity. We have recently prepared the stable diastereoisomers of 3-hydroxy-oxindolyl-L-alanine and, indeed, find the same opposing specificity for tryptophan enzymes as with dihydrotryptophan. Since the proton at C-3 in the indolenine tautomer is known to be accessible to a basic site in the enzyme, we plan to convert the C-3 hydroxyl into leaving groups in order to generate affinity lables for the basic site.

Fluorine-19-nuclear magnetic resonance and differential absorption spectroscopy have been used to study the binding and reactions of the D and L isomers of 5-fluorotryptophan, tryptophan and of (3S)- and (3R)-2,3- dihyro-5-fluorotryptophan. Tryptophan synthase specifically and tightly binds the (3S) diastereoisomer of both 2,3-dihydro-5-fluoro-D-tryptophan and 2,3-dihydro-5-fluoro-L-tryptophan, whereas it binds 5-fluoro-D-tryptophan more tightly than 5-fluoro-L-tryptophan. Unexpectedly, we find that the D and L isomers of 5-fluorotryptophan, tryptophan, and (3S)-2,3-dihydro-5-fluorotryptophan are slowly interconverted by isomerization reactions. These isomerization reactions are much slower than β-replacement and the β-elimination reactions catalyzed by tryptophan synthase. Since pyridoxal phosphate itself slowly catalyzes many reactions of amino acids in model systems, our results raise the interesting question of whether tryptophan synthase itself serves a catalytic role in these slow reactions or whether the enzyme simply binds the substrate and pyridoxal phosphate stereospecifically and thus promotes the intrinsic catalytic activity of pyridoxal phosphate. Our results further define the stereochemistry of the substrate binding site of tryptophan synthase.

ANTIDIABETIC DRUGS: ALDOSE REDUCTASE INHIBITORS

Inhibition of the enzyme aldose reductase represents a new pharmacological approach toward the treatment of late-onset diabetic complications. These complications affect the eye, kidney, nervous system and circulation; they are thought to result from the hyperosmotic effects of high concentrations of sorbitol, in turn resulting from the reduction of the excess glucose symptomatic of diabetes. Our methods for the synthesis of inhibitors of tryptophan-metabolizing enzymes involve intermediates (XI) which are fairly similar in overall structure to compounds (e.g., Kyorin, XII) now in clinical trials as aldose reductase inhibitors. Furthermore, our kinetic and mechanistic studies have shown that the lactone ring of XI is opened gradually at mildly alkaline pH; should XI bind to aldose reductase, the possibility then exists that the

compound might serve as a covalent affinity label for the tyrosine phenolic group present in the inhibitor-binding site and believed to be critical for activity.



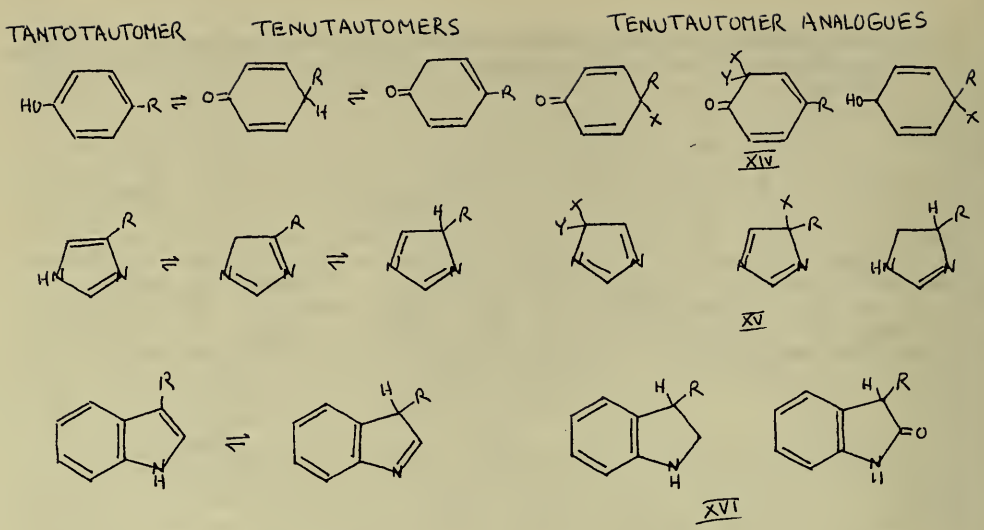
XI XIII XII

The first series of compounds evaluated as inhibitors showed the spirolactone (XI) to be active only at concentrations 100 times that of commercial inhibitors; on the other hand, the hydroxyacids (XIII) resulting from ring opening were ca. ten times more active than the lactones, providing a totally new direction for the design of inhibitors.

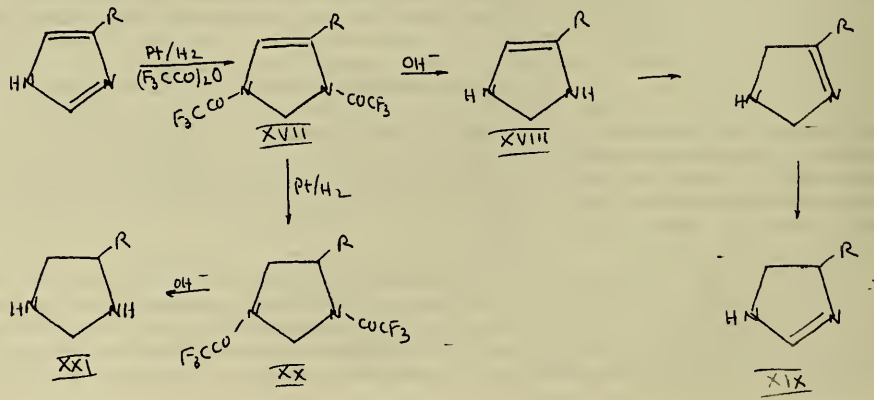
TENUTAUTOMER ANALOGUES

Our studies in tryptophan chemistry and biochemistry have revealed that the molecules present in the active sites of tryptophanase and tryptophan synthase are not the common NH tautomers (tantotautomers) of tryptophan but the higher energy, minor tautomers (tenutautomers). A variety of biological metabolites have similar major and minor capabilities - phenols, catechols, imidazoles, purines, etc. It is conceivable, therefore, that a variety of enzymes utilize an ability to bind and stabilize tenutautomers as a means of activating the substrate for a chemical transformation. There is now ample evidence that tenutautomers are the active species in a number of test-tube reactions of both phenols and imidazoles; furthermore, the experimental data for some enzyme-catalyzed reactions might become more intelligible if the substrates were viewed as their tenutautomers.

Since it is still impossible to examine the detailed structure of a substrate within a binding site, arguments for the tenutautomer concept must be based on evidence and inference from the behavior of stable tenutautomer analogues (XIV, XV). This approach was highly successful and very convincing in the case of tryptophan (XVI). We have now undertaken analogous studies for the other tautomeric systems.



The imidazole ring is considerably more refractory to reduction than even benzene. We have found that catalytic hydrogenation can be achieved in the presence of trifluoroacetic anhydride, leading to the acylated dihydro derivative (XVII). The trifluoromethyl groups of XVII can be removed at pH 12-13 to give the free amino acid (XVIII). We then hope to isomerize the double bond of XVIII to form XIX. Continued reduction of XVII gives the fully reduced ring. Both XVIII and the tetrahydro derivative of histidine are expected to serve as inhibitors of tetrahydrofolate reductase.



GENERAL PRINCIPLES OF ENZYME CATALYSIS AND SIMULATION

In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation of free energy (the energy hill which must be surmounted to get from starting material to product) is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereopopulation control). The compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. Enzymes catalyze many reactions which cannot be observed under mild laboratory conditions. We have shown that our "locked" test-tube analogs can undergo a number of these reactions under physiological conditions of temperature and pH. Thus, one can demonstrate such difficult processes as hydride transfer and displacement of aromatic halogens. Recent work has involved the synthesis of compounds designed (1) to evaluate the flexibility of conformationally frozen carbon chains by ring-ring interconversion and (2) to study steric and electronic effects on ^{1}H and ^{13}C nmr spectra through space rather than through covalent bonds.

As part of our studies of practical applications of stereopopulation control, we are currently exploring the use of o-nitroaryl derivatives of biogenic amines and antibiotics as prodrugs. The intent is to facilitate passage from gut to circulatory system and from circulatory system to brain by temporary masking of charge within the molecule.

SECTION ON CARBOHYDRATES

The Section is continuing its work on the molecular interaction between antigens and monoclonal antibodies. Elucidation of the nature of this interaction is of importance not only in immunology but for a general understanding of the interaction between receptors and haptens. Our approach is three fold:

A. The interaction of ligands (natural or synthetic) with monoclonal antibodies is evaluated and (possibly) correlated with epitopes on both antigen and protein.

B. Rearranged immunoglobulin genes are cloned with the object of site specific mutagenesis so that specifically altered antibodies may be obtained. These can then be evaluated for their altered binding properties.

C. The preparation of cold, and radioactively labeled affinity labels, and their reaction with monoclonal antibodies.

RECENT WORK

Sub A. There is one antipolysaccharide monoclonal immunoglobulin known capable of binding to the terminus of its dextran antigen only (see J. Exp. Med. 142, 435, 1975; Carbohydr. Res. 72, 315, 1979). This type of binding has been referred to as "cavity" type binding. In order to evaluate hydrogen bonding and thus map the subsites of this kind of antibody we have prepared a number of deoxyfluoro α-D-glucoside derivatives and we are studying the interaction of these ligands and protein.

Sub B. In the past we have been able to specifically assign binding affinities to each of four subsites each capable of binding a single galactosyl residue of a galactan for a set of monoclonal anti-galactan antibodies. In correlation with their known amino acid sequences we were able to propose certain amino acid contact residues. These will become the most likely candidates for site specific alteration. Thus we are cloning the rearranged heavy and light chain genes for IgA X-24, one of the two genes from which all anti-galactan genes are derived by somatic mutation.

Sub C. We have prepared a number of galacto (oligo)saccharides with the aglycon carrying a reactive group such as an epoxypropyl or diazirino group (radioactively labeled). The latter has been covalently linked to IgA X-24 by photochemical activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19001-15 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Reactions and Immunochemistry of Carbohydrates

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

PI: Cornelis P.J. Glaudemans, Chief, Section on Carbohydrates NIDDK LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Carbohydrates

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Section is continuing its work on the molecular interaction between antigens and monoclonal antibodies. Elucidation of the nature of this interaction is of importance not only in immunology but for a general understanding of the interaction between receptors and haptens. Our approach is three-fold:

A. The interaction of ligands (natural or synthetic) with monoclonal antibodies is evaluated and (possibly) correlated with epitopes on both antigen and protein.

B. Rearranged immunoglobulin genes are cloned with the object of site specific mutagenesis so that specifically altered antibodies may be obtained. These can then be evaluated for their altered binding properties.

C. The preparation of cold, and radioactively labeled affinity labels; and their reaction with monoclonal antibodies.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19002-14 LC

PERIOD COVERED: October 1, 1986 to March 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: B.Cell Proliferation:Mechanism of Triggering & Regulating Activation

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

PI:	Milton Kern	Research Chemist	NIDDK-LC
OTHERS:	Sibghat Ullah	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Chemistry

SECTION: Section on Carbohydrates

INSTITUTE AND LOCATION: NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been discontinued since the death of Dr. Milton Kern in March of 1987.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19200-37 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Biological-Pharmacological Investigation of Opioids and Stimulants/Depressants

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

PI:	A. E. Jacobson	Research Chemist	NIDDK-LC
Other:	M. Mattson	Technician	NIDDK-LC

COOPERATING UNITS *(if any)* Univ. of Michigan Med. School, and the Medical College of Va., Johns Hopkins Univ. Med. School, Univ. of Chicago Med. School and the Committee on Problems of Drug Dependence, Inc.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK-NIH-Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19202-14 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Synthesis and Evaluation of Potential CNS, Antiinflammatory & Anticancer Agents

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

PI:	K. C. Rice	Research Chemist	NIDDK-LC
	T. R. Burke,Jr.	Senior Staff Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19216-11 LC

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Structure-Activity Relationships of Colchinoids Based on Tubulin Binding

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Peter Kerekes	Visiting Scientist	NIDDK-LC
	Raymond Dumont	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)* C. Chignell, Res. Tri. Park, N.C.; M. Suffness/F. Quinn, NCI/NIH; M. Banwell, University of Auckland, New Zealand; J. Wolff, NIDDK, NIH; P. Sharma, School of Pharmacy, Univ. of Kansas, Lawrence; Pierre Potier, Gif,CNRS, France; M. Ravid, Dept. of Medicine, Meir Gen. Hosp., Israel; Roussel-UCLAF Co., Paris, France

LAB/BRANCH: Laboratory of Chemistry

SECTION: Section on Medicinal Chemistry

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19226-09 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Pharmacological Probes of the Benzodiazepine Receptor

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

PI:	K. C. Rice	Research Chemist	NIDDK-LC
Others:	A. Hauck-Newman	Guest Worker	NIDDK-1c

COOPERATING UNITS *(if any)*

NIMH-CP (S. Paul, R. Weber, M. Goldman), NIDDK-LBC (Phil Skolnick, H. Luddens)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19233-08 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Total Synthesis of Opioids via Dihydrothebainone and Derivatives

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

PI:	K. C. Rice	Research Chemist	NIDDK-LC
Other:	A. Hauck-Newman	Guest Worker	NIDDK-LC

COOPERATING UNITS *(if any)*

Research Triangle Institute, (F. I. Carroll); University of Minnesota (P. S. Portoghese)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19235-06 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Characterization of Opiate Receptors Using Nonrigid Irreversible Inhibitors

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

PI:	K. C. Rice	Research Chemist	NIDDK-LC
Others:	A. E. Jacobson	Research Chemist	NIDDK-LC

COOPERATING UNITS *(if any)*

NIMH (W. Klee, W. Simonds, R. Rothman, C. Pert, M. Herkenham)
Walter Reed Army Institute of Research (J. Holaday)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

This project has been transferred to the Laboratory of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19236-06 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Characterization of Opioid Receptors Using Rigid Probes

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

P.I.:	A. E. Jacobson	Research Chemist	NIDDK-LC
Others:	R. Lessor	Staff Fellow	NIDDK-LC
	K. C. Rice	Research Chemist	NIDDK-LC

COOPERATING UNITS *(if any)*

W. A. Klee; LGCB-NIMH-ADAMHA

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19241-06 LC

PERIOD COVERED

October 1, 1986 to September 30, 1986

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

Agonists and Antagonists for the Phencyclidine Receptor

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

PI:	A. E. Jacobson	Research Chemist	NIDDK-LC
OTHER:	M. V. Mattson	Technician	NIDDK-LC
	K. C. Rice	Research Chemist	NIDDK-LC
	R. A. Lessor	Staff Fellow	NIDDK-LC
	A. Thurkauf	NRSA Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

Experimental Therapeutics Branch, NINCDS, NIH (Drs. T. O'Donohue, P. Contreras), Department of Pharmacology, University of Michigan Medical School (Drs. J. Woods)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK-LC, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19243-05 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Pyrrolidine Ant Toxins

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Wieslaw Gessner	Visiting Associate	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19246-05 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Characterization of Opiate Receptors Using Positron Emission Transaxial Tomography

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

P.I.:	K. C. Rice	Research Chemist	NIDDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIDDK-LC
	A. Newman	Guest Worker	NIDDK-LC

COOPERATING UNITS *(if any)*

NIMH (C. Pert, A. Pert, N. Ostrowski), CC (S. Larson, R. Finn, M. Channing)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Behtesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Neuroscience.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19247-05 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Perhydrohistrionicotoxins

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

PI:	Wieslaw Gessner	Visiting Fellow	NIDDK-LC
Others:	Arnold Brossi	Visiting Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

J. Daly, NIDDK, NIH; E. X. Albuquerque, U. of Maryland, Baltimore, MD.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19249-04 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies on the Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Wieslaw Gessner	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

C. W. Abell, Department of Biochemistry, University of Texas Medical Branch at Galveston; S. P. Markey, M LCS, NIH; J. P. N. Rosazza, Univ. of Iowa, Ames.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19250-04 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Chemistry and Metabolism of Qinghaosu, A Chinese Antimalarial Drug

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	B. Venugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

D. Klayman, Walter Reed Research Institute; P. Buchs, SAPEC SA., Lugano, Switzerland; P. Trigg, SWG-CHEMAL, WHO, Geneva, Switzerland

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK-LC, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19252-03

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Synthesis of Morphine in Animal Tissue from Intermediates of its Plant Biosynthesis

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
OTHERS:	Kenner C. Rice	Research Chemist	NIDDK-LC
	Raymond Dumont	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

Dr. Sidney Spector, Roche Institute of Molecular Biology, Nutley, New Jersey; V. Toome, Physical Chemistry Department, Roche, Nutley, New Jersey.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19253-03 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Physostigmine and Analogs

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Bernhard Schönenberger	Visiting Fellow	NIDDK-LC
	Bernhard Witkop	Chief, LC	NIDDK-LC

COOPERATING UNITS *(if any)*

Prof. E.X. Albuquerque, Univ. of Maryland, Baltimore; Dr. R. Ray, Pharmacology Branch, U.S. Army Medical Research, Aberdeen Proving Ground; S. Rapoport, NIA LN, NIH.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19255-03

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

8-Aminoquinoline antimalarials

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	Wieslaw Gessner	Visiting Fellow	NIDDK-LC
	B. Venugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

H. Rupp, Hoechst India, Research Institute Mulund; I. Landau, Laboratoire des Vers, Paris, C. W. Abell, U. Texas Medical Center, Galveston.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19256-02 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Mammalian Alkaloids

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	B. Schönenberger	Visiting Fellow	NIDDK-LC
	C. Schoenberger	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

H. Thomas, Dept. of Physiology, Univ. of Ulm, West Germany; C. W. Abell, Univ. of Texas Medical Center, Galveston; J. Flippen-Anderson, Naval Research Laboratory, Dept. of the Navy, Washington, D. C.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19257-02 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

1-Phenylisoquinolines

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

PI:	Arnold Brossi	Visiting Scientist	NIDDK-LC
Others:	B. Benugopalan	Guest Scientist	NIDDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Medicinal Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19401-22 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Natural Products as Agonists, Antagonists, Desensitizers & Probes for Receptors

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

PI: Dr. Bernhard Witkop Chief, NIDDK-LC

Cooperating Units: H. Pollard, Chief, Lab. Cell. Biol. Genetics, NIDDK, I.L. Karle, U.S. Naval Res. Lab., Wash. D. C.; E. X. Albuquerque, C. Spivak & M.P. Blaustein, Univ. MD Med. Sch.; T. Gund, N. J. Inst. Tech., Newark, N. J.; Prof. Gabor Fodor, Dept. Chem. W. Va. Univ.; R. Aronstam, Univ. GA.

Foreign: Boris Khodorov, Vishnevsky Inst. Surgery, Moscow; O. Yonemitsu, Y. Kanaoka & T. Iwakum, Univ. Hokkaido; E. Gössinger, Univ. Vienna, Austria; E.M. Kosower, Tel-Aviv Univ.; Shin-Chi-yi, Univ. Peking.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The Chief Investigator - largely with extramural support - has kept up a widely diversified program, international and interdisciplinary in character, involving binding studies of agonists, electrophysiology of ion flux, photochemistry of psychoactive drugs, modeling of nicotinic and muscarinic agonists, consultation on protective measures against organophosphorous agents--and support function for a clinical program on degenerative diseases of the rain. In addition the Chief Investigator is active in international scientific exchange and collaboration with most countries of Western Europe, China, Japan and Taiwan. He is Editor of FEBS Letters (Federation of European Biological Societies) for North America, Member of the Paul Ehrlich Foundation in Frankfurt, Germany, and as (honorary) Member of Academies and Learned Societies of Europe and Japan participates in the formulation of research aims and policies.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19402-14 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Interferon Induction and Action. The Antiviral Activity of Nucleoside Analogs

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

PI:	Paul F. Torrence	Research Chemist	NIDDK-LC
Others:	Alice Wong	Technician	NIDDK-LC
	David Alster	NRSA Fellow	NIDDK-LC
	Yukio Kitade	Visiting Fellow	NIDDK-LC
	Danuta Brozda	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)*

Foreign: J.-L. Imbach, U. Montpellier, France; W. Dawson, U. Cal.; J. Mond, USUHS, C. Altona, Univ. Leiden, Netherlands; W. Pfleiderer, U. Konstanz, W. Germany

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to the Laboratory of Analytical Chemistry.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERV

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19603-11 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Histidine Analogs

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

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIDDK
Others: Kazuyuki Takahashi, Guest Researcher, Virender Labroo, Visiting Associate, Eric Chang, Summer Student, Nelly Kolodny, Visiting Fellow, Nana Nikoi (Guest Researcher), Ludwig Thierfelder, Visiting Fellow (term 12/15/86), Stefan VonHof, Visiting Fellow, and Shelly Grisaru, Guest Researcher, NIDDK-LC.

COOPERATING UNITS *(if any)*

G. Feuerstein, Dept. of Pharmacology, USUHS
E. De Clercq, Louvain, Belgium
H. Kimoto, Nagoya, Japan
R. Howard, LPD, NIAID

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 2.1	PROFESSIONAL: 1.4	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.)*

TRH Analogs: In addition to governing the release of thyrotropin and prolactin in the pituitary gland, TRH (L-pyroglutamyl-L-histidyl-L-proline amide) is known to possess a wide variety of effects on both the central nervous sytem (CNS) and the cardiovascular system (CVS). TRH has shown promise for use in the treatment of shock, as an analeptic and antidepressant, and as a promoter of the regeneration of injured spinal cord. However, the great variety of its biological effects presents a serious drawback to its use as a specific drug. Our early studies with synthetic analogs of TRH (involving modification of the imidazole ring of histidine) has suggested that the peptide hormone elicits each of its physiological responses at a different receptor and that appropriate analogs may achieve some of the desired specificity of action.

It is now quite clear that at least four of the biological activities of TRH involve uniquely different receptors and that, after a decade of effort in various laboratories, the separation of these activities has at last been achieved. Thus, 4-NO_2-Im-TRH, highly selective for CVS activity, may be useful in the treatment of various forms of shock without a concomittant enhancement of thyroid activity or of prolactin release. On the other hand, 2,4-I_2-Im-TRH or Nva^2-TRH may be useful as diagnostic tools for the assessment of pituitary function without the risk of increased blood pressure and tachycardia induced by TRH. The iodinated analog is particularly useful since it can be prepared readily with radioactive iodine. Furthermore, each of these selective agonists should provide a useful research tool for the study of the role and mechanism of TRH involvement in the respective function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 19604-17 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

General Principles of Enzyme Catalysis and Simulation

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

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIDDK

Other: Michael King Guest Worker GWU

COOPERATING UNITS *(if any)*

Eugene Man, University of Miami
Yoshio Ueno, Nagoya, Japan
Wieslow Antkowiak, Poznan, Poland
Yoshio Takeuchi, Toyama, Japan

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

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

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

In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation free energy is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of the bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant, and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereopopulation control). These compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. The protein raises both the entropic and enthalpic components of the substrate by binding it in a single, rigid conformation.

Recent work has involved the synthesis of compounds designed (1) to evaluate the flexibility of conformationally frozen carbon chains by ring-ring interconversion and (2) to study steric and electronic effects of ^{1}H and ^{13}C nmr spectra through space rather than through covalent bonds.

As part of our studies of practical application of stereopopulation control, we are currently exploring the use of o-nitroaryl derivatives of biogenic amines and antibiotics as prodrugs. The intent is to facilitate passage from gut to circulatory system and from circulatory system to brain by temporary masking of charge within the molecule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19605-11 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Chemistry of Substituted Imidazoles

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

P.I.	Louis A. Cohen, Chief,	Section on Biochemical Mechanisms,	NIDDK, LC
Others:	Robert Jerussi	Guest Worker (FDA)	NIDDK-LC
	Stuart Cohen	Guest Worker	NIDDK-LC
	Virender Labroo	Visiting Associate	NIDDK-LC
	Kazuyuki Takahashi	Guest Worker	NIDDK-LC
	Bianca Avramovici	Visiting Fellow	NIDDK-LC

COOPERATING UNITS *(if any)* H. Kimoto, Industrial Res. Inst., Nagoya, Japan; R. Henkin, Georgetown Univ. Hosp., Wash, D.C.; E. De Clercq, Louvain Univ., Belgium; A. Shanzer, Rehovot, Israel; W. Nagai, Nagoya, Japan.

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Ring-trifluoromethylated imidazoles show the unique property of losing hydrogen fluoride above pH 8 to form metastable difluorodiazafulvenes, which then react with any available nucleophile to form new covalent bonds. Such intermediates, derived from trifluoromethylhistamine or histidine, may be able to serve as covalent affinity labels for specific binding sites, both in vitro and in vivo. It would be desirable, therefore, to have available a series of trifluoromethyl analogs with a range of reactivities, and to be able to correlate reactivity with some substituent parameter. Our discovery of a simple photochemical method for the trifluoromethylation of imidazoles has made available a large series of analogs for study. We have now developed procedures for the conversion of aminohistidines into azido and nitrohistidine, of amino to chloro, bromo and iodo, of trifluoromethyl into methyl, cyano, carboxy, carbomethoxy, etc. Recently, we synthesized 2- and 4-(pentafluoroethyl)-histidines by photochemical radical substitution. These compounds are converted by base into the corresponing (trifluoroacetyl)histidines, which have such reactive carbonyl groups that they may serve as affinity labels for histidine-binding sites. The trifluoroacetylimidazoles can be reduced to the secondary alcohols, also obtainable by direct condensation of imidazoles with trifluoroacetaldehyde. In turn, the secondary alcohols can be oxidized to the trifluoroacetyl ketones. Upon treatment with methanolic base, (trifluoromethyl)histidine can be converted into (trimethoxymethyl)histidine and pentafluoroethyl into the corresponding ketal. There ortho functionalities are also of interest as potential covalent affinity labels.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19606-11 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Halogenated Biogenic Amines in Biochemistry and Pharmacology

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

P.I.: Kenneth L. Kirk, Research Chemist,NIDDK, LC

Other: Adeboye Adejare, Visiting Fellow, NIDDK,LC
David Furlano, IRTA Fellow, NIDDK, LC
Kenneth A. Jacobson, Senior Staff Fellow, NIDDK, LC
Silvia Calderon, Guest Worker, NIDDK, LC
George Chen, Guest Worker, NIDDK, LC

COOPERATING UNITS *(if any)* J. Daly, C.R. Creveling, F. Gusovsky, (LBC,NIDDK): M. Channing, D. Kiesewetter, R. Finn, S. Larson (CC, Dept. of Nuclear Medicine); D. Thakker, C. Boehlert (CDB, FDA): C.C. Chieuh (NIMH), K.A. Muszkat (Weizmann Institute, Israel).

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Biogenic amines play key roles in neurotransmision, metabolism, and in control of various physiological processes. Ring-Fluorinated analogs have proved to be powerful tools for the study of the mechanisms of transport, storage, release, metabolism, and modes of action of these amines since they simulate the geometries of the natural compounds so well. By virtue of its very small size and high electronegativity, fluorine is a very favorable replacement for hydrogen in these analogs. In 1970, we developed novel methods for the introduction of fluorine into organic molecules and have applied these methods to the syntheses of a wide variety of biogenic amines with fluorine at various ring-positions. The biological properties and usefulness of these ring-fluorinated biogenic amines have proved to be extremely rewarding and continue to find applications in a multitude of studies. Perhaps the most significant finding, to date, is that 6-fluoronorepinephrine is a selective α-adrenergic agonist and 2-fluoronorepinephrine is a selective β-adrenergic agonist. Various explanations for the role of fluorine in creating such selectivity have been considered and discarded. Proposals under current consideration include a critical dipole-dipole repulsion between the benzylic hydroxyl group and fluorine in the 2- and 6-positions. This interaction could lead to side-chain conformational preferences favorable for interaction with the β- and α-adrenergic receptors, respectively. Effects of fluorine on the electronic properties of the aromatic ring are considered also to be important in defining selectivities. Experiments to differentiate between conformational and electronic effects have been initiated. Biological properties of new analogs, including fluorinated epinephrines have mechanistic significance.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 19607-05 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Chemistry, Biochemistry and Pharmacology of Bioindole Analogs

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

P.I. Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC/NIDDK

Other: Rita Labroo Guest Worker GWU

COOPERATING UNITS *(if any)* Edith Miles, LBP,NIDDK
Robert Phillips, University of Georgia
Peter Kador, LMOD, NEI

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH,NIDDK, Bethesda, Maryland 20892

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.)*

The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine tautomer. An enzyme carboxyl group should also promote indolenine formation, suggesting the indolenine to be the true substrate for certain tryptophan enzymes.

The first conclusive support for this concept is found in the demonstration that 2,3-dihydro-L-tryptophan and oxindolyl-L-alanine, analogs of the indolenine tautomer of tryptophan (tetrahedral carbon at C-3), are potent competitive inhibitors of tryptophan synthase and tryptophanase. Furthermore, the two enzymes show opposing specificity for the C-3 diastereoisomers of 2,3-dihydroxy-L-tryptophan, suggesting that these enzymes catalyze their reactions via enantiomeric indolenine intermediates.

Inhibition of the enzyme aldose reductase represents a new pharmacological approach toward the treatment of late-onset diabetic complications. These complications affect the eye, kidney, nervous system and circulation; they are thought to result from the hyperosmotic effects of high concentrations of sorbitol, in turn resulting from the reduction of the excess glucose symptomatic of diabetes. Our methods for the synthesis of inhibitors of tryptophan-metabolizing enzymes involve spirolactone intermediates which are fairly similar in overall structure to compounds now in clinical trials as aldose reductase inhibitors.

The first series of compounds evaluated as inhibitors show the spirolactones to be active only at concentrations 100 times that of commerical inhibitors; on the other hand, the hydroxyacids resulting from ring opening were ca. ten times more active than the lactones, providing a totally new direction for the design of inhibitors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 19608-04 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Functionalized Congeners of Bioactive Compounds

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

PI:	K. Jacobson	Staff Fellow	NIDDK-LC
Other:	K. Kirk	Research Chemist	NIDDK-LC
	J. Zimmet	Student Volunteer	NIDDK-LC
	S. Barone	Special Volunteer	NIDDK-LC
	J. Daly	Chief	NIDDK-LBC
	G. Evoniuk	PRAT Fellow	NIDDK-LNS

COOPERATING UNITS *(if any)*

T. Seale (U. Oklahoma), B. Fredholm (Karolinska Inst.), J. Carney (U. Oklahoma), H. Fales (NHLBI), P. Churchill (Wayne State Univ.), R. Olsson (Univ. So. Fla.), G. Stiles (Duke Univ.), P. Marangos (NIMH), M. Williams (CIBA-GEIGY), D. Kiesewetter (NM-CC)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.7	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.)*

Recent work in our laboratory and in others has demonstrated that certain drugs may be attached to well-defined "carrier" molecules and still retain the ability to bind to the receptor site and effect biological activity. This synthetic strategy for the attachment of drugs to carriers is termed the "functionalized congener" approach. The "carrier" molecule may be many times larger than the parent drug; indeed there is practically no maximum size limitation for a fully potent analog. Unlike the prodrug approach or the immobilization of drugs for slow release, the "functionalized congener" approach is designed to produce analogs for which no metabolic cleavage step is necessary for activation. Moreover, the attachment of the drug to a "carrier" such as a peptide may result in the enhanced affinity at an extracellular receptor site and an improvement in the pharmacological profile of the parent drug.

The extracellular adenosine receptor has a modulatory role in the nervous, circulatory, endocrine, and immunological systems. The prospect of harnessing these effects specifically for therapeutic purposes is attractive, but efforts have not met with much success in the past.

The functionalized congener approach has been applied to the adenosine receptor to produce analogs of agonists and antagonists which have promise as therapeutic agents and as receptor probes. In the antagonist series new analogs which combine potency, water solubility, and A_1-adenosine receptor selectivity in the same compound are now being evaluated in in vivo testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 19609-03 LC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Determination of Amines and Amine Metabolites in Biological Samples

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

P.I. Kenneth L. Kirk, Research Chemist, NIDDK, LC

Other: Kenneth A. Jacobson, Senior Staff Fellow, NIDDK, LC

COOPERATING UNITS *(if any)*

M. Linnoila, NIAAA, NIH
T. Marshall, NIAAA, NIH
G. Gusovsky, NIDDK, NIH
A. Gjerris (Copenhagen)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

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

The importance of biogenic amines in neurotransmission, metabolism, and in control of various physiological processes has spurred intense interest in the develpment of precise and sensitive methods for the quantitation of these amines and their metabolites present in cebrospinal fluid, plasmas and urine.

We have developed precise, operationally simple procedures for the analyses of certain of these substances based on HPLC separation using highly sensitive electrochemical detection, coupled with the use of internal standards for accurate quantitation. The utility of the approach has been extended greatly by selective and efficient acylation of the endogenous amines which permits ready isolation from the biological sample. Use of an electroactive acylating reagent further extends this approach to the accurate detection of amines which, themselves, are not amenable to electrochemical detection (e.g., histamine and phenethylamine).

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

ANNUAL REPORT OF THE LABORATORY OF CELL BIOLOGY AND GENETICS
NATIONAL INSTITUTE OF DIABETES, DIGESTIVE AND KIDNEY DISEASES

The Laboratory of Cell Biology and Genetics carries on a broad program of investigation into hormone and transmitter secretion and the molecular events regulating these processes. Four specific tissues are used: chromaffin cells, which secrete adrenaline, ATP and endogenous opiates; pancreatic beta cells, which secrete insulin; the frog neuromuscular junction, in which acetylcholine is the principal secreted substance, and bovine submucosal glands from the trachea, which secrete mucins.

Membrane fusion is a key event in numerous biological processes, including neurotransmission and exocytosis, and may depend on calcium and other ions and factors. Much of our work this year has been devoted to biological signals which regulate this process in chromaffin and B cells, as well as to studies on the calcium binding protein synexin. At present we consider synexin, to be at least one likely mediator of membrane contact and fusion during exocytosis.

This year we have been very fortunate to finally begin understanding how synexin goes about fusing membranes to one another. The first hints came from studies on the primary structure of synexin, derived from cloning the synexin gene. These studies showed that the structure of synexin was very similar to structures previously shown for many typical intrinsic membrane proteins. By this we mean that the protein was constructed from long stretches of hydrophobic amino acids, punctuated by short regions of charged or neutral amino acids. Since synexin has both water-soluble and water-insoluble forms, depending on the absence or presence of calcium, this meant to us that synexin must exist in at least two conformations. In the absence of calcium the hydrophobic sequences must be hidden while in the presence of calcium the same hydrophobic sequences might be more exposed, and the synexin molecule might be able to enter membranes and behave just like an intrinsic membrane protein.

This expectation was verified by circular dichroism studies, by electrophysiological studies involving capacity measurements, and by the discovery that synexin could enter membranes and exhibit calcium channel activity. These data meant that synexin not only could enter the membrane, but could also span the membrane. The concept of spanning the membrane was a necessary consequence of the fact that in order to be a channel synexin must connect both sides of a target membrane with an aqueous space of "channel." From these facts we have developed a hypothesis to explain the mechanism of synexin-driven membrane fusion, and have called it the "hydrophobic bridge hypothesis." This hypothesis explains membrane fusion by presuming that calcium-activated, hydrophobic synexin polymers enter both fusing membrane partners simultaneously and provide a bridge over which phospholipids from either juxtaposed membrane leaflet can cross and mix. The hypothesis is also based on our recent observation that membrane mixing precedes volume mixing in a synexin-driven membrane fusion system. We anticipate that this hypothesis may explain membrane fusion in other types of systems besides that of exocytosis from chromaffin cells.

In chromaffin cells and islets of Langerhans, an increase in cytosolic calcium concentration seem to be a prerequisite for hormone secretion. We have found that calcium enters chromaffin cells through both voltage-sensitive calcium channels as well as through nicotinic receptor gated channels. Then using a sensitive luciferin/luciferase based detector we have been able to measure the concurrent kinetics of granule-localized ATP release from the cell. These data show that while barium and calcium enter the cell through membrane channels, the consequent secretion is additive once the two ions enter the cell. This indicates that separate mechanisms for calcium and barium evoked secretion must operate coincidentally within the cell, and renders explicable the common observation that many calcium-sensitive proteins within the cell, such as synexin, calmodulin or protein kinase C, that are possibly involved in secretion, are nonetheless also insensitive to barium. Apparently, an independent, barium activated secretory mechanism may also be found in these cells.

Of likely clinical relevance are our recent experiments showing that bovine chromaffin cells can be transplanted into regions of the central nervous system in rats and monkeys involved in sensitivity to pain. Upon stimulating the implanted cells by nicotine administration to the intact animal, profound suppression of pain sensation was observed. This suppression could be reversed by blockers of morphine, such as nalaxone, and of alpha receptor action such as phentolamine. These data thus indicate that transplanted chromaffin cells can secrete opioid peptides and catecholamines in response to physiologic stimuli, and portend possible applications to treatment of humans for intractable pain.

New discoveries have also recently been made regarding the physical and chemical biology of the secretion event using rapid freezing electron microscopy and in situ optical microscopy. By freezing cells within a millisecond time range, masses of potentially diffusable elements such as calcium, magnesium, chloride, sodium and potassium can be measured with confidence in specific regions of the cell. However, the concentrations of these elements can be measured only if the water content can also be measured. We found a way of measuring the water content of discrete regions with accuracy, and can now precisely calculate these concentrations. One application is to use the relative chloride concentration across membranes to estimate the electrical potential, an application previously reserved to electrophysiology, and in that case for only large membrane-enclosed compartments. The technique has been used to test predictions of the chemiosmotic hypothesis for exocytosis, proposed by ourselves some years ago to explain aspects of the secretory process in endocrine cells.

Finally, recent studies on endothelial cell biochemistry have revealed at least one function of the ATP co-stored and co-secreted with many hormones. Endocrine cells such as chromaffin and beta cells of the pancreas secrete their hormones directly into the circulation through closely juxtaposed endothelial cells. Because of the physiologic importance of endothelial cells we have isolated and studied those from the easily obtained adrenal medulla. We found that substances secreted from the chromaffin cells, such as catecholamines and ATP, had profound

effects on endothelial cell function. For example, ATP combines with P_2 purinergic receptors on the endothelial cell, to activate phospholipase C, which in turn release IP3 and causes an increase in cytosolic free calcium concentration. This calcium activates phospholipase A2, which in turn increases the levels of arachidonic acid. The latter substance is converted to prostacyclin, a vasodilating substance, which is then released from the cell. The purposes to which ATP may be used therefore seem to include vasodilitation of the vascular pathway through which hormones, some of them vasoconstrictive in their own right, must pass on their way to the periphery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 21008-21 LCBC

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Cytogenetics

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

P.I.: J.H. Tjio Chief, Section on Cytogenetics LCBG:NIDDK

COOPERATING UNITS *(if any)* UCB (Dr. G. Brecher), Albany Med. College, Albany, NY (Dr. E. Scott Raveche), Ernst Moritz Arendt Universitat, Institut fur Medizinische Genetik, Greifswald DRR, Germany (Dr. W. Knoll), Karl Marx Universitat, Institut fur Klinische Immunologie, Leipzig, DRR, Germany (Prof. Dr. H. Storch), Humbold Univ. Zoologiches Museum, East Berlin, (Prof. Dr. S. Koref-Santibanez and Dr. H.J. Paepke

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Cytogenetics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Fish Sex Chromosomes

In general, no sex chromosomes can be identified in fish spp. A study has been started to identify sex chromosomes in Sphaerichtys osphromenoides which has only 16 chromosomes, medium size and biarmed. Thus far only females have been studied. As they are import fishes from Malaya, it is difficult to get specimens.

A study in sex reversal in Xiphophorus sp and Beta splendeus which has been reported to show sex reversal (female male) showed that this is not true. In five generations we have only found late maturing males that look like females until late age when they showed their male morphology. Sex hormone treatments did not induce sex reversal. Only sterile fishes were produced. In Tilapia sp, interspecific crosses produce up to 100% male progeny, as published by others. But they never follow up the progeny from the embryonic stage. All were raised and studied in pond cultures. An experiment is planned to study fertilized eggs with sperm in vivo in containers and follow up the embryos to find out why only females survive. Another plan is to study intergeneric crosses of sunfish, which have been claimed to produce mainly female progeny. Collaborative studies are being carried out with Prof. Dr. S. Koref-Santibanex and Dr. H.J. Paepke on the chromosomes of five macropodus spp and Osphronemus gorami.

Human Cytogenetics

A collaborative study has been started with cytogeneticisits of the Ernst Moritz Arendt University in Greifswald, East Germany on chromosomes of chorionic villi and aminocentesis cells as well as a clinical immunogenetic study on plasmacytes of human bone marrow cells with Prof. H. Storch, Karl Marx University Department of Clinical Immunology, Leipzig, East Germany.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 21009-20 LCBG

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI: B. J. White Director, Cytogenetics Unit LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

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

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

This project was transferred to the Laboratory of Chemical Biology. The new project number is Z01 DK 25064-01 LCB.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 21019-05 LCBG

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Mechanism of hormone and transmitter secretion

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

P.I.: Harvey B. Pollard, Chief, Laboratory of Cell Biology and Genetics, NIDDK

Others: R. Ornberg, Ph.D., Elec. Microscopist; G. Lee, Ph.D., Res. Chemist; E. Rojas, Ph.D., VS; I. Atwater, Ph.d., Expert; R. Santos, VA; M. Levine, M.D. RA; K. Brocklehurst, Ph.D., VF; P. Lelkes, Ph.D., VS; L. Rosario, Ph.D.,VA; E. Forsberg Ph.D.., SF; A. Burns, Ph.D., Expert; I. Cabantchik, Ph.D., SV; Stutzin, Ph.D., VF; J. Bitran, MD, VF: M. Srivastava, Ph.D., VF; C. McCutchen, Ph.D., Res. Phys; G. Kuijpers, Ph.D., VF; K. Magendzo-Weinberger, Ph.D., SV; P. Mathias, M.D., VF; A. Munoz, M.D., SP; V. Cena, Ph.D., M.D., VA; M. Li, M.D., VF, Y.Shi, Ph.D., SV; G. Goping, EM Tech.; B. Cheung, SV; Carroll, P., NRSA, SV; Bdolah, A., Ph.D., SV; Vanek, P., SV; Buckley, N., SV; D. Tombaccini, MD., SV; W. Hartzell,SV; P. Washko,SV

COOPERATING UNITS *(if any)*

Dipak Banerjee, Ph.D., University of Puerto Rico

LAB/BRANCH

Laboratory of Cell Biology and Genetics

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
24.5	24	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our recent work has focussed on the processes leading to fusion between granule and plasma membranes during exocytosis in cells such as chromaffin cells, beta cells in islets of Langerhans, frog neuromuscular junction, and tracheal submucosal gland cells. Synexin, a calcium-binding membrane fusion protein found in many tissues, was found to fuse membranes by a mechanism involving membrane mixing occurring prior to volume mixing. Synexin was also found to enter membranes and change the capacitance, and simultaneously to exhibit calcium channel activity. From these and other data we have formulated a hydrophobic bridge hypothesis for membrane fusion driven by synexin. The fast-freeze electron microscopy technique has been used to measure distribution of elements and their concentrations in discrete regions of cytosol. By this method we have measured transmembrane electrical potentials across organelles in cells frozen within a millisecond. Bovine chromaffin cells have been transplanted into regions of brain involved in pain. Stimulation of these implanted cells by nicotine administration causes a reduction in pain sensed by the recipient rats and monkeys. Cytosolic pH may be important in stimulus-secretion coupling in chromaffin and beta cells, resulting in release of adrenaline and insulin, respectively. Transient acidification is followed by promot return to ambient pH using a Na^{+}/H^{+}-exchange pump in the plasma membrane. Ascorbate, an important vitamin, is critically required for biosynthesis of noradrenaline in chromaffin granules within intact chromaffin cells.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

I. POLYAMINES

Polyamines (such as putrescine, spermidine, and spermine) are major cellular components, and have been shown to be involved in many systems related to growth and differentiation. Our studies have been directed at learning how these polyamines are synthesized and regulated, and their physiological function. To this end we have carried out a wide variety of genetic and biochemical studies. We have: (1) established the pathways for the biosynthesis of these amines; (2) isolated the enzymes involved in this biosynthetic pathway; (3) identified the genes responsible for each of these steps and constructed mutants lacking the coded enzymes; (4) constructed plasmids that contain these genes, and that permit overproduction of the various enzymes; (5) studied the physiological effects of amine deprivation *in vivo* on ribosome action and on protein biosynthesis; (6) in current studies we have shown that the gene coding for spermidine synthase (*speE*) and the gene coding for adenosylmethionine decarboxylase (*speD*) form an operon at 2.7 minutes on the *Escherichia coli* chromosome. We have sequenced and characterized this operon. We have shown that adenosylmethionine decarboxylase is formed as proenzyme which is then processed by a post-translational cleavage at a lysyl-serine peptide to form two subunits, one of which contains the pyruvoyl group that is found in the mature enzyme and is essential for enzymatic activity.

. . . . . Drs. H. Tabor, C. W. Tabor, and Q.-W. Xie

II. YEAST RNA VIROLOGY

There are five families of double-stranded RNA virus-like particles (L-A, L-BC, M, T, and W) and two distinct single-stranded RNA virus-like entities (20S RNA and 23S RNA) that replicate in cells of *Saccharomyces cerevisiae*. We have studied how these genomes replicate in yeast with emphasis on the role of the host. Highly purified virus-like particles (VLPs) carry out both (+) strand and (-) strand synthesis of L-A, L-BC, or M RNA *in vitro* in a conservative, sequential reaction. We can open L-A double-stranded RNA (dsRNA) VLPs by dialysis. They release their dsRNA, but now can use exogenous (+) strand of L-A or M as a template to make the corresponding dsRNAs *in vitro* by synthesis of (-) strands. Analysis by Western blots reveals a 180,000 dalton VLP protein that specifically binds L-A or M (+) single-stranded RNA. We have isolated, cloned, and sequenced a deletion mutant of the 4.5 kb L-A, called X, that is 530 bp long. X dsRNA is in VLPs with an L-A encoded coat and is transcribed and replicated in these VLPs. Thus the *cis* signals for these processes are in the X sequence. X has the same ends as the parent L-A molecule and lacks most of the center sequences. Unlike L-A, X is incompatible with M_1 and requires many chromosomal genes that M_1, but not L-A, needs for its replication. Like M_1, X represses the L-A copy number. We suggest that molecules encoding the coat protein (L-A parent) need fewer *MAK* genes to protect them from *SKI* products than do

molecules borrowing their coat protein from L-A (like M_1 and X). We find that [B], a cytoplasmic gene suppressing M's requirement for many MAK genes, is located on certain L-A natural variants.

Sequence data and gene fusion studies indicate that the MAK11 product is a membrane-associated protein. The MAK16 gene is involved in the yeast cell division cycle and is necessary for passage through the "start point" at which cells are arrested by the mating pheromones. Our sequence of the CDC16 gene, which is involved in chromosome segregation, shows that the protein has three apparent zinc-binding--nucleic acid-binding "fingers."

. . . . . Drs. R. B. Wickner, T. Fujimura, R. Esteban, T. Icho, and H. Uemura

III. NUCLEIC ACIDS

L Transposons

Introduction. All mammals contain several families of repetitive DNA sequences that comprise a substantial portion of the genome. Our studies on one of these families, the rat long interspersed repeated DNA family (LINE or L family) of the rat have provided, among other things, the most direct evidence to date that a mammalian highly repeated DNA family consists of mobile DNA elements; the presence or absence of L members causes allelic variation at a number of single copy loci. For this reason and because it appears as if L elements of mammals quite likely are the mammalian analogs of the I elements of Drosophila, a family of bonafide mobile elements, we think it appropriate to refer to the mammalian L family as L transposons. The L transposon of rats contains about 40,000 members and accounts for about 10% of the rat genome. Most members are full length (6.7 kb), 5 kb of which is devoted to protein encoding sequence. A promoter-like sequence for the transcription of the open reading frames (ORFs) is at the left end of the element, and G-rich homopurine stretches are at the other end. L elements terminate about 35 bp 3' of the G-rich (GHP) stretches in an A-rich region of variable length.

Current Findings. Although 5 kb or so of the rat and mouse L DNA is very highly conserved, their promoter-like sequences are so divergent that they could not have been derived from the same ancestral DNA sequence. This means that novel species-specific promoter sequences have been repeatedly acquired during the evolution of L families, which we suggest may account for the recurring amplification of these transposable elements that occurred concurrent with mammalian speciation.

. . . . . Drs. A. V. Furano, S. M. Robb, and F. T. Robb

We found that the rat promoter sequence is of the type that is affected by DNA methylation and have devised a method to completely demethylate essentially all of the L DNA genomic promoter sequences. Since demethylation of L transposons is probably necessary, but not necessarily

sufficient, to activate L transposons, we anticipate that an attempt to trap an active L transposon will require the use of cells containing demethylated L DNA. In other studies we have analyzed the activity of cloned L promoters fused to the test gene, chloramphenicol acetylase. We have so far found that this L promoter is active in various cell lines and exhibits significant synergy with the SV40 promoter.

. . . . . Drs. I. Nur and A. V. Furano

In contrast to the complete lack of homology among their promoter-like sequences, all mammalian L elements contain at their right end GHP stretches. We have recently found that these sequences have the remarkable property of inducing the unpairing of contiguous duplex DNA such that this DNA can now take up (hybridize) complementary DNA sequences. Furthermore, the hybridized DNA sequence can be elongated by added DNA polymerase. Both of these phenomena are essential intermediates in well-documented models for certain types of recombinational and transpositional events. This suggests that the L GHP stretches may be very important for these properties of L DNA.

. . . . . Drs. K. Usdin and A. V. Furano

We are studying the E. coli bacteriophage T4 as a model system for duplex DNA replication. Efficient DNA replication in vitro is achieved with seven purified proteins encoded by T4 phage: T4 DNA polymerase (gene 43 product), gene 32 DNA helix-destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, and the genes 41 and 61 priming proteins.

Primase-Helicase. The 61 and 41 proteins function as a complex with primase and DNA-unwinding (helicase) activities. The coupling of these two activities is important for coordinating DNA synthesis on the leading and lagging strands of the replication fork. The proteins act as a helicase to open the duplex ahead of the nascent leading strand, and stop periodically to make the pentanucleotide primers needed to initiate new chains on the lagging strand. Using a series of synthetic forked helicase substrates with different lengths of single strands ahead of the duplex, we have shown that the 41-61 helicase interacts with a region of greater than 50 nucleotides on the lagging strand template and 40 nucleotides on the leading strand template. The weak helicase activity of 41 protein alone requires ATP or GTP and is not affected by CTP or UTP. In contrast, the rate of unwinding by the 41 and 61 proteins together is greatest with all four rNTP needed for primer synthesis.

The 61 and 41 proteins together make mainly the pentamer primers which initiate new T4 DNA strands in vivo. 61 protein alone has a weak primase activity making predominantly dimers and traces of longer products. In the absence of 41 protein, the gene 32 single-stranded DNA (ssDNA) binding protein strongly stimulates the synthesis of very long RNA ($n > 300$) by high concentrations of 61 protein. Our studies suggest that 41 protein specifically stimulates the synthesis of pentamers

beginning with A, prevents the 61 and 32 proteins from elongating the pentamers, but facilitates their elongation into DNA by T4 DNA polymerase and its accessory proteins.

Using a gel-DNA retardation assay, we have shown that 41 protein binds tightly to ssDNA only in the presence of both 61 protein and an NTP. Binding is greatest with ATP or GTP, which serve as cofactors for the 41 protein helicase.

. . . . . Drs. R. W. Richardson and N. G. Nossal

RNase H. We have shown that RNase H activity increases 10-fold after T4 infection and have purified this activity from T4 infected cells. This RNase H efficiently removes the pentamer primers synthesized by the 41 and 61 proteins.

. . . . . H. C. Hollingsworth and N. G. Nossal

T4 RNA Polymerase Binding Protein. A new RNA polymerase binding protein (rbsA or 45.1) has recently been shown by Karam and Geiduschek and associates to be encoded just upstream of the T4 polymerase accessory proteins in a region of a putative origin of replication. We have purified this protein to test its possible effect on T4 DNA replication and transcription.

. . . . . Dr. N. G. Nossal

Bacteriophage T4 Gene Expression. Bacteriophage T4 provides a model for examining developmental regulation of gene expression. The phage uses the host RNA polymerase to transcribe its genome, but as infection proceeds, different classes of genes are expressed. In order to understand how the phage regulates its transcription, the expression of 4500 bp of T4 DNA which includes the genes uvsX (recombination protein), 40 (stimulates head formation), and 41 (primase-helicase component) is being studied. This DNA has been inserted into a multicopy vector, giving the plasmid pDH428.

S1 nuclease protection experiments indicate that phage infection alters the specificity of the host RNA polymerase in its transcription of the T4 uvsX, 40, and 41 genes. As early as 2 minutes after infection T4 transcripts from this region differ significantly from plasmid transcripts made by uninfected RNA polymerase in vivo. Two or 6 minutes after infection, RNA start sites 900 and 160 bp upstream of uvsX are obtained. In contrast, RNA made from pDH428 in an uninfected cell in vivo begins 800, 700, and 450 bp upstream of uvsX. Two of these starts (800 and 700 bp upstream) correspond to transcription from promoters previously identified after in vitro transcription using uninfected RNA polymerase. In addition, evidence has been obtained for a factor-dependent transcription termination or processing site between uvsX and 41 which is used in an uninfected cell but not early after infection. In an uninfected cell, approximately half of the plasmid transcripts stop 70 bases downstream of the uvsX gene. This stop is

factor-dependent since it is not observed after transcription by host RNA polymerase in vitro. In contrast, 6 minutes after phage infection, most uvsX transcripts extend past this stop, indicating that phage infection alters the 3' end of this transcript.

In an effort to identify all of the T4 genes present on the plasmid pDH428, plasmids containing portions of pDH428 have been transcribed and translated in vitro using an uninfected extract. These experiments confirm the previous genetic assignment of gene 40 between uvsX and 41 and identify a new 17,000 dalton protein, called X.1, which lies upstream of uvsX.

. . . . . Dr. D. M. Hinton

Hepatitis Non-A, Non-B. Hepatitis non-A, non-B (HNANB) is a world-wide problem, and 90% of the transfusion-related hepatitis cases in the United States (and 80-90% in several other countries) are diagnosed as HNANB. Approximately 50% of all acute HNANB patients develop chronic HNANB (an estimate of 4 million persons). They remain as potential sources of infection. Recent publications suggest a correlation between certain hepatocellular carinomas and chronic HNANB infections.

Based on biochemical, immunlogical, and morphological evidence, we suggested that the HNANB agent is a mammalian type C retrovirus. Recently, using an in vitro focus-induction assay developed for mammalian type C viruses, we observed that pelleted material from HNANB sera (transfusion-related) induced foci formation. This result is consistent with the presence of a mammalian type C virus in HNANB sera.

A DNA probe of 780 base pairs isolated from HNANB-infected chimpanzee liver and selected by subtractive hybridization with normal chimpanzee liver was shown to hybridize with liver sections from three HNANB-infected chimpanzees but not with liver from two HBV-infected animals. This DNA fragment has been cloned, completely sequenced, and placed under the control of the Sp6 promoter.

. . . . . Drs. W. G. Coleman, Jr., L. Chen, and B. P. Seto (HL, DBBP, Center for Drugs and Biologics)

IV. MEMBRANE STUDIES OF MACROPHAGES AND OF ESCHERICHIA COLI

Aldoheptose Biosynthesis. Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. The rfaD gene codes for ADP-L-glycero-D-mannoheptose-6-epimerase, an enzyme required for lipopolysaccharide (LPS) core biosynthesis. The nucleotide ADP-D-glycero-D-mannoheptose accumulates in rfaD mutant strains. The rfaD phenotype includes increased permeability to a large number of hydrophobic drugs and dyes, and the formation of mucoid colonies. A 9-kilobase DNA EcoRI fragment carrying the rfaD gene was initially identified in the Clarke-Carbon Colony Bank cloned in pBR322,

and subsequently smaller restriction fragments were cloned into several expression plasmid vectors. The proteins expressed by RfaD$^+$ plasmids, using several in vivo and in vitro expression systems, have been examined by SDS gel electrophoresis. RfaD$^+$ plasmids express a protein with a molecular weight of 37,000. One of these plasmids, pJP5, which contains a 1.8-kilobase EcoRI-NruI fragment, expresses the rfaD gene product and complements all of the phenotypes associated with the rfaD mutation. Finally ADP-L-glycero-D-mannoheptose-6-epimerase has been purified to homogeneity by 60% ammonium sulfate precipitation, followed by ion exchange and gel filtration. The gel filtration profile also indicates that the rfaD gene product has a molecular weight of 37,000.

. . . . . Drs. J. C. Pegues and W. G. Coleman, Jr.

V. ENZYME MECHANISMS AND PROTEIN STRUCTURE

Three-Dimensional Structure of the Tryptophan Synthase $\alpha_2\beta_2$ Complex from Salmonella at 2.8 Å Resolution. The three-dimensional structure of the tryptophan synthase $\alpha_2\beta_2$ complex has been determined by x-ray crystallography. The atomic model shows the arrangement of the subunits in the $\alpha_2\beta_2$ tetramer, the folding pattern of each of the subunits, and the relative positions of the active sites of the α and β subunits. Since these active sites are 25 Å apart, the indole which is produced at the active site of the α subunit must be channeled through a cleft between the two domains of the β subunit to the pyridoxal phosphate cofactor at the active site of the β subunit. X-ray data recently collected in the presence of ligands is allowing us to identify active site residues in each of the subunits.

. . . . . Drs. C. C. Hyde, E. A. Padlan, S. A. Ahmed, E. W. Miles, and D. R. Davies

Microcrystals of the Tryptophan Synthase $\alpha_2\beta_2$ Complex from Salmonella Are Catalytically Active. Microcrystals of tryptophan synthase $\alpha_2\beta_2$ complex have been prepared in order to determine the enzymatic activity of the crystalline form of the enzyme. Scanning electron microscopy demonstrates that these crystals are fairly uniform in size and have the same crystal habit as the larger crystals being used for x-ray crystallography. Suspensions of these microcrystals are almost fully active in several reactions catalyzed by the active sites of the α and β subunits. Thus the larger crystals being used for x-ray crystallography are a functional form of the enzyme and should form complexes with substrates and analogs which will allow us to identify active site residues in each of the subunits.

. . . . . Drs. S. A. Ahmed, C. C. Hyde, G. Thomas, and E. W. Miles

Single Crystal Polarized Absorption Microspectrophotometry of Tryptophan Synthase from Salmonella typhimurium. The pyridoxal phosphate cofactor of the β subunit of tryptophan synthase forms complexes with substrates and analogs which have distinctive spectral properties. The spectra are sensitive to conformational changes which may result from intersubunit

interactions and reactions with ligands. Polarized absorption spectra of single crystals in the presence and absence of ligands have been measured by microspectrophotometry in order to compare the functional and dynamic properties of tryptophan synthase in solution and in the crystalline state. Some observed differences between the spectral properties of the two states of the enzyme may be the consequence of a different distribution of intermediates in the two states.

. . . . . Drs. A. Mozzarelli, G. L. Rossi, C. C. Hyde, S. A. Ahmed, and E. W. Miles

Stereochemistry and Mechanism of a New Single-Turnover, Half-Transamination Reaction Catalyzed by the Tryptophan Synthase $\alpha_2\beta_2$ Complex. Tryptophan synthase is a versatile enzyme that catalyzes a wide variety of pyridoxal phosphate-dependent reactions that are also catalyzed in model systems. The discovery that this enzyme will also catalyze a new stereospecific single-turnover, half-transamination reaction between pyridoxamine phosphate and indole-3-pyruvic acid extends the understanding of the stereochemistry and mechanism of this enzyme. The results clarify the relationship between enzyme-catalyzed and model reactions.

. . . . . Dr. E. W. Miles

Site-Specific Mutagenesis of the α Subunit of Tryptophan Synthase from Salmonella typhimurium. Site-specific mutagenesis of the α subunit of tryptophan synthase has been initiated in order to investigate the effects of structure on the functional properties of tryptophan synthase. A mutagenesis system has been developed which is useful for preparing mutants at any desired position in the α or β subunit. The tryptophan synthase $\alpha_2\beta_2$ complex in which arginine-179 of the α subunit has been changed to leucine was engineered by site-specific mutagenesis, expressed, purified, and crystallized. The mutant enzyme was partially active but had changed properties in response to ligands, suggesting that the mutation altered the reciprocal transmission of substrate-induced conformational changes between the α and β subunits in the $\alpha_2\beta_2$ complex.

. . . . . Drs. E. W. Miles, H. Kawasaki, S. A. Ahmed, and R. Bauerle

The Tryptophan Synthase α Subunit Glutamic Acid-49 Is Essential for Activity: Studies with 19 Mutants at Position 49. Glutamic acid-49 of the α subunit of tryptophan synthase of *E. coli* has been changed to 19 different amino acids by site-specific mutagenesis. All of the mutant α subunits and the native α subunit associate with the β_2 subunit to form an $\alpha_2\beta_2$ complex which catalyzes the β reaction. However, the 19 mutant $\alpha_2\beta_2$ complexes are completely devoid of activity in reactions normally catalyzed by the active site of the α subunit. The mutant α subunits bind ligands normally and transmit ligand-dependent changes to the β subunit. The results are strong evidence that glutamic acid-49 is an essential base in reactions catalyzed by the α subunit.

. . . . . Drs. K. Yutani and E. W. Miles

An automated method for the rapid measurement of diffusion coefficients has been developed. Using this method, the diffusion coefficients of each of several dilute tracer proteins have been measured as a function of the concentrations of each of several unlabeled background proteins at concentrations of up to 200 g/l.

. . . . . Drs. N. Muramatsu and A. P. Minton

A microcomputer program for simulation of sedimentation in a centrifuge has been generalized to treat substantial deviations from thermodynamic ideality, multiple solutes, and self-associating solutes with arbitrarily specified association and dissociation rate constants.

. . . . . Drs. R. C. Chatelier and A. P. Minton

Sedimentation equilibrium experiments conducted on three different proteins over a broad range of concentrations have revealed that two of them exhibit previously undetected self-association at high total protein concentration.

. . . . . Drs. N. Muramatsu and A. P. Minton

The competition of labeled and unlabeled ligands for common acceptors has been analyzed for the case of multiple classes of acceptors, cooperative binding, and multivalent ligands.

. . . . . Dr. R. C. Chatelier

Extensive data on the indirect interaction between the neurotransmitter acetylcholine, which binds to muscarinic receptors, and batrachotoxin, which binds to sodium channels, have been analyzed quantitatively using a model postulating that each of the ligands modulates the affinity of its respective acceptor species for limited amounts of two different effector species, tentatively identified as different forms of the G-protein.

. . . . . Dr. A. P. Minton

A route to definitive characterization of an oxido-reductive mechanism for regulating protein biosynthesis was opened by the finding that the reductive activation of a complex-bound valyl-tRNA synthetase can occur in 50-55% ethylene glycol. In this reagent the activation, which probably occurs normally in microseconds, is slowed so that its completion requires more than an hour, and it can thus be studied in the manner of an enzymatic reaction. In the ethylene glycol test the reaction is found to require a heat-stable aresenite-binding protein. The protein has been purified from phosphate-containing heat extracts, after addition of arsenite and precipitation from 50% alcohol, by DEAE-cellulose chromatography, and was obtained as a crystalline product from a frozen suspension at -20°C in the presence of 4 mM mercuric chloride. In addition to the protein, three dialyzable, coenzyme-like substances promote the enzyme's activation. These can be separated on

columns of Sephadex G10. A prominent component of the coenzyme mixture is an unusual flavin which the most recent experiments suggest binds to the heat-stable protein to form a labile aresenite-sensitive disulfide-reducing protein-coenzyme pair.

. . . . . Dr. S. Black

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems.

Amino acid sequences of proteins are analyzed primarily with the Monte Carlo techniques to evaluate the uniqueness and homology of these sequences. The property of uniqueness (the occurrence of a small peptide at a frequency considerably less than that expected) has been quantified, and speculations on this quantity and the immune response have been presented.

. . . . . Drs. H. A. Saroff and E. Mihalyi

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 23,140-29 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Biochemistry of Sulfur-Containing Compounds

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

PI: Simon Black, Ph.D. Biochemist and Assistant Chief, LBP LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	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.)*

A route to definitive characterization of an oxido-reductive mechanism for regulating protein biosynthesis was opened by the finding that the reductive activation of a complex-bound valyl-tRNA synthetase can occur in 50-55% ethylene glycol. In this reagent the activation, which probably occurs normally in microseconds, is slowed so that its completion requires more than an hour, and it can thus be studied in the manner of an enzymatic reaction. In the ethylene glycol test the reaction is found to require a heat-stable aresenite-binding protein. The protein has been purified from phosphate-containing heat extracts, after addition of arsenite and precipitation from 50% alcohol, by DEAE-cellulose chromatography, and was obtained as a crystalline product from a frozen suspension at -20°C in the presence of 4 mM mercuric chloride. In addition to the protein, three dialyzable, coenzyme-like substances promote the enzyme's activation. These can be separated on columns of Sephadex G10. A prominent component of the coenzyme mixture is an unusual flavin which the most recent experiments suggest binds to the heat-stable protein to form a labile arsenite-sensitive disulfide-reducing protein-coenzyme pair.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,230-37 LBP

PERIOD COVERED

October 1, 1986 through October 31, 1986

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

Chemotherapy of Mouse Leprosy

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

PI: Yao Teh Chang, M.D. Research Pharmacologist LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated due to the retirement of Dr. Yao Teh Chang on October 31, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 23,330-09 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Aldoheptose Biosynthesis and Its Regulation

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

PI:	William G. Coleman, Jr., Ph.D.	Research Microbiologist	LBP NIDDK
Others:	Joyce C. Pegues, Ph.D.	Staff Fellow	LBP NIDDK
	Lishi Chen, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)*

Belinda P. Seto, Ph.D., HL, DBBP, Center for Drugs and Biologics

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.5	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.)*

Aldoheptose Biosynthesis. Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. The rfaD gene codes for ADP-L-glycero-D-mannoheptose-6-epimerase, an enzyme required for lipopolysaccharide (LPS) core biosynthesis. The nucleotide ADP-D-glycero-D-mannoheptose accumulates in rfaD mutant strains. The rfaD phenotype includes increased permeability to a large number of hydrophobic antibiotics, and the formation of mucoid colonies. A 9-kilobase DNA EcoRI fragment carrying the rfaD gene was initially identified in the Clarke-Carbon Colony Bank cloned in pBR322, and subsequently smaller restriction fragments were cloned into several expression plasmid vectors. RfaD+ plasmids express a protein with a molecular weight of 37,000, and all complement all phenotypes associated with the rfaD mutation. Finally ADP-L-glycero-D-mannoheptose-6-epimerase has been purified to homogeneity.

Hepatitis Non-A, Non-B. Hepatitis non-A, non-B (HNANB) is a world-wide problem, and 90% of the transfusion-related hepatitis cases in the United States (and 80-90% in several other countries) are diagnosed (by exclusion) as HNANB. Approximately 50% of all acute HNANB patients develop chronic HNANB (an estimate of 4 million persons).

Biochemical, immunological, and morphological evidence suggested that the HNANB agent is a mammalian type C retrovirus. Recently, using an in vitro focus-induction assay developed for mammalian type C viruses, we observed that pelleted material from HNANB sera (transfusion-related) induced foci formation.

A DNA probe of 780 base pairs isolated from HNANB-infected chimpanzee liver and selected by subtractive hybridization with normal chimpanzee liver was shown to hybridize with liver sections from three HNANB-infected chimpanzees but not with liver from two HBV-infected animals. This DNA fragment has been cloned, completely sequenced, and placed under the control of the Sp6 promoter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 23,580-24 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Mammalian Transposons (was Gene Expression in the Rat and Other Organisms)

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

PI:	Anthony V. Furano, M.D.	Medical Officer (Research) and Chief, Section on Genomic Structure and Function, LBP	LBP NIDDK
Others:	Israel Nur, Ph.D.	Visiting Fellow	LBP NIDDK
	Esterina Pascale, Ph.D.	Visiting Fellow	LBP NIDDK
	Karen Usdin, Ph.D.	Visiting Fellow	LBP NIDDK
	Anne Salemme, B.A.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	4.0	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The L transposon family (long interspersed repeat DNA or LINE family) of rats contains about 40,000 members and accounts for about 10% of the rat genome. Most members are full length (6.7 kb), 5 kb of which is devoted to protein encoding sequence. A promoter-like sequence for the transcription of the open reading frames (ORFs) is at the left end of the element, and G-rich homopurine (GHP) stretches are at the other end. Although 5 kb or so of the rat and mouse L DNA is very highly conserved, their promoter-like sequences are so divergent that they could not have been derived from the same ancestral DNA sequence. This means that novel species-specific promoter sequences have been repeatedly acquired during the evolution of L families. The rat promoter sequence is of the type that is repressed by DNA methylation and we have devised a method to completely demethylate essentially all of the L DNA genomic promoter sequences. Since demethylation of L transposons is probably necessary, but not necessarily sufficient, to activate L transposons, we anticipate that attempts to trap an active L transposon will require the use of cells containing demethylated L DNA. In other studies we have analyzed the activity of cloned L promoters fused to the test gene, chloramphenicol acetylase. We have so far found that this L promoter is active in various cell lines and exhibits significant synergy with the SV40 promoter. In contrast to the complete lack of homology among their promoter-like sequences, all mammalian L elements contain at their right end GHP stretches. We have recently found that these sequences have the remarkable property of inducing the unpairing of contiguous duplex DNA such that this DNA can take up (hybridize) complementary DNA sequences. Furthermore, the hybridized DNA sequence can be elongated by added DNA polymerase. Both of these phenomena are essential intermediates in well-documented models for certain types of recombinational and transpositional events. This suggests that the L element GHP stretches may be very important for these properties of L DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 23,600-17 LBP

PERIOD COVERED
October 1, 1986 through March 14, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Dynamic Properties of Cell Membranes and Related Systems

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

PI: Norman L. Gershfeld, Ph.D. Research Chemist LBP NIDDK

COOPERATING UNITS *(if any)*

Dr. Ralph J. Nossal, PSL, DCRT, and Dr. Robert L. Berger, LTD, NHLBI

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Physical Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

This project was transferred to the Section on Macromolecular Biophysics, Laboratory of Physical Biology, NIAMS. The new project number for the period March 15, 1987 to September 30, 1987 is Z01 AR 27,004-18 LPB. Please see Project Number AR 27,004-18 LPB for Summary of Work and Project Description.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,750-01 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Bacteriophage T4 Gene Expression

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

PI: Deborah M. Hinton, Ph.D. Research Chemist LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Bacteriophage T4 provides a model for examining developmental regulation of gene expression. The phage uses the host RNA polymerase to transcribe its genome, but as infection proceeds, different classes of genes are expressed. I am studying the expression of 4500 bp of T4 DNA which includes the genes uvsX (recombination protein), 40 (stimulates head formation), and 41 (primase-helicase component). This DNA has been inserted into a multicopy vector, giving the plasmid pDH428.

S1 nuclease protection experiments indicate that phage infection alters the specificity of the host RNA polymerase in its transcription of the T4 uvsX, 40, and 41 genes. As early as 2 minutes after infection T4 transcripts differ significantly from plasmid transcripts made by uninfected RNA polymerase in vivo. After infection, RNA start sites 900 and 160 bp upstream of uvsX are obtained. In contrast, RNA made from pDH428 in an uninfected cell in vivo begins 800, 700, and 450 bp upstream of uvsX. Two of these starts (800 and 700 bp upstream) correspond to transcription from promoters previously identified after in vitro transcription using uninfected RNA polymerase.

Furthermore, I have obtained evidence for a factor-dependent transcription termination or processing site between uvsX and 41 which is used in an uninfected cell but not early after infection. In an uninfected cell, approximately half of the plasmid transcripts stop 70 bases downstream of the uvsX gene. This stop is factor-dependent since it is not observed after transcription by host RNA polymerase in vitro. In contrast, early after phage infection, most uvsX transcripts extend past this stop, indicating that phage infection alters the 3' end of this transcript.

In an effort to identify all of the T4 genes present on the plasmid pDH428, I have transcribed and translated plasmids containing portions of pDH428 in vitro using an uninfected transcription/translation extract. These experiments confirm the previous genetic assignment of gene 40 between uvsX and 41 and identify a new 17,000 dalton protein, called X.1, which lies upstream of uvsX.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 23,860-27 LBP

PERIOD COVERED

October 1, 1986 through March 14, 1987

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

Biophysical Studies of Metabolic Activity and Control

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

PI: Ellis S. Kempner, Ph.D. Physicist, and Chief, Section on Physical Biology LBP NIDDK

COOPERATING UNITS *(if any)*

Drs. M. J. McCreery (Letterman Army Institute of Research); S. Pestka (Roche Institute); R. Wood (University of Georgia); R. Salovey (University of Southern California)

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Physical Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project was transferred to the Section on Macromolecular Biophysics, Laboratory of Physical Biology, NIAMS. The new project number for the period March 15, 1987 to September 30, 1987 is Z01 AR 27,003-28 LPB. Please see Project Number Z01 AR 27,003-28 LPB for Summary of Work and Project Description.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 24,140-21 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Tryptophan Synthase: Structure and Function and Relationship to Tryptophanase

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

PI:	Edith Wilson Miles, Ph.D.	Research Chemist	LBP NIDDK
Others:	Syed A. Ahmed, Ph.D.	Visiting Fellow/Visiting Associate	LBP NIDDK
	Haruhiko Kawasaki, Ph.D.	Visiting Fellow	LBP NIDDK
	Katsuhide Yutani, Ph.D.	Visiting Scientist	LBP NIDDK
	Haroshi Morita, Ph.D.	Guest Researcher	LBP NIDDK
	Hatsue Morita, B.S.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

Drs. D. R. Davies, C. C. Hyde, and E. A. Padlan, LMB, NIDDK; G. Thomas, LOP, NEI; R. Bauerle, Department of Biology, Univ. of Virginia, Charlottesville; A. Mozzarelli and G. L. Rossi, Univ. of Parma, Italy; K. Yutani, Osaka Univ., Japan

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	3.9	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are investigating the structure and function of the bacterial tryptophan synthase α2β2 complex by use of x-ray crystallography, site-specific mutagenesis, and spectrophotometric studies. The tryptophan synthase multienzyme complex catalyzes the final reaction of the biosynthesis of L-tryptophan and has been the subject of many genetic and biochemical studies. Our recent determination of the three-dimensional structure of this multienzyme complex by x-ray crystallography allows us to locate the active sites of both the α and β subunits and to understand how the indole produced at the active site of the α subunit is channeled a distance of 25 A to the active site of the β subunit. The pyridoxal phosphate-dependent reaction of indole with L-serine at the active site of the β subunit can be studied spectrophotometrically. A comparison of the kinetics of reaction and of the spectral properties of the enzyme in solution and in the crystalline state shows that the crystalline form is catalytically active. Site-specific mutagenesis of tryptophan synthase has been initiated in order to investigate the effects of structure on the functional properties of tryptophan synthase. The tryptophan synthase α2β2 complex in which arginine-179 of the α subunit has been changed to leucine was engineered by site-specific mutagenesis, expressed, purified, crystallized, and characterized. The mutant enzyme was partially active but had changed properties in response to ligands, suggesting that the mutation altered the reciprocal transmission of substrate-induced conformational changes between the α and β subunits in the α2β2 complex. Studies of α subunits in which glutamic acid-49 was changed to 19 different amino acids indicate the glutamic acid-49 is an essential base in reactions catalyzed by the α subunit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 24,150-16 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Noncovalent Intermolecular Interactions in Biochemistry

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

PI:	Allen P. Minton, Ph.D.	Research Chemist	LBP NIDDK
Others:	Ronald C. Chatelier, Ph.D.	Visiting Fellow	LBP NIDDK
	Nobuhiro Muramatsu, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)*

J. H. Shelhamer, Critical Care Medicine Department, Clinical Center, NIH
M. Sokolovsky, Tel Aviv University, Tel Aviv, Israel

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.8	0.2

CHECK APPROPRIATE BOX(ES)

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

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

An automated method for the rapid measurement of diffusion coefficients has been developed. Using this method, the diffusion coefficients of each of several dilute tracer proteins have been measured as a function of the concentrations of each of several unlabeled background proteins at concentrations of up to 200 g/l.

A microcomputer program for simulation of sedimentation in a centrifuge has been generalized to treat substantial deviations from thermodynamic ideality, multiple solutes, and self-associating solutes with arbitrarily specified association and dissociation rate constants.

Sedimentation equilibrium experiments conducted on three different proteins over a broad range of concentrations have revealed that two of them exhibit previously undetected self-association at high total protein concentration.

The competition of labeled and unlabeled ligands for common acceptors has been analyzed for the case of multiple classes of acceptors, cooperative binding, and multivalent ligands.

Extensive data on the indirect interaction between the neurotransmitter acetylcholine, which binds to muscarinic receptors, and batrachotoxin, which binds to sodium channels, have been analyzed quantitatively using a model postulating that each of the ligands modulates the affinity of its respective acceptor species for limited amounts of two different effector species, tentatively identified as different forms of the G-protein.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 24,260-21 LBP

PERIOD COVERED: October 1, 1986 through September 30, 1987

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

Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System

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

PI:	Nancy G. Nossal, Ph.D.	Research Chemist and Chief, Section on Nucleic Acid Biochemistry, LBP	LBP NIDDK
Others:	Ross W. Richardson, Ph.D.	Staff Fellow	LBP NIDDK
	Helen C. Hollingsworth, B.A.	Guest Researcher	LBP NIDDK

COOPERATING UNITS (if any)

Dr. David Ollis, Department of Biochemistry, Northwestern University, Evanston, Illinois

LAB/BRANCH: Laboratory of Biochemical Pharmacology

SECTION: Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are studying the E. coli bacteriophage T4 as a model system for duplex DNA replication. Efficient DNA replication in vitro is achieved with seven purified proteins encoded by T4 phage: T4 DNA polymerase (gene 43 product), gene 32 DNA helix-destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, and the genes 41 and 61 priming proteins.

Primase-Helicase. The 61 and 41 proteins function as a complex with primase and DNA-unwinding (helicase) activities. The proteins act as a helicase to open the duplex ahead of the nascent leading strand, and stop periodically to make the pentanucleotide primers needed to initiate new chains on the lagging strand. We have shown that the 41-61 helicase interacts with a region of greater than 50 nucleotides on the lagging strand template and 40 nucleotides on the leading strand template. The rate of unwinding by the 41/61 helicase is greatest with all four rNTP needed for primer synthesis.

The 61 and 41 proteins together make mainly the pentamer primers which initiate new T4 DNA strands in vivo. 61 protein alone has a weak primase activity making predominantly dimers and traces of longer products. In the absence of 41 protein, the gene 32 ssDNA binding protein strongly stimulates the synthesis of very long RNA (n more than 300) by high concentrations of 61 protein. Our studies suggest that 41 protein specifically stimulates the synthesis of pentamers beginning with A, prevents the 61 and 32 proteins from elongating the pentamer, but facilitates their elongation into DNA by T4 DNA polymerase and its accessory proteins. Using a gel-DNA retardation assay, we have shown that 41 protein binds tightly to ssDNA only in the presence of both 61 protein and an NTP. Binding is greatest with ATP or GTP, which serve as cofactors for the 41 protein helicase.

RNase H. We have shown that RNase H activity increases 10-fold after T4 infection and have purified this activity from T4 infected cells. This RNase H efficiently removes the pentamer primers synthesized by the 41 and 61 proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 24,590-16 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Structure and Interactions of Biologically Important Macromolecules

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

PI:	Harry A. Saroff, Ph.D.	Research Chemist (Intermittent) and Scientist Emeritus	LBP NIDDK
Other:	Elemer Mihalyi, M.D., Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

A. Patchornik, Weizmann Institute of Science, Rehovot, Israel
Clinical Endocrinology Branch, NIDDK, NIH, and National Center for Drugs and Biologics

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

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

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

Cooperative binding systems are being studied taking into account site or subunit interactions, ligand interactions, aggregation and redistribution in proteins, and model systems. Methods are being developed to evaluate reasonable values for the parameters describing these systems.

Amino acid sequences of proteins are analyzed primarily with the Monte Carlo techniques to evaluate the uniqueness and homology of these sequences. The property of uniqueness (the occurrence of a small peptide at a frequency considerably less than that expected) has been quantified, and speculations on this quantity and the immune response have been presented.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 24,709-06 LBP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polyamine Biosynthesis and Function (was Biochemical and Genetic Studies on . . .

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

PI:	Celia White Tabor, M.D.	Medical Director, USPHS	LBP NIDDK
Others:	Herbert Tabor, M.D.	Supervisory Medical Officer (Research); Chief, Section on Pharmacology, LBP; and Chief, Laboratory of Biochemical Pharmacology	LBP NIDDK
	Qiao-Wen Xie, Ph.D.	Visiting Fellow	LBP NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.1	3.0	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.)*

Polyamines (such as putrescine, spermidine, and spermine) are major cellular components, and have been shown to be involved in many systems related to growth and differentiation. Our studies have been directed at learning how these polyamines are synthesized and regulated, and their physiological function. To this end we have carried out a wide variety of genetic and biochemical studies. We have: (1) established the pathways for the biosynthesis of these amines; (2) isolated the enzymes involved in this biosynthetic pathway; (3) identified the genes responsible for each of these steps and constructed mutants lacking the coded enzymes; (4) constructed plasmids that contain these genes, and that permit overproduction of the various enzymes; (5) studied the physiological effects of amine deprivation in vivo on ribosome action and on protein biosynthesis; (6) in current studies we have shown that the gene coding for spermidine synthase (speE) and the gene coding for adenosylmethionine decarboxylase (speD) form an operon at 2.7 minutes on the Escherichia coli chromosome. We have sequenced and characterized this operon. We have shown that adenosylmethionine decarboxylase is formed as proenzyme which is then processed by a post-translational cleavage at a lysyl-serine peptide to form two subunits, one of which contains the pyruvoyl group that is found in the mature enzyme and is essential for enzymatic activity.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 24,710-37 LBP

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Polyamine Biosynthesis and Function in Escherichia coli

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

PI: Herbert Tabor, M.D. Supervisory Medical Officer (Research); Chief, Section on Pharmacology, LBP; and Chief, Laboratory of Biochemical Pharmacology LBP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been discontinued as a separate entry, since it is now combined with Project No. Z01 DK 24,709-06 LBP.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 24,940-14 LBP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Yeast RNA Virology (was "The Killer Double-Stranded RNA Plasmids of S. cerevisiae")

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

PI:	Reed B. Wickner, M.D.	Medical Director, USPHS, and Chief, Section on Genetics of Simple Eukaryotes, LBP	LBP NIDDK
Others:	Tsutoma Fujimura, Ph.D.	Visiting Associate	LBP NIDDK
	Tateo Icho, Ph.D.	Visiting Associate	LBP NIDDK
	M. Rosa Canibano Esteban, Ph.D.	Visiting Fellow	LBP NIDDK
	Hiroshi Uemura, Ph.D.	Visiting Fellow	LBP NIDDK
	Yang-Ja Lee, Ph.D.	Guest Researcher	LBP NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.5	5.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

There are five families of double-stranded RNA virus-like particles (L-A, L-BC, M, T, and W) and two distinct single-stranded RNA virus-like entities (20S RNA and 23S RNA) that replicate in cells of Saccharomyces cerevisiae. We have studied how these genomes replicate in yeast with emphasis on the role of the host. Highly purified virus-like particles (VLPs) carry out both (+) strand and (-) strand synthesis of L-A, L-BC, or M RNA in vitro in a conservative, sequential reaction. We can open L-A double-stranded RNA (dsRNA) VLPs by dialysis. They release their dsRNA, but now can use exogenous (+) strand of L-A or M as a template to make the corresponding dsRNAs in vitro by synthesis of (-) strands. Analysis by Western blots reveals a 180,000 dalton VLP protein that specifically binds L-A or M (+) single-stranded RNA. We have isolated, cloned, and sequenced a deletion mutant of the 4.5 kb L-A, called X, that is 530 bp long. X dsRNA is in VLPs with an L-A encoded coat and is transcribed and replicated in these VLPs. Thus the cis signals for these processes are in the X sequence. X has the same ends as the parent L-A molecule and lacks most of the center sequences. Unlike L-A, X is incompatible with M1 and requires many chromosomal genes that M1, but not L-A, needs for its replication. Like M1, X represses the L-A copy number. We suggest that molecules encoding the coat protein (L-A parent) need fewer MAK genes to protect them from SKI products than do molecules borrowing their coat protein from L-A (like M1 and X). We find that [B], a cytoplasmic gene suppressing M's requirement for many MAK genes, is located on certain L-A natural variants.

Sequence data and gene fusion studies indicate that the MAK11 product is a membrane-associated protein. The MAK16 gene is involved in the yeast cell division cycle and is necessary for passage through the "start" point at which cells are arrested by the mating pheromones. Our sequence of the CDC16 gene, which is involved in chromosome segregation, shows that the protein has three apparent zinc-binding--nucleic acid-binding "fingers."

LABORATORY OF CHEMICAL BIOLOGY
NATIONAL INSTITUTE OF DIABETES, AND DIGESTIVE AND KIDNEY DISEASES
ANNUAL REPORT: October 1, 1986 to September 30, 1987

The Laboratory of Chemical Biology conducts research on structure, function and dynamics of proteins; on fundamental problems in molecular interactions; and on molecular biology and genetics, especially as related to genetic disease. The Laboratory has recently initiated several major new areas of research. There is currently a large program to identify the cis-sequences of globin genes that control transcription and to isolate the trans-acting factors in the nuclei of human erythroid cells that control the ontogeny of hemoglobin synthesis. A related research endeavor focuses on ascertaining the way in which genetic variables, especially fetal hemoglobin levels, affects the manifestations of sickle cell disease. This study involves Clinical Center trials of drugs, such as hydroxyurea, which clinically increase fetal hemoglobin levels. Also at the molecular genetic level, are studies to identify new genes coding components of the human T-cell receptor. One component of the work on protein folding is now concentrating on the production of monoclonal antibodies to yeast cytochrome c so as to be able to study the forces stabilizing antigen-antibody interactions. At the biophysical level are analyses of hydration forces and configurational entropy on the interactions of macromolecules, and of the flexibility and conformation of oligonucleotides, DNA-gyrase complexes and of myosin. A long term project has been initiated to develop a true animal model of sickle cell anemia, using transgenic techniques. Most recently, a program to study the tat gene of the HIV has been initiated as part of the NIH Intramural AIDS Research Program. We are attempting to produce large amounts of the tat protein for characterization and to study its molecular mechanism of action.

During the last year further reorganization of the Laboratory occured. There are now three sections: the Section on Protein Chemistry and Conformation, the Section on Molecular Forces and Assembly, and the Section on Molecular Biology and Genetics. The Section on Protein Chemistry and Conformation, under Dr. Hiroshi Taniuchi, is devoted primarily to the study of protein folding and dynamics, in particular to the origin of forces stabilizing the three dimensional structure of globular proteins. The Section on Molecular Forces and Assembly, is concerned with biophysical studies of the forces between DNA, protein and carbohydrate molecules. The Section on Molecular Biology and Genetics, under Dr. Alan N. Schechter, is concerned primarily with the molecular genetic basis of the developmental control of gene expression, especially in human erythroid and lymphoid cells, and its relevance to the understanding of the molecular basis of disease states and possible approaches to their therapy. New programs on cytogenetics (in conjunction with the NIH Inter-Institute Genetics Program) and on AIDS research have recently been initiated in this Section.

During the last year, Dr. Beverly White has been transferred to this Laboratory in a joint effort with the Clinical Center to establish a research and clinical Cytogenetics Unit. The responsibilities and resources for the program on molecular forces are now shared with the Laboratory of Biochemistry and Metabolism, NIDDK. Dr. Constance Tom Noguchi has established a major research group in the Laboratory of Chemical Biology. Dr. Patricia Berg, a Senior Staff Fellow, also has a group working on globin molecular genetics. Dr. David I. Cohen has joined the Laboratory as a Senior Medical Staff Fellow to establish a program in the molecular genetics of normal and abnormal human lymphoid cells. Dr. Griffin Rodgers is now serving as a Robert Wood Johnson Fellow; while Dr. Donald Rau has become an Expert Consultant. Dr. C.B. Anfinsen, who is a Scientist Emeritus in this Laboratory, visits here several days each month, in part in his additional capacity as senior advisor to the medical students in the Howard Hughes Medical Research Institute Program at the NIH.

Extensive research collaborations exist within this Laboratory and with other Laboratories in this Institute, in NIH, and nationally and internationally as outlined in the individual Research Project Reports. A formal collaboration has been established with the Clinical Center's Inter-Institute Medical Genetics Program to fund a clinical and research cytogenetic program. Clinical collaborations also exist with the Clinical Hematology Branch of NHLBI and other units. In addition, a formal collaboration has been established involving the exchange of personnel and resources with Dr. David Hankins of the Laboratory of Experimental Hematology of the Armed Forces Radiobiological Research Institute at the National Naval Medical Center. The participation of this Laboratory in the NIH Inter-Institute Medical Genetics Program and the NIH-George Washington University Hematology Training Program continues to grow. The Laboratory is now also a major part of the recently established Intramural AIDS Research Program.

Section on Protein Chemistry and Conformation

The recently completed studies on folding and fragment complexes of staphylococcal nuclease, RNase A, cytochrome c and certain chemically synthesized derivatives of cytochrome c has led to the hypothesis of the importance of globally coupling forces mediated through a line of contacting atoms, forming a close curve in three dimensional space, in the folding of proteins. It is suggested that these forces do not correspond to the conventional ones studied in protein physical chemistry but constrain individual atomic residues in the three dimensional structures of proteins and are detected by changes in the same direction of both enthalpy and entropy upon substitutions of specific amino acids. These coupled forces are being studied in analyses of derivatives of cytochrome c which undergo an intermolecular "flip." They could constitute a significant new way of examining the structure and dynamics of proteins.

An outgrowth of the above work is an extensive project on the total chemical synthesis of cytochrome c and its derivatives, including a postulated ancestral sequence. A variety of synthetic strategies have been developed for coupling large and small fragments of the protein made by Merrifield solid phase methods and for the eventual covalent linking of the heme group with a specific enzyme that has already been characterized.

Recent results suggest strongly that this structure should fold correctly. Another related project is the production and characterization of a number of monoclonal antibodies to cytochrome c. Six monoclonal Ab molecules have characterized as to their exact sites of binding to the protein in preparation for studies of the dynamics of their interaction with native protein as well as to chemical derivatives and fragment systems.

Section on Molecular Forces and Assembly

The work of this Section has involved both theoretical and experimental analyses of forces stabilizing DNA, proteins, lipids, and carbohydrates. Theoretical analyses have clarified effects of mechanical motion on the long range forces acting between neighboring linear macromolecules. Hydration forces between DNA molecules have been measured using the osmotic stress technique with X-ray diffraction measurements of molecular spacing. These results show that the changes in entropy in the energetics of these interactions are determined by the structure of water of hydration surrounding these molecules. This has also been shown to be true for carbohydrates, including neutral sugars which cannot have Coulombic interactions.

Other experimental studies show that the bending of oligonucleotides is due to the base tilting from B. It has also been shown, using electric dichroism measurments, that ATP binding to DNA-gyrase complexes causes the DNA tails to fold back across the complex; myosin II has also been studied in this manner. These studies are of major importance in emphasizing the role of water and hydration forces in macromolecular interactions. New studies are underway in evaluating the role of configurational entropy on macromolecular interactions and in developing a technique of photochemical electric dichroism to identify the exact orientations of different parts of DNA sequences interacting with proteins.

Section on Molecular Biology and Genetics

The major part of this Section's work is devoted to clarifying the molecular genetic basis by which the developmental switch from embryonic to fetal to adult hemoglobins occurs in the human. Understanding of the control of globin gene expression would be a very important general point with respect to developmental biology, but might also have specific therapeutic relevance for the diseases of hemoglobin. The project is being pursued for the most part by trying to understand the phenotype of a cell line, the K562 cells, which appears to be arrested in the late embryonic stage of globin gene expression. Evidence has been obtained that there are intranuclear factors, trans-acting factors, that determine which genes are expressed and which are silent in these cells. During the last year, a broad range program to identify and isolate these factors and to understand their mechanism of action has been developed. To this end studies are underway of nuclease hypersensitivity in the chromatin structure around active and inactive globin genes, of the structure and function of the globin promoter regions (cis-acting sequences) by fusing families of deletion mutants to the gene for the enzyme chloramphenicol transferase (CAT) and assaying CAT activity in cells transfected with various promoter-CAT fusion genes, of in vitro transcription systems to provide a direct assay for trans-acting factors, and of the effects of known viral trans-acting factors (such as the SV40 T antigen, the adenovirus E1A

protein, the HTLV I tat-1 gene and the products of various oncogenes) to clarify the mechanism and specificity of trans-activation. In addition direct binding assays (footprinting and gel shift) and subtractive cloning techniques are being used in order to isolate the protein or the gene for one or more of these trans-acting factors. Although these goals are not simple, the elucidation of the control of this biologically and medically important human gene system would be a potentially major step in molecular and developmental biology and in applied medical molecular genetics.

This Section also continues its work on the pathophysiology of sickle cell anemia. During the last year the role of fetal hemoglobin levels in determining disease severity and expected response to therapy has been clarified. Studies of non-invasive methods to evaluate blood flow in sickle cell anemia patients also continue to offer the potential of developing objective measures of disease severity. A project to develop an animal model of sickle cell disease by using transgenic methods to introduce the β^S and the human α gene into mice has been initiated. Methods to remove the endogenous mouse globins, including the use of α- and/or β-thalassemic mice or the use of anti-sense globin genes, are also being studied. This work is regarded as a long term project to develop a true model of the disease for study of sickle cell rheology, pathophysiology, and treatment. A Clinical Center program to treat select patients with hydroxyurea has been initiated and seven patients have been treated so far.

Another program in the Section is the study of genes in human lymphoid tissues that code for the T-cell receptor (TCR), including the α, β, γ and the newly postulated δ genes. A new genetic element, TEA, has been identified in early human T cells and suggests a novel rearrangement of the TCR gene and may relate to the δ gene. Work on mice shows that the CY gene repertoire is very large and rearranges easily, with detectable polymorphisms. These genes are postulated to play a major role in the mechanism by which an organism distinguishes between "self" and "non-self."

The cytogenetics group has studied high resolution chromosome preparations in a large number of Clinical Center patients and has shown a very high frequency (29%) of abnormal karyotypes. Several hypotheses concerned with karyotype abnormalities in Alzheimer's disease are being tested.

The NIH Intramural Research Program on AIDS has established a program in this Section to analyze transcriptional mechanisms related to the tat gene of HIV. The cloned tat gene has been obtained and is being inserted into an expression vector for large scale production of the tat protein in E. coli and, possibly, eukaryotic cells. The protein will be purified to allow detailed structure function studies, including X-ray crystallography and high resolution NMR. The tat gene is also being transfected into heterologous cells to examine its interaction with other promoters so as to clarify its molecular mechanisms of action. We are trying to develop structural (binding) and functional assays to allow systematic study of potential inhibitors of tat function. We hope these studies may lead to a new approach to the treatment of AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25008-24 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Synthesis of Ancestral Form of Cytochrome c: Protein Evolution

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

PI:	Hiroshi Taniuchi,	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Other:	Alice Fisher	Chemist	LCB, NIDDK
	Xuan Truong	Biological Aid	LCB, NIDDK

COOPERATING UNITS *(if any)*

University of Padova, Padova, Italy

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Protein Chemistry and Conformation

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	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.)*

Synthesis of the ancestral cytochrome c deduced by W. Fitch and E. Margoliash serves two purposes, i.e. to see whether the structure-function of this theoretical protein is similar to cytochrome c and if so, to study how invariant amino acids and the specific covalent attachment of heme are related to the structure-function. We have found and solubilized cytochrome c synthetases from both yeast and beef heart mitochondria which catalyze covalent attachment of heme to yeast apo-iso-1- or horse apocytochrome c (see previous reports). To see whether such an ancestral form could exhibit the structure-function of cytochrome c, we studied two forms type I and II of hybrid fragment complexes formed among different cytochrome c species. The discontinuity of the polypeptide chain occurs between residues 23 and 25 for type I form and between residues 38 and 39 for type II. The structural integrity was based on the 695nm absorption band. The heme- and apofragments (or apoprotein) were found to be completely exchangeable between candida and horse cyts. c for both types of complexes. (Previously the heme- and apofragments of horse and tuna cyts. c were found to be completely exchangeable for complex I.) The present studies also show that for complex I the heme- and apoprotein are completely exchangeable between yeast iso-1- and horse cyts. c. Complex II can also be formed between the heme fragment of yeast iso-1-cyt. c and the apofragment of horse, tuna or candida cyt. c or between the heme fragment of candida cyt. c and the apofragment of tuna, horse or candida cyt. c. However, no type II complex is formed between the apofragment of yeast iso-1-cyt. c and the heme fragment of horse, tuna, candida or yeast iso-1-cyt. c nor between the heme fragment of tuna cyt. c and the apofragment of candida cyt. c. The results suggest that the polypeptide chains (no discontinuity) containing hybrid amino acid sequences even between phylogenetically distant cyts. c, if synthesized chemically or through recombinant DNA, would likely fold to the cyt. c-fold. Thus, the ancestral form would also be likely to assume the cyt. c fold possibly with the function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25011-13 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Principles That Govern Protein Folding: The Second Half of the Genetic Code

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

PI:	Hiroshi Taniuchi	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Others:	Boleslaw Picur	Visiting Fellow	LCB, NIDDK
	Marek Lisowski	Visiting Fellow	LCB, NIDDK

COOPERATING UNITS *(if any)*
Research Department, Nestec Ltd., Vevey, Switzerland

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry and Conformation

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

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

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

Based on the previous studies, we hypothesize that after folding of, for example, staphylococcal nuclease the interatomic interactions would be globally coupled to generate extra force for shifting the equilibrium between folding and unfolding in favor of folding and that such coupling would occur on the basis of a line of contacting atoms forming a closed curve in the three-dimensional structure. A result of modulation of this global coupling would be that transformation from one of the conformational energy states to another would involve concerted changes of forces constraining the atomic positions throughout the structure. We have tested this hypothesis using a model system of two isomeric complexes type I and II formed from heme fragment (1-38)H and apoprotein of horse cytochrome c. We investigated 1) kinetics and thermodynamics of interconversion between type I and II forms of complex ferro(1-38)H•(1-104); 2) the CO-binding population; 3) the rate of dissociation of complexes ferri- and ferro(1-38)H•(39-104) (mimicking type II form); and 4) thermal transition of the 695nm absorption band and biological activity. The results indicate a) interconversion between the two forms of complex ferro(1-38)H•(1-104) occurs without going through dissociation and is associated with enthalpy change favoring type I and entropy change favoring type II; b) the CO-binding population correlates with type II; and c) the redox state of heme appears to influence the thermodynamic relationship between the two forms. The results suggest that "intra-molecular" flip between ferro-type I and ferro-type II forms would establish the Boltzmann distribution of these two distinctly different energy states, type I form having more strengthened interatomic interactions and type II more pronounced internal motion. Since this model is an exact two-state system by virtue of isomeric complexes, the measured enthalpy and entropy changes can be unambiguously interpreted as relating to changes of internal motion, i.e. changes of forces constraining atomic positions distributed in the structure, revealing new major sources of enthalpy and entropy changes for protein folding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25016-14 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trans-Acting Factor(s) Controlling Globin Gene Expression in K562 Cells

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

PI:	Pablo Gutman	Visiting Fellow	LCB, NIDDK
Others:	Shi-Xian Cao	Visiting Fellow	LCB, NIDDK
	Helena Mishoe	Senior Staff Fellow	LCB, NIDDK
	Patricia Berg-Lovett	Senior Staff Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

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

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

K562 is an erythroleukemic cell line widely used as a model for the study of the control of human globin gene expression. These cells do not support transcription of the beta-globin gene (human adult pattern of expression) but do express transcripts of epsilon- and gamma-globin genes (human embryonic and fetal pattern) at very high levels when exposed to a number of inducing agents. Results from this and other laboratories suggest that the control of this pattern of expression is mediated by the presence and/or absence of trans-acting factors which exert their action on sequences corresponding to the promoters of these genes. Sequence specific DNA binding proteins acting on cis-regulatory control elements have been hypothesized to be key elements in eukaryotic gene transcription, and even though considerable progress has been made in their isolation, DNA binding proteins with affinity for the human globin gene promoters have not yet been identified. We have chosen to study the interaction of these factors with DNA sequences belonging to the epsilon-gene promoter. The methodology used included DNase footprinting and the gel retardation assay. By the former, two protective patterns have been detected surrounding the -500 and -260 nucleotide regions, and by the latter multiple DNA binding activities have been shown, some of which possess specificity. Experiments leading to the detection of the exact sites to which these elements bind are now under course using a combination of these two methods. Further studies will be undertaken to show the functional significance of these factors. Characterization of such factors is crucial in understanding the mechanisms underlying the transcriptional control of human globin genes.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25021-12 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sickle Cell Anemia: The Intracellular Polymerization of Hemoglobin S

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

PI:	Constance Tom Noguchi	Research Physicist	LCB, NIDDK
Others:	Griffin P. Rodgers	Guest Researcher	LCB, NIDDK
	Barbara Torain	Biological Lab Technician	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
LCDB, NIDDK (J. Blanchette-Mackie); University of Birmingham, U.K. (Dr. J. Stuart); Johns Hopkins University, Baltimore (Drs. G. Dover and S. Charache); MRC Unit, Kingston, Jamaica (Dr. G. Serjeant).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

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

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

The extent of intracellular polymerization of hemoglobin S is primarily determined by oxygen saturation, hemoglobin concentration and hemoglobin composition. We have examined the filterability of sickle erythrocytes to determine whether sufficient sickle hemoglobin polymer forms at arterial oxygen saturation to adversely affect cell deformability. Progressive reduction of oxygen tension within the arterial range caused a sudden loss of filterability of sickle erythrocytes through 5 micron diameter pores at a critical pO2 which correlated significantly with the polymerization tendency for each patient. This loss of filterability was reversible upon reoxygenation and occurred at a higher pO2 than did morphological sickling. Impairment of erythrocyte filterability at high oxygen saturation suggests that small changes in oxygen saturation within the arterial circulation cause rheological impairment of sickle cells.

It has been appreciated that fetal hemoglobin has a specific "sparing" effect in inhibiting polymerization of sickle hemoglobin, however, the exact amounts of fetal hemoglobin necessary to ameliorate the various manifestations of the sickle cell syndromes have been uncertain. Epidemiological analyses of sickle cell disease severity and studies of the biophysics of intracellular polymerization were used to estimate potential clinical benefit of various levels of fetal hemoglobin for use as guideposts for therapeutic goals in studies designed to increase fetal hemoglobin levels in sickle cell disease.

Erythrocytes containing hemoglobin Setif can undergo pseudosickling in the laboratory when incubated under select buffer conditions. Corresponding aggregation of hemoglobin lysate from these erythrocytes was detected when incubated in phosphate buffered saline at either 290 mOsm or 459 mOsm. However, changing buffer conditions reversed the hemoglobin aggregation. Detailed studies of hemoglobin Setif aggregation may suggest alternate strategies of the inhibition of sickle hemoglobin aggregation.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25025-11 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Origin of the Specificity of Antigen-Antibody Interaction

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

PI:	Hiroshi Taniuchi	Chief, Section on Protein Chemistry and Conformation	LCB, NIDDK
Others:	Ida Silvestri	Visiting Fellow	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry and Conformation

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)

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

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

The previous studies have led to the hypothesis that interaction between spatially distant regions of proteins would be mediated by some unknown factor which would operate on the basis of a line of contacting atoms forming a closed curve in the three-dimensional structure. On the basis of this theory, we hypothesize that binding of an antibody to its antigen would establish such a closed curve of a line of contacting atoms in the antibody to generate extra force for the binding. As a step for testing this hypothesis, we prepared 6 hybridoma cell lines 4.74.6, 4.128.6, 4.145.10, 2.96.12, 2.34.19 and 10-28-86 each producing IgG1, and one cell line 39-14 producing IgM. All of the monoclonal antibodies (Abs) are directed to yeast iso-1-cytochrome c with the exception that Ab 10-28-86 is directed to apo-iso-1-cyt. c. To map the epitopes, yeast iso-1- and iso-2-cyts. c, cyts. c from C. krusei, tuna, pigeon, chicken, rabbit, rat, dog, bovine, porcine, sheep and horse, yeast apo-iso-1-cyt. c, C. krusei and horse apocyts. c, hybrid fragment complexes between yeast iso-1- and horse cyts. c and fragments of yeast iso-1-cyt. c were tested for reaction with the monoclonal Abs. Further, horseradish peroxidase conjugated monoclonal Abs were prepared (except for Ab 39-14) to test the antigen binding inhibition between monoclonal Abs. The results have permitted tentative assignment of the specifically recognized residues of yeast iso-1- cyt. c: 67 and 68 for 4-74-6, 58 or 61 for 4-128-6 or 4-145-10, 97 for 2-96-12, 88 for 2-34-19, 30 and 31 for 39-14 and 62, 70 or 77 for 10-28-86. Residues 30 and 31, 58 or 61 and 88 represent new epitopes for cyt. c. Abs 39-14 and 10-28-86 recognize both yeast iso-1 native and apocytochrome c and the rest only native cyt. c. Abs 2-96-12 and 2-34-19 appear to be more sensitive for the epitope conformation than the others in that either Ab does not react with any hybrid complexes between yeast iso-1- and horse cyts. c in contrast with Abs 4-74-6, 4-128-6 and 4-145-10 reacting with hybrid complexes between the heme fragment of horse cyt. c and apoprotein or apofragment of yeast iso-1-cyt. c.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25028-09 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development of Non-Invasive Methods to Assess Sickle Cell Patients

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

PI:	Griffin P. Rodgers	Guest Researcher Robert Wood Johnson Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Senior Investigator	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Clinical Hematology Branch, NHLBI (A.W. Nienhuis); Clinical Branch, NEI (M. Roy); Transfusion Medicine, CC (H. Klein); BEIB (Eli Walker); Biometry Branch, NEI (M. Podgor); MRC Laboratory, Kingston, Jamaica (G. Serjeant).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Despite recent insights into the molecular and cellular pathophysiology of the sickle cell syndromes, our understanding of the relationship of these subcellular events to the variable clinical expression of sickle cell disease remains largely speculative. We have sought to develop quantitative ways to clarify disease pathogenesis, as well as to assess severity and progression. Using calibrated phthalate ester separation method, which we previously described, we have now shown that there are at least three cellular processes contributing to the extensive red cell heterogeneity that is commonly observed in the sickle cell syndromes. Ocular studies of the patients show striking correlations between the extent of erythrocyte heterogeneity with conjunctival and retinal vessel pathology. As predicted by biophysical studies of polymer formation, we find that treatment of steady state sickle cell patients with selective arteriolar vasodilators results in a significant resolution of both acute conjunctival and retinal abnormalities, as well as an improvement in color vision performance. These salutary effects occurred in the absence of a direct drug-induced change in polymer formation, and suggests that inappropriate vasospasm or frank vasoconstriction, perhaps in response to the altered rheology of red cell containing polymerized sickle hemoglobin is a significant contributing factor to the pathogenesis of sickle cell disease. This conclusion is also supported by our recent observation that "relative" hypertension is a significant risk factor for the occurance of stroke in sickle cell patients. Using the technique of Laser-Doppler velocimetry, we have found that forearm cutaneous microcirculatory flow undergoes a unique characteristic periodic pattern, which may become more "normalized" depending upon the fraction of non-S hemoglobins. We hope that these cellular and physiological measurements will allow us to understand better the extreme spectrum of disease manifestations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25038-07 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of HTLV-I Tat-I Product on Globin Gene Expression

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

PI:	Henry B. Fox	Staff Fellow	LCB, NIDDK
Others:	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Metabolism Branch, NCI (Drs. T. Waldmann and W. Greene); LTCB, NCI (Drs. H. Streicher and R. Gallo)

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Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

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 control of human globin gene expression in erythroid cells involves trans-factors (substances active at distant locations in the genome), which have yet to be identified or clearly described. One experimental approach to their identification is to study the effects on globin gene expression of well-described trans-factors from tumor viruses. We have shown that the HTLV-I trans-factor tat-I stimulates both beta- and epsilon-promoters fused to a CAT gene, resulting in roughly 20-fold increase in CAT enzyme activity. In the case of beta-globin, only 185 bp of 5' flanking sequence is required for this effect. There is relatively little trans-activation when the globin promoter already has an SV40 enhancer in cis.

Further studies will involve characterization of the tat-I induced trans-activation of globin promoters, including studies of mRNA levels and rate of transcription. While tat-I has been shown not to bind directly to DNA, we hypothesize that this trans-activation of globin genes involves interaction with other proteins that do bind to cellular DNA. Our ultimate objective is to identify such cellular proteins that interact with tat-1 to trans-activate globin genes. Study of such proteins would increase understanding of trans-activation of globin genes and may clarify the developmental regulation of globin gene expression in human erythroid cells.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25045-04 LCB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Regulation of Globin Gene Expression by 5' DNA Sequences

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

PI:	Patricia Berg-Lovett	Senior Staff Fellow	LCB, NIDDK
Others:	Ruo-Lan Qian	Visiting Fellow	LCB, NIDDK
	Shi-Xian Cao	Visiting Fellow	LCB, NIDDK
	Donna Williams	Staff Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Chemical Biology

SECTION: Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION: NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Although a number of human genetic diseases have been defined as mutations in or near the human beta-globin gene locus, regulation of gene expression of the human beta-globin gene is not well understood. To study this regulation, we are using a mutant human erythroleukemia cell line, K562. These cells can synthesize embryonic and fetal globins but not adult beta-globin, although they contain a structurally normal beta-globin gene which can be induced to express in transient heterokaryons. Thus, the molecular defect in K562 cells is most likely due to differences in trans-acting factors between K562 cells and normal erythroid cells such as continuous synthesis of a repressor, lack of synthesis of an activator molecule, or both. If there is a negative regulatory factor in K562 cells, deletion of its DNA binding site might then allow expression. On the other hand, deletion of DNA containing a binding site for a positive acting factor should be seen as decreased expression if the gene were active.

In an attempt to understand regulation of expression of the human beta-globin gene we are first studying its 5' DNA sequences. We have fused the 5' flanking region to a heterologous gene, chloramphenicol acetyl transferase (CAT). Consistent with other reports from this laboratory, we found no expression of this gene in uninduced K562 cells. Our deletion analysis of this DNA suggest there are at least three regulatory regions 5' to the beta-globin gene, two negative control regions (NCR) and one positive control region (PCR). Only the PCR appeared to be specific for K562 cells when these deletions were studied in a Chinese hamster and a mouse erythroleukemia cell line. Analysis of these plasmids in hemin-induced K562 cells showed expression of the original CAT plasmid for the first time. This plasmid could also be expressed in uninduced K562 cells in the presence of the SV40 enhancer. Preliminary experiments suggest there may be protein(s) binding to both NCR1 and NCR2, as well as to the region between them.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25046-03 LCB

PERIOD COVERED

Terminated as of September 30, 1986

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

Control of Exocytosis in Sea Urchin Eggs by Osmotic Stress

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

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Others:	V.A. Parsegian	Chief, Section on Molecular Forces and Assembly	LCB, NIDDK

COOPERATING UNITS *(if any)*

University of London, UK (Dr. M. Whitaker); Harvard University, Cambridge, MA (Dr. J. Liu).

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Laboratory of Chemical Biology

SECTION

Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25047-03 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Hydration Forces and Applications of the Osmotic Stress Technique

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

PI:	Donald C. Rau	Expert	LCB, NIDDK
Others:	Rudi Podgornik	Visiting Fellow	LCB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Physical Sciences, DCRT and Laboratory of Biochemistry and Metabolism, NIDDK (Dr. V. Adrian Parsegian).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

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

The observation of hydration forces as the dominant interaction between macromolecules at close approach has been extended to another class of important biopolymers, polysaccharides. The two helical carbohydrates studied were xanthan and schizophyllan. In both cases, the observed forces between helices are characteristic of hydration forces as defined by previous work on DNA and lipid bilayers, a force that varies exponentially with distance between surfaces with a 3A decay length, that is independent of ionic strength and composition. Polysaccharides offer a wide variety of surface groups for study. By measuring forces between a large number of these surfaces, we can begin to assign surface hydration parameters to chemical groups. These parameters will determine the strength and polarity (attraction or repulsion) of hydration interactions between any two surfaces. This is a necessary step toward developing a predictive hydration force framework. The utility of xanthan for these measurements is that a number of surface groups can be chemically removed without disturbing structure and changes in hydration forces measured. The polymer schizophyllan is important for another reason. This carbohydrate is a triple helix composed entirely of glucose, with no formal charge in the structure. The observation of hydration forces without added salt precludes that these forces are in any way due to screened Coulombic interactions.

In a second project, the effect of configurational entropy on intermolecular forces is being evaluated. A decrease in macromolecular freedom is an important component of all assembly reactions and the interplay of this with intermolecular forces is as yet not well understood for molecules with internal degrees of freedom. At present, we are observing changes in DNA configurational freedom for arrays of helices under osmotic stress. It appears very strongly that this internal motion significantly affects the interactions between DNA helices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25048-03 LCB

PERIOD COVERED

Terminated as of September 30, 1986

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

Molecular Forces

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

PI: V.A. Parsegian Chief, Section on Molecular Forces and Assembly LCB, NIDDK

COOPERATING UNITS *(if any)*

Brock University, Canada (R.P. Rand); Univ. British Columbia, Canada (E.A. Evans); Princeton Univ. (S.M. Gruner); Clarkson Univ. (E. Barouch).

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25049-03 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Trans-activation of Globin Promoters by SV40 T-Antigen and Adenovirus E1A

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

PI:	Shi Xian Cao	Visiting Fellow	LCB, NIDDK
Other:	Helena Mishoe	Senior Staff Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Chemical Biology

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Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Regulation of gene expression in eukaryotic cells is largely controlled at the level of transcription. This control is mediated by interactions of trans-acting factors and cis-regulatory sequences. To gain some insights into the mechanisms of trans-activation, we have undertaken studies to examine the ability of two viral early gene products, T antigen of SV40 and E1A protein of adenovirus, to trans-activate human globin gene promoters. Since T antigen and E1A possess both similar and distinct properties in gene regulation, we thought it is important to know the relationship between the two viral proteins in trans-activation of gene expression. This may help us to understand the trans-activation mechanisms of polymerase II transcription in eukaryotic cells. During the past year, we have compared the trans-activation effect of T antigen and E1A by co-transfecting a testing plasmid p-epsilon-GLCAT with either pRSV-T (plasmid expressing the SV40 T antigen) or pE1A (plasmid expressing the E1A protein of adenovirus) into CV-1 cells and COS-1 cells. By transient assay, we found that while both T antigen and E1A trans-activated the epsilon-globin promoter, they required different 5' flanking sequences for trans-activation. We also demonstrated that T antigen can produce additional stimulatory effect on p-epsilon-GLCAT-SV (enhancer+) in CV-1 cels, but E1A has no any effect on this plasmid. Furthermore, whereas introduction of pRSV-T into COS-1 cells had no effect on p-epsilon-GLCAT expression, the presence of pE1A produced great increase in CAT activity, compared with transfection carried out with p-epsilon-GLCAT alone. Our results, therefore, suggest that T antigen and E1A trans-activate the epsilon-globin promoter by using different mechanisms, probably mediated by different cellular transcription factors.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25050-03 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure and Physical Properties of DNA and DNA-Protein Complexes

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

PI: Donald C. Rau — Expert — LCB, NIDDK

COOPERATING UNITS *(if any)*
George Mason University, Fairfax, VA (Dr. H. Chen); LMB, NIDDK (J. Nickol); LCP, NIDDK (Drs. S.S. Wijemga and E. Charney); LCB, NHLBI (Drs. M.A.L. Atkinson and E.D. Korn).

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SECTION
Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Three different projects have been initiated within the past year. Major emphasis has been on developing a modification of the classical electric dichroism experiment. This new technique, called photochemical electric dichroism, will allow a straight-forward determination of DNA wrapping, folding, or looping topology in DNA-protein complexes. It is basically a hybrid technique, uniting the methodology of electric dichroism with the sensitivity and selectivity of DNA footprinting techniques. The link is the formation of photochemical dimers between stacked pyrimidines, which is a marker for an absorption event. DNA helices can be cleaved chemically and enzymatically at the sites of these photodimers and probabilities analyzed by electrophoresis. Comparing frequencies of photodimer formation at a particular region between unoriented complexes and complexes oriented by an electric field gives the dichroism of the DNA at that region. We have recently visualized the loop of DNA in the DNA-DNA gyrase complex by this technique. Future experiments are planned for studying the structure of both bulk and active gene chromatin to deduce the effect of specific sequence protein binding on DNA structure.

Statically bent DNA is now thought to result from the juxtaposition of sequences of DNA with different base pair tilting properties. A project has been initiated to evaluate this proposal. A fragment of DNA with a biphasic B-A form transition has been uncovered. These two forms have very different base pair tilting and the rotational hydrodynamics of this fragment at the transition midpoint are consistent with a bent rod of DNA. We are now quantitating this effect.

Finally, the sensitivity of rotational motion to molecular dimensions is allowing us to determine the structure and flexibility of Acanthameoba myosin II in a variety of structures, monomeric, dimeric, and bipolar filamentous, with both native and phosphorylated myosin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25051-03 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trans-Acting Factors Involved in Globin Gene Expression in K562 Cells.

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

PI:	Helena Mishoe	Senior Staff Fellow	LCB, NIDDK
Others:	Donald Rau	Expert	LCB, NIDDK
	Pablo Gutman	Visiting Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

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

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

Many genes expressed by multicellular organisms are expressed in one cell type but not another. Two cis-acting elements, promoters and enhancers have been shown to be resonsible for tissue specificity. However, the biochemical basis for this cell type specificity is not clearly understood. We are using the K562 leukemic cell line as a model system to increase our understanding of events associated with tissue specific and developmental expression in normal marrow progenitor cells.

The human globin genes exhibit a high degree of sequence conservation not only in their coding region but also in their 5'-flanking regions. Despite the considerable degree of sequence homology, the globin genes are expressed in a distinct developmental manner. Therefore, this is an interesting system for studying the co-evolution of cis- and trans-acting elements in addition to investigating the molecular mechanisms which control tissue and developmental specific gene expression. To this end we are using techniques which will enable us to identify and characterize trans-acting factors which interact with the (epsilon) embryonic globin gene, which is the predominate hemoglobin message in K562 cells. We have used a labelled DNA fragment containing the epsilon promoter in a DNA-protein binding assay. We have been able to detect a DNA-protein complex using a 0.3M KCl nuclear extract and the epsilon promoter fragment. We have determined that the complex is specific by competition assays. At present we are attempting to footprint the DNA-protein complex and identification of the protein is being attempted by UV cross-linking experiments.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25052-03 LCB

PERIOD COVERED
Terminated as of September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Control of Membrane Transport by Osmotic Stress

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

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Others:	V.A. Parsegian	Chief, Section on Molecular Forces and Assembly	LCB, NIDDK

COOPERATING UNITS *(if any)*
Lab. Theoretical Biology, NCI (Dr. A. Walter); Johns Hopkins University, Baltimore, MD (Dr. A. Harris); UCLA, CA (Dr. F. Bezanilla).

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SECTION
Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25053-03 LCB

PERIOD COVERED

Terminated as of September 30, 1986

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

Histamine Release from Beige Mouse Mast Cells

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

PI:	Joshua Zimmerberg	Guest Researcher	LCB, NIDDK
Others:	M. Curran	Guest Researcher	LCB, NIDDK

COOPERATING UNITS *(if any)*

Rush Medical College, Chicago, IL (Dr. F.S. Cohen); University of Texas, Galveston, TX (Dr. M. Brodwick).

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Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25054-02 LCB

PERIOD COVERED

Terminated as of September 30, 1986

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

G-protein Diffusion During Muscarinic Activation

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

PI: Joshua Zimmerberg Guest Researcher LCB, NIDDK

COOPERATING UNITS *(if any)*

Laboratoire de Neurobiology, Ecole Normale Superieure, Paris, France (Dr. A. Marty).

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Forces and Assembly

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25055-02 LCB

PERIOD COVERED

Terminated as of September 30, 1986

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

Survey of Human Lymphoid Diseases for Human Pathogenic Retroviruses

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

PI:	David I. Cohen	Medical Staff Fellow	LCB, NIDDK
Others:	Jean-Pierre deVillartay	Guest Researcher	LCB, NIDDK

COOPERATING UNITS *(if any)*

BRMPDS, NCI (Dr. D. Longo); LMM, NIAID (Drs. M. Martin and H. Gendelman); ARB, NIDDK (Drs. G. Tsokos and F. Steinberg).

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25056-02 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Human T Cell Receptor Alpha and Delta Genes

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

PI:	Jean-Pierre deVillartay	Guest Researcher	LCB, NIDDK
Others:	David Cohen	Medical Officer	LCB, NIDDK
	David Coran	Guest Researcher	LCB, NIDDK
	Ellen Bernstein	Biologist	LCB, NIDDK

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI (Drs. S. Korsmeyer, T. Waldmann); LGG, NIAID (Dr. J. Coligan); Lab. of Immunogenetics, NIAID (Dr. R. Sekaly); Lab. of Tumor Cell Biology (Dr. I. Tschachler)

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Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.6	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.)*

Two properties of T lymphocytes are the ability to respond to foreign antigens in association with molecules of the self major histocompatibility complex (MHC) and the unresponsiveness towards self MHC molecules alone. Important progress has been made in understanding the process by which T lymphocytes recognize antigen through the cloning and characterization of the alpha and beta genes of the T cell receptor for antigen (TCR). Despite this progress, the precise mechanisms of thymic education, in particular the self-tolerance education, remain poorly understood. Beside the alpha/beta TCR for antigen, a second TCR called gamma/delta has been described at the protein level. The gamma/delta TCR has frequently been found in immature T cells and thymocytes and is therefore thought to play a role during the early events of thymic T cell development. This project aims to attempt to clone the human delta chain gene as well as to study its relationship with the other TCR genes, in particular the alpha gene.

In the course of characterizing immature human T cell tumors, we detected a novel 2 kb alpha-related transcript. cDNA clones corresponding to this transcript established that a new genetic element (T early alpha = TEA), had been spliced to the alpha constant region. The expression of TEA is highest in early fetal thymus and declines during T cell maturation. TEA is present in the germline configuration in gamma/delta expressing cells and is deleted in mature alpha/beta expressing cells. Surprisingly the TEA probe detected a limited number of rearranged band in a non-clonal thymic DNA. We are currently characterizing these rearrangements, and their relationship to immature and mature TCR gene expression.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25057-02 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Genetic Influences on the Diversity of the Gamma Chain of the T-Cell Receptor

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

P.I.:	Jeffrey N. Siegel	Medical Staff Fellow	LCB, NIDDK
Others:	David I. Cohen	Medical Officer	LCB, NIDDK

COOPERATING UNITS *(if any)*

Massachusetts Institute of Technology (Dr. D. Raulet); NIAID, NIH (Drs. J. Coligan and E. Shevach).

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The antigenic repertoire of T lymphocytes is generated primarily by the combinatorial diversity of the various V (variable), D (diversity) and J (joining) gene segments which rearrange to a constant region (C) to form the functional genes for the T cell receptor for antigen (TCR). In most mature T lymphocytes the TCR consists of a cell surface heterodimer composed of two molecules (alpha and beta) associated with a second molecular complex, termed CD3. An alternative TCR has also been described which is expressed on a subpopulation of T lymphocytes in thymus and in peripheral lymphoid organs. This receptor represents the major expressed TCR early in fetal development. This alternative receptor appears to contain a different heterodimer or set of related heterodimers termed gamma/delta which is also associated in the membrane with CD3. One unique feature of TCR/gamma is the limited number of observed rearrangements compared to either TCR/alpha or beta.

We undertook studies designed to probe what genetic influences determine the size of the expressed repertoire of gamma chain genes in the mouse. By examining rearrangements in a variety of mouse strains, we have determined that the type of V-J-C gene rearrangements and their relative frequency is determined in large part irrespective of the H-2 haplotype of the mouse. We have also established that there is an additional gamma gene rearrangement which joins V2 to C4 and is present in most strains examined. The presence of this rearrangement is not correlated with the major histocompatibility haplotype of the mouse but is in fact associated with a major polymorphism at the gamma locus. This work establishes that the gamma gene repertoire is larger than previously described, demonstrates that the C/gamma 4 gene rearranges more actively than previously thought, and describes a polymorphism of the murine C/gamma gene locus.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25058-02 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Laboratory and Clinical Models for the Study of Globin Gene Expression

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

PI:	Griffin P. Rodgers	Guest Researcher Robert Wood Johnson Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Research Physicist	LCB, NIDDK
	Nadera Ahmed	Guest Researcher	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

Lab. Mol. Genetics, NICHD (Dr. H. Westphal); MRC Unit, Univ. of West Indies, Kingston, Jamaica (Dr. G. Serjeant); Jackson Labs, Bar Harbor, ME (Dr. J. Barker)

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.4	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We are investigating the molecular mechanisms which control the individual and total concentrations of hemoglobins in human erythrocytes. In addition, we are studying the effects of functional alpha globin gene number, fetal hemoglobin (HbF) levels and the extent of red cell heterogeneity on the various manifestations of sickle cell disease and its genetic variants. The levels of each of the normal hemoglobins (A, A2, F) are determined by controls at the level of transcription and/or translation of the globin genes, as well as by factors that regulate protein degradation. The study of the control of hemoglobin levels has direct relevance to various hemoglobinopathies, especially thalassemia and sickle cell disease. In addition, these studies are of potential relevance to the more general question of control of gene expression in eukaryotic cells. For our experimental system, we are using the K562 human leukemic cell line, as well as peripheral blood from individuals with sickle cell disease. We are studying the effects of short-term and long-term exposure of these cells to 5-azacytidine on their phenotype and the factors that control globin gene transcription. The *cis*-acting sequences in the promoter regions of the beta-like globin genes which interact with putative *trans*-acting factors are being delineated by a variety of molecular approaches. Concurrently, we are also attempting to develop a sickle cell mouse model by the introduction of a cloned human sickle globin gene into the mouse germ line by the microinjection of DNA into the pronuclei of fertilized eggs. The establishment of such a model would allow for basic and fundamental questions to be asked about the molecular, cellular and physiologic aspects of the disease, as well as provide an *in vivo* system to monitor the effects of potential therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25059-02 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trans-activating Factors and Globin Gene Expression: A Direct Approach

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

PI:	Harish Dave	Visiting Fellow	LCB, NIDDK
Others:	Pablo Gutman	Visiting Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

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

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

Humans undergo two developmental switches in their hemoglobin phenotype. The embryonic to fetal switch early in gestation and the fetal to adult switch around the time of birth. The K562 human leukemia cell line expresses all globin genes other than the adult beta-globin. Previous work from this laboratory has shown that the K562 beta-globin gene functions normally in a heterologous expression system. Elucidation of the mechanism of failure of beta-globin gene expression in K562 cells may provide an insight into globin gene expression and switching in normal erythroid cells.

The direct isolation of trans-activating gene(s) will be attempted using the strategy that led to the isolation of several oncogenes. Hybrid beta-Neo plasmids, which do not express in K562 cells, will be cotransfected with another selectable marker (RSV-GPT). Stable tranformants will be obtained by selecting for GPT and the presence of beta-Neo confirmed by Southern blotting. High molecular weight genomic DNA from K562 and MEL cells will be transfected into these clones and the activation of beta-Neo sought by G418 selection. The genomic DNA will be fractionated until the gene(s) of interest is/are isolated. A "rescue" strategy will be used when studying MEL cell genomic DNA.

C-myc has been studied as putative trans-acting factor for beta and epsilon globin genes. Expression was not detected in heterologous transient assay systems using CAT activity as a marker. Further studies using stable K 562 cell transformants containing c-myc are in progress as are studies looking for a potential repressor effect of c-myc.

No suitable human cell lines expressing beta globin are available. It has not proven possible to immortalize marrow erythroid progenitors using combinations of c-myc, c-Ha-ras, and E1A oncogenes. This is felt to reflect suboptimal transfection and/or growth conditions resulting from a scarcity of human material. Optimization will be attempted using murine marrow prior to returning to human marrow studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25060-02 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

In Vitro Transcription of Human Globin Genes With K562 Nuclear Extracts

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Neme, title, leboretory, and institute affilietion)*

PI:	Yuko Wada	Visiting Fellow	LCB, NIDDK
Others:	Barbara Torain	Biological Lab Technician	LCB, NIDDK
	Constance T. Noguchi	Research Physicist	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have prepared extracts from nuclei of hemin-induced and uninduced K562 cells for use in a soluble cell-free in vitro system. Cloned human epsilon-, A/gamma- and beta-globin genes, the insulin gene and the adenovirus 2 major late promoter (Ad2MLP) were employed for transcription in vitro with K562 nuclear extracts. Nuclear extracts could direct accurate initiation of transcription in vitro from epsilon-globin and A/gamma-globin genes and Ad2MLP without supplement of a whole cell extract. A clear dependence of protein concentrations of nuclear extracts on transcriptional enhancement was observed on the epsilon-globin gene. A/gamma-globin gene and Ad2MLP could be transcribed with nuclear extracts at higher concentrations, however, beta-globin gene and the insulin gene were not transcribed under any concentrations of nuclear extracts, either from induced or uninduced cells.

To determine the effect of the length of the promoter region (upstream from the cap site) and the relative importance of specific regions from the promoter on gene transcription, studies of the epsilon-globin gene using truncated DNA templates in the K562 in vitro transcription assay are in progress.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25061-02 LCB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Isolation of Embryonic Globin Transcriptional Factors by Subtractive cDNA Cloning

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

PI:	Yongji Wu	Visiting Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Research Physicist	LCB, NIDDK
	Ellen Bernstein	Biologist	LCB, NIDDK

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Chemical Biology

SECTION: Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION: NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.2	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.)*

The specific transcription of human globin genes may involve the complex interaction of a variety of factors. For the study of globin gene expression, we use the K562 human erythroleukemia cell line as a model system for globin gene expression. The K562 cell line can be induced by hemin to accumulate embryonic and fetal hemoglobin, but not adult hemoglobin. It has been demonstrated that the beta-globin gene is intact but inactive in these cells. The zeta-globin gene promoter functions after microinjection into oocytes but not after transfection into HeLa or COS cells, also suggesting that there might be transcriptional factors specific for embryonic globin genes. The goal of the present study is trying to clone and characterize such factors.

The current study assumes that induced K562 cells contain transcriptional factors specific for embryonic and fetal globin genes, which are absent or present only at very low levels in uninduced K562 cells. We have prepared cDNA from the mRNA of induced K562 cells and cloned it into a vector (lambda gt10) to replicate enough copies for differential screening with 32-P-cDNA probes, from both the induced and uninduced K562 cells. Those cDNA clones which are differentially expressed will be further screened with human embryonic and fetal globin gene DNA probes as well as 32-P-cDNA probes from mRNA of HL-60 cells (human promyelogenous leukemia cell line, a non-hemoglobin producing hematopoetic cell line) to subtract cDNA clones corresponding to embryonic and fetal globin as well as to proteins present in both the induced K562 cells and HL-60 cells. The remaining cDNA clones will be further characterized by transfecting back into K562 cells, other hemoglobin or non-hemoglobin producing cell lines, or by inserting into a protein expression vector (lambda gt11) so that the protein product can be examined for its functional activity on globin and non-globin genes. The protein product will also be examined by protein-specific DNA sequence binding assay or for its activity in an in vitro transcription system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25062-02 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Affecting Mouse Beta-Globin Gene Expression

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

PI:	Donna M. Williams	Staff Fellow	LCB, NIDDK
Others:	Patricia Berg-Lovett	Senior Staff Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Molecular Hematology, NHLBI (Drs. D. Kuebbing and W.F. Anderson).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

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

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

In order to analyze the sequence requirements for induction of the beta-globin gene, we have developed a transient assay system in mouse erythroleukemia (MEL) cells. These cells can be chemically induced to undergo terminal differentiation during which transcription of endogenous alpha and beta globin genes is greatly increased. Our objective was to mimic this induction effect on transiently expressed genes in order to quickly and conveniently analyze plasmid constructions with varying amounts of DNA 5' or 3' to the beta-globin promoter. We succeeded in optimizing transient assay conditions for both uninduced and induced MEL cells but observed no induction of the beta-globin gene or beta-globin fusion genes located on transfected plasmids. We have developed similar assays in parallel (project Z01 DK 25045-04) in an inducible human erythroleukemia cell line, K562, where we do detect clear effects of induction upon transiently expressed beta-globin fusion genes.

We have previously shown that DNA sequences known as enhancers increase the activity of the mouse beta-globin promoter in transient assays. Enhancers are cis-acting DNA sequences which act at the level of transcription to increase gene expression. They can function in either orientation both 3' and 5' to the target gene and their level of activation is relatively independent of position. Other than two categories of short "core" regions, no high degree of sequence homology among the presently identified enhancers has been observed. Our analysis of the sequences of several enhancers indicated that they contained regions of dyad symmetry. We were interested in determining if this was a common feature of enhancer sequences and, if so, whether it might suggest possible mechanisms of enhancer action. Our results indicate that while dyad symmetry is a feature of some enhancers, it does not appear to be present in others. Dyad symmetry is therefore unlikely to relate to a generalized mechanism of enhancer function, although it may play a role in the activity of enhancers that exhibit this trait.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25063-01 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Effect of Hydroxyurea on Fetal Hemoglobin Synthesis in Sickle Cell Patients

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

P.I.	Griffin P. Rodgers	Guest Researcher Robert Wood Johnson Fellow	LCB, NIDDK
Others:	Constance T. Noguchi	Research Physicist	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*

CHB, NHLBI (A.W. Nienhuis); CB, NEI (Dr. M. Roy); BEIB (Mr. E. Walker); Depts. of Medicine, Pediatrics & Pathology, Johns Hopkins University, Baltimore, Md. (Drs. G. Dover and S. Charache).

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have initiated a project to broaden the available fund of knowledge related to the effects of hydroxyurea (HU) on fetal hemoglobin synthesis in patients with sickle cell anemia by studying the acute and chronic responses associated with its administration to such individuals. These studies should provide insight into the pharmokinetics of HU, optimal dosage regimens, and predictive factors associated with the F-reticulocyte response. Preliminary results on 6 patients suggest that the F-reticulocyte count and fetal hemoglobin levels can be universally increased following HU administration, although the magnitude of the response is a complex function of the rate of F-cell production, enrichment and preferential survival. We are now examining the DNA haplotype around the gamma-delta-beta genes of these patients in order to look for genetic predictive factors of the F-cell response. In addition, we plan to enumerate the sequential molecular, cellular and physiological consequences resulting from HU treatment. Should a significant sustained F-cell response be observed in select patients while on HU, it may be possible to increase further the magnitude of the response by simultaneously administering short courses of cloned human erythropoietin or cloned granulocyte-macrophage colony stimulating factor. In this fashion, one may approach fetal hemoglobin levels consonant with those observed in the benign HbS-HPFH phenotypes.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25064-01 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Cytogenetic Investigations of Patients with Genetically Determined Diseases

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

PI:	Beverly J. White	Director, Cytogenetics Unit	LCB, NIDDK
Others:	Margarita Collins	NRSA Fellow	LCB, NIDDK
	Mary Graham	Medical Technologist	OD, CC

COOPERATING UNITS *(if any)*
Interinst. Med. Genetics Program, CC, (J. Mulvihill, D. Parry, J. Green); Genetics Dept., Children's Hospital Natl. Med. Ctr., Washington, D.C. (K. Rosenbaum); LN, NIA (M. Schapiro, J. Luxenberg, S. Rapoport); LTIB, NCI (H. Cooper).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Cytogenetics Unit

INSTITUTE AND LOCATION
CC, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A wide range of cytogenetic methods were utilized for patient investigations in order to correlate specific chromosomal variations with phenotypic abnormalities. Patients with Alzheimer's disease, trisomy 21 and other recognized syndromes, and parents of children with trisomy 21 were studied. We began cytogenetic evaluations of other Clinical Center patients, when the Medical Genetics Program assumed this responsibility in December, 1986.

Our controlled investigations of Alzheimer's disease and older Down syndrome patients with dementia are nearly completed. The only specific variation correlated with dementia was typical constitutional trisomy 21; secondary aberrations observed were probably age- rather than disease-specific. These results are consistent with recent molecular analyses, which indicate that microduplications or other genetic abnormalities of chromosome 21 are associated with dementia of the Alzheimer type. Our analysis of ribosomal DNA gene expression of the nucleolus organizing regions (NOR) of these subjects will soon be completed; the results will be correlated with age and clinical status and compared with control subjects. Our analysis of the NOR in parents of Down syndrome patients is complete; NOR duplication (dNOR variant) reported by others to be frequent in such parents, predisposing to trisomy 21, was not observed.

High-resolution collaborative studies of several recognized syndromes with suspected deletions were recently initiated; data will be collected during the coming year. In situ hybridization experiments with high-resolution chromosome preparations and previously localized probes are in progress, and collaborative experiments with unmapped DNA probes will soon be attempted. Among clinical center patients evaluated with the Medical Genetics Program, abnormal karyotypes and conspicuous variants were frequent (29%). This suggests that many active clinical protocols concern subjects with chromosomal abnormalities or require differentiation between cytogenetic and other genetic etiologies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 25065-01 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Transcriptional Control of Globin Genes in Human Erythroleukemia K562 Cells

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

PI:	Constance Tom Noguchi	Research Physicist	LCB, NIDDK
Others:	Griffin Rodgers	Guest Researcher Robert Wood Johnson Fellow	LCB, NIDDK
	Nadera Ahmed	Guest Researcher	LCB, NIDDK
	Barbara Torain	Biological Lab Technician	LCB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION

NIDDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.2	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 developmental switch which occurs in hemoglobin production during embryonic, fetal and adult life serves as a model for studying the spatial and temporal specificity of gene expression. The K562 human erythroleukemia continuous cell line is a useful tool for examining globin gene expression as these cells express constitutively low amounts of embryonic and fetal hemoglobins and can be further induced for hemoglobin production by various chemical stimuli such as hemin. The structural requirements for active gene transcription are being assessed by examining the binding characteristics of nuclear protein extracts from K562 cells to regions of globin-DNA such as the epsilon promoter, using techniques such as gel-retardation electrophoresis, DNA-footprinting and ion exchange and affinity chromatography. To examine the functional requirements for transcription, a globin-hybrid gene system has been designed to facilitate the analysis, separation and recovery of viable cells in which the epsilon globin gene is actively transcribed.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 25066-01 LCB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
AIDS: Transcriptional Regulation by the TAT Gene and Protein of HI

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

PI:	Constance Tom Noguchi	Research Physicist	LCB, NIDDK
Others:	Henry B. Fox	Staff Fellow	LCB, NIDDK
	Jian-gang Yuan	Visiting Fellow	LCB, NIDDK
	Alan N. Schechter	Chief	LCB, NIDDK

COOPERATING UNITS *(if any)*
LTCB, NCI (Drs. Gallo and Streicher); Kabigen, Stockholm, Sweden (Prof. Hartmanis).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Molecular Biology and Genetics

INSTITUTE AND LOCATION
NIDDK, Bethesda, Maryland

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

The HIV retrovirus is the etiologic agent for AIDS. The tat (trans-activating transcriptional) protein encoded by HIV has the ability to autoregulate the expression of HIV by promoting the transcriptional activity of the HIV LTR promoter. The molecular cloning of HIV has provided the isolation of tat coding DNA from other HIV coding sequences and has facilitated studies of the protein activity independent of other retroviral proteins. We are studying the transcriptional activity of the tat protein on the HIV LTR promoter and on other constitutively expressed cellular genes. The effect of the tat genes and proteins on tissue specific expression as well as the role of cellular proteins on tat activity are being examined using model systems based on human continuous cell lines, such as the K562 erythroleukemia cell line which expresses specific globin genes. Both structural and functional assays are being used to characterize the transcriptional activity of the tat gene and protein. Large scale production, isolation, and purification, of the tat protein from prokaryotic and eukaryotic cells, using cloned tat DNA in suitable expression vectors, is being done to be able to study structure-function relations (by protein chemical methods including NMR and X-ray crystallography) and to develop assays for the study of potential inhibitors of the protein. This work could lead to a new approach to the prevention or treatment of AIDS.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHYSICS
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Research in this laboratory is concerned with the application of modern physical methods to a wide range of problems in molecular and cellular biophysics. A variety of spectroscopic techniques are employed in these investigations, including nuclear and electron magnetic resonance, Raman and Fourier transform infrared spectroscopies, electric-field-induced linear dichroism, ultraviolet and visible microspectrophotometry, and time-resolved optical spectroscopy using nanosecond lasers. There is also a major effort in theoretical studies to complement the experimental work, including both analytic methods and the use of high speed computers in large scale calculations. The systems under study include nucleic acids, proteins, intact and model membranes, retinal photoreceptors, and various small prototypical biological molecules. Current research focusses on: the development of new methods in NMR; the structure of macromolecules in solution by two-dimensional NMR; the structure and dynamical behavior of nucleic acids and nucleoproteins; conformational, dynamical, and functional characteristics of model membrane systems; the dynamics of ligand binding and conformational changes in proteins; theoretical analysis of kinetics and dynamics in macromolecules; computer simulations of atomic motions in proteins; rheological properties of cell membranes; the molecular mechanism of excitation in photoreceptor cells and ionic processes in cell membranes; the gelation of hemoglobin S and its relation to the pathophysiology of sickle cell disease; the analysis of excited electronic states of polyenes in the vapor phase and in molecular beams; and the asymmetric synthesis and structure of metabolites. The following gives a brief summary of the major findings over the past year.

Earlier developments of new methods for correlating proton chemical shifts with shifts of low-gamma nuclei have been continued. For the first time, it has been shown possible to record proton-carbon and proton-nitrogen shift correlation of small proteins (<15 kD) at natural isotopic abundance. A quite different approach has been developed for correlating proton and phosphorous chemical shifts and applied to the study of oligonucleotides. (Bax, Sklenar)

New methods have been developed for recording phase-sensitive two-dimensional proton NMR spectra in water solution without the need for presaturation. In contrast to existing techniques, the new methods accomplish the water suppression in two stages: in the first stage a relatively low suppression is obtained, sufficient to overcome dynamic range problems in the receiver, in the second stage phase cycling removes the water signal from the spectrum almost completely. The new methods have been demonstrated for the important NOE, spin-locked NOE and homonuclear Hartmann-Hahn experiments. (Sklenar, Bax)

A new procedure has been developed for measurement of previously unresolvable coupling constants. By suppressing the effect of all scalar couplings apart from the interaction of interest in a two-dimensional experiment it becomes possible to extract the coupling constants of interest. The procedure has been applied to measurement of J(C3'H-O-P) couplings in the oligonucleotide d(CGCGAATTCGCG)2. The corresponding dihedral epsilon angles show significant differences with X-ray crystallographic work. (Sklenar, Bax)

The analysis of transient and steady-state electro-optic measurements has been refined to the point where rather detailed information on the structure and physical properties of polyelectrolytes can be determined on DNA and the nucleic acids in solution. The solution structure of A-DNA and of B-DNA has been shown to be essentially the same as that of the crystal structures. Current studies are underway on the flexibility of A-DNA with preliminary results that indicate A-DNA to be considerably stiffer than that of B-DNA. Work is also underway on DNA containing specific nucleotide sequences. Early results indicate that the triplet sequence CAC/GTG is probably statically bent. (Charney, Rau, Chen)

Vibrational Raman and infrared spectroscopy have been used to probe the dynamical, conformational, functional and thermodynamic properties of both model and intact membrane assemblies. Emphasis has been placed on elucidating both lipid-lipid and lipid-protein interactions within the membrane bilayer complex. For example, the association between ferricytochrome c, a mobile electron-transfer protein which diffuses between the inner and outer mitochondrial membranes, and cardiolipin was studied using resonance Raman spectroscopic techniques. The observed data exhibit the accepted spectral frequency and intensity markers for ferrocytochrome c. That is, the data imply that the iron atom is reduced, but no obvious reductant exists to effect this change. Visible spectra and electron paramagnetic resonance studies indicate a slightly perturbed low spin Fe(III) (ferricytochrome c) species. Raman spectra for the acyl chain C-H stretching mode region show increased cardiolipin chain disorder and the involvement of the chain cis-double bond regions upon complexation. Interaction of the protein with cardiolipin changes both the porphyrin ring conformation and heme coordination to mimic the reduced cytochrome c system with no electron transfer occurring. (Levin, Vincent)

The membrane effects of ethanol were monitored in model dipalmitoyl-phosphatidylcholine liposomes by spatially resolving Raman spectra across a concentration gradient. Deuterated ethanol was used as the perturbant since its unique spectral signature allows the alcohol concentration to be specified quantitatively. Both Raman spectral frequency and intensity data provided a detailed characterization of the bilayer membrane as the lipid acyl chains pass, as a function of alcohol concentration, from weakly interacting monolayers to a completely interdigitated phase. (Lewis, Levin)

Integrated intensity analysis of spectra obtained by temperature programmed Raman spectroscopy of artificial phospholipid membranes shows that thermal history (prior to spectroscopy) of the specimens determines the course, rate and intensity of configurational alterations associated with the subtransition (crystal to gel state) of 12-18 carbon chain preparations. The more subtle spectral changes within the 2800-3100 cm^{-1} region (CH stretch), indicative of packing characteristics, we feel demonstrate that reorganization of packing occurs by domains rather than randomly. (Adams, Levin)

Time resolved optical spectroscopy with nanosecond lasers and molecular dynamics calculations have been employed to investigate ligand rebinding and conformational changes in hemoglobin subsequent to photodissociation of the carbon monoxide complex. In order to precisely measure the time course of the changes in the conformation of the deoxy photoproduct, which produce small spectral changes, as well as to determine the kinetics of ligand

rebinding, an automated, sensitive nanosecond spectrometer has been developed to measure time-resolved spectra. The spectra have been analyzed using singular value decomposition to produce a set of orthonormal basis spectra and the time course of their amplitudes. With these techniques the kinetics of ligand rebinding and conformational changes have been studied for the alpha subunit of an iron-cobalt hybrid hemoglobin initially in the R or T quaternary structure. The R to T quaternary transition is observed for the completely unliganded R state molecule to occur at about 20 μs, while both R and T state molecules show tertiary conformational relaxations at about 50 ns and 500 ns. The 50 ns relaxation is simultaneous with geminate rebinding, suggesting that it is caused by motion of the ligand out of the heme pocket. Using the simplest kinetic model, a comparison of the geminate kinetics for R and T state molecules indicate that the difference in the factor of about 50 in the overall rate of ligand binding to the R and T states can be explained by differences in binding rates to the heme from within the heme pocket. Changes in the barriers to motion of the ligand inside the protein or between the protein and the solvent appear to play a minor role in determining the difference in overall rates between the two quaternary structures. (Hofrichter, Murray, Henry, Eaton)

Time resolved spectra of trout I hemoglobin following photodissociation of the carbon monoxide complex have been measured as a function of temperature between 2 and 60°C. The results show that the rate of binding of carbon monoxide to the heme from within the heme pocket and the rate of escape of the ligand from the heme pocket into the solvent are essentially temperature independent. The increase in the overall binding rate with increasing temperature must therefore result from an increase in the rate at which the ligand enters the heme pocket from the solvent. The amplitudes of the spectral changes associated with both tertiary and quaternary conformational changes are highly temperature dependent, decreasing with increasing temperature. This decrease could be a spectroscopic effect, resulting from the multifold-degenerate ground state, or it could represent the fact that the R and T structures become more similar at elevated temperatures, as indicated by the decrease in the allosteric equilibrium constant. (Murray, Hofrichter, Henry, Eaton)

The photodissociation process is being simulated using the technique of molecular dynamics, which describes the motion of the individual atoms. Calculations on a complete tetramer in vacuo show that the heme conformation change is a sub-picosecond process and that the excess vibrational energy of the heme is deposited in the surrounding protein in about 20 ps, via channels that appear to increase the temperature of all parts of the protein simultaneously. The trajectories are being analyzed to determine the response of the globin conformation to the change in heme conformation. (Henry, Eaton)

Molecular dynamics simulations of atomic motions in sperm whale myoglobin have also been performed. The simulations predict the existence of multiple distinct conformations accessible to each tryptophan sidechain in the protein. Further analysis has shown that this structural heterogeneity can account for the fluorescence intensity and anisotropy decays observed for the tryptophans in myoglobins from sperm whale and other species. Our final molecular dynamics study has addressed the dissipation into the protein matrix of excess vibrational energy deposited in the heme by photo-excitation. (Henry)

Gas-phase normal mode analyses, that have been used to study the frequencies and amplitudes of collective motions in macromolecules, have been generalized to the liquid phase where frictional forces play a important role. Within the framework of the Langevin equation, the problem has been reduced to solving an eigenvalue equation involving supermatrix constructed from the force constant and friction matrices and computationally convenient expressions have been obtained for the relevant experimentally accessible correlation functions. Preliminary calculations indicate that this approach provides a viable means of determining the influence of solvent on the dynamics of collective motions in macromolecules. The transient electric birefringence (TEB) of polyelectrolytes such as DNA, reflects not only the rotational motion of the macroions but also the dynamics of the surrounding ion atmosphere. By correctly treating the coupling between the rotational and counterion dynamics, rigorous expressions for the TEB when an external electric field is turned on, reversed or oscillates were obtained and used to successfully analyze recent experimental data on short DNA restriction fragments. The theory of the current to microelectrodes with band and ring geometries has been developed and applied to the analysis of electrochemical measurements using such devices. (Szabo, Lamm)

Erythrocyte ghosts, resealed hypotonically and isotonically have been shown by an EPR technique developed in the last few years in this lab, to have markedly different deformability and flow characteristics despite the fact that the ghost shapes (biconcave discoid) are virtually indistinguishable. In the hypotonically resealed ghosts, the cytoskelatal network shows an enhanced segmental mobility that correspond to an altered state of spectrin hetero-dimer association. (Kon, Ito)

The hypothesis that sensory transduction in retinal rods requires the hydrolysis of cytoplasmic 3',5' cyclic guanosine monophosphate (cGMP) has been tested by measuring the heat produced when rods give electrical responses to light flashes. Using a new technique that simultaneously measures the electrical and thermal responses of rods, it has been found that a small pulse of heat is released during transduction. This heat is equivalent to hydrolysis of less than a 2 micromoles of cGMP per liter of rod cytoplasm, a quantity much too small to fit the currently popular model of phototransduction. Other heats associated with activation of the intermediate steps in the chain reactions leading to cGMP hydrolysis have been identified. The driving forces for the sensory dark current of retinal rods under various functional conditions have been measured by improved methods of electron-probe microanalysis. The activity of free calcium ions in rod cytoplasm that is in equilibrium with sodium-calcium exchange has been estimated to be greater that 2 micromolar in dark-adapted rods, a value much higher than free calcium in most neurons. Fluorescent dyes with molecular sizes similar to cGMP diffuse freely within rod outer segments. (Yoshikami, Foster, Hagins)

A quantitative description of the role of gelation in the pathophysiology of sickle cell disease is being formulated to aid in the development of agents that can be used in the treatment of patients. A new laser photolysis technique has been developed to assess the quantitative significance of the delay time of hemoglobin S gelation to the pathophysiology. The saturation at which polymers first form in individual sickle erythrocytes upon deoxygenation is much lower than the saturation at which polymers disappear upon reoxygenation. The results indicate that at

physiological saturations with oxygen, gelation takes place in the large majority of cells at equilibrium, but is prevented from occurring in vivo because the delay times are sufficiently long that most cells return to the lungs and are reoxygenated before polymerization has begun. These techniques are being extended to measure the delay time as a function of saturation on physiological times scales over a wide range of hemoglobin S concentrations and saturations. With this data it will be possible to provide a more accurate description of gelation in vivo. The measurement of the delay time on single cells in these experiments can also be used as a very sensitive method to assess the potential efficacy of agents that are potential drugs for the treatment of sickle cell disease. (Hofrichter, San Biagio, Eaton)

Continuing the study of the excited electronic states of the precursors of multiple conjugated unsaturated molecules, a series of spectroscopic measurements have been initiated with the specific object of determining the states responsible for the observed emission of 1,1,4,4-tetramethyl butadiene, the first diene from which any emission has been observed. The resonant multiphoton ionization (RMPI) of the diene has been measured, the analysis of which shows the molecule, unlike butadiene itself, is not centrosymmetries, the RMPI spectrum is sufficiently different in the region of the long wavelength pi-pi* transitions to indicate that states other than these exist from which the emission takes place. (McDiarmid)

Antiviral and antitumor compounds and inhibitors of enzymes that promote certain types of cataracts have been studied by computerized molecular modeling techniques. Possible three-dimensional structures of the anti-AIDS agent, AZT, have been identified and the transition energies between them have been estimated. (Sharpless)

The enantioselective hydrolysis of esters by the mold Rhizopus nigricans have been examined in the course of developing methodologies for the preparation of chiral alcohols of a predictable configuration. Early studies focused on configurational assignments of the alcohols formed, while recent work has been directed toward quantitative predictions of the enantiomeric excess (e.e.) of the alcohol. To improve the reliability of the e.e. determinations it was necessary to develop an analytical non-optical method. The method adopted involves preparation of a diastereomeric ester which is analyzed by capillary gas chromatography. As it was also important to be able to resolve mg quantities of some alcohols for pharmacological studies, the use of an HPLC based method of separating these diastereomeric esters were also examined. The elution order of enantiomers on a chiral column or diastereomers on achiral columns have been used to make tentative configurational assignments. Similar correlations in the groups of compounds, 2-cycloalkenols and 1,2-benzocycloalken-3-ols, are being investigated. In addition to enzymically mediated hydrolyses, the regio- and stereo-selectivity of a hydroxylating group of enzymes in Beauvaria sulfurescens are being studied. Although alcohols and amines have proved to be poor substrates, the N-phenyl carbamates of several alcohols are hydroxylated. However, for the reaction to be of synthetic utility the low yields of hydroxylated materials had to be increased. Ways of elevating the level of the hydroxylating enzyme and developed a successful approach have been examined. This method will be used to study the regio- and stereo-chemical preferences of the enzyme. In a separate study the structure and stereochemistry of a photodimer obtained from the irradiation of methyl p-nitrocinnamate was assigned from a detailed NMR study of the material. (Ziffer, Hu)

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29001-15-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular dynamics and vibrational characteristics of membrane assemblies

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

P.I. :	Ira W. Levin	Research Chemist	LCP-NIDDK
Others:	Neil E. Lewis	Visiting Associate	LCP-NIDDK
	Peter M. Green	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)* R. Adams, LCP-NIDDK; Clifford J. Steer, LBM-NIDDK; C. Huang, School of Medicine, Univ. of VA; William C. Harris, Natl. Science Foundation; James S. Vincent, Univ. of MD; W.H. Kirchhoff, National Bureau of Standards; S.F. Bush, Univ. of North Carolina; T.J. O'Leary, AFIP

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

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NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

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

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

Vibrational Raman and infrared spectroscopy are used to probe the dynamical, conformational, functional and thermodynamic properties of both model and intact membrane assemblies. Emphasis is placed on elucidating both lipid-lipid and lipid-protein interactions within the membrane bilayer complex. For example, the association between ferricytochrome c, a mobile electron-transfer protein which diffuses between the inner and outer mitochondrial membranes, and cardiolipin was studied using resonance Raman spectroscopic techniques. The observed data exhibit the accepted spectral frequency and intensity markers for ferrocytochrome c. That is, the data imply that the iron atom is reduced, but no obvious reductant exists to effect this change. Visible spectra and electron paramagnetic resonance studies indicate a slightly perturbed low spin Fe(III) (ferricytochrome c) species. Raman spectra for the acyl chain C-H stretching mode region show increased cardiolipin chain disorder and the involvement of the chain cis-double bond regions upon complexation. Interaction of the protein with cardiolipin changes both the porphyrin ring conformation and heme coordination to mimic the reduced cytochrome c system with no electron transfer occurring.

The membrane effects of ethanol were monitored in model dipalmitoylphosphatidylcholine liposomes by spatially resolving Raman spectra across a concentration gradient. Deuterated ethanol was used as the perturbant since its unique spectral signature allows the alcohol concentration to be specified quantitatively. Both Raman spectral frequency and intensity data provided a detailed characterization of the bilayer membrane as the lipid acyl chains pass, as a function of alcohol concentration, from weakly interacting monolayers to a completely interdigitated phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29002-14-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry of natural compounds, and synthetic organic chemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: Ulrich Weiss Research Chemist (Scientist Emeritus) LCP-NIDDK

COOPERATING UNITS *(if any)*
Prof. James M. Cook, Department of Chemistry, University of Wisconsin-Milwaukee

LAB/BRANCH
Laboratory of Chemical Physics

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Office of the Chief

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NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

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

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

In continued cooperation with Prof. J.M. Cook and his coworkers at the University of Wisconsin-Milwaukee, the synthesis of di- and polycyclic ring systems composed of fused cyclopentane rings has been developed further. The approach chosen is based on the ready stereospecific formation of derivatives of cis-bicyclo[3.3.0] octane-3,7-dione (1) from 1,2-dicarbonyl compounds and esters of 3-oxoglutaric acid (the "Weiss reaction").

In 1986, the first synthetic derivative of a particular tetracyclic system has been obtained using this approach. One compound derived from this system has been encountered as a transformation product of a natural terpenoid, but no previous synthetic work in this series seems recorded. The synthesis required solution of two non-trivial problems: (1) modification of only one of the two chemically identical carbonyls in an intermediate of type (1), achieved by selective monoketalization with a bulky diol, and (2) prevention of an undesirable 1,2-migration of a double bond during a Wolff-Kishner reduction by addition of silver carbonate.

A review of the chemistry of natural perylenequinones, written by U. Weiss, L. Merlini, and G. Nasini, has been completed and is in press. It is the first comprehensive review of this class of substances, which includes many powerful photosensitizers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29005-13-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Asymmetric synthesis; structure, stereochemistry and NMR

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

P.I. :	Herman Ziffer	Research Chemist	LCP-NIDDK
Other:	Yulin Hu	Visiting Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

Prof. Marvin Charton, Chemistry Department, Pratt Inst., Brooklyn, N.Y.
Prof. Paul F. Schuda, Chemistry Department, Univ. of Md., College Park, MD

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

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

We have continued to examine the enantioselective hydrolysis of esters by the mold Rhizopus nigricans in the course of developing methodologies for the preparation of chiral alcohols of a predictable configuration. Early studies focused on configurational assignments of the alcohols formed, while recent work has been directed toward quantitative predictions of the enantiomeric excess (e.e.) of the alcohol. To improve the reliability of the e.e. determinations it was necessary to develop an analytical non-optical method. The method adopted involves preparation of a diastereomeric ester which is analyzed by capillary gas chromatography. As it was also important to be able to resolve mg quantities of some alcohols for pharmacological studies, we also examined the use of an HPLC based method of separating these diastereomeric esters. The elution order of enantiomers on a chiral column or diastereomers on achiral columns have been used to make tentative configurational assignments. Similar correlations in the groups of compounds, 2-cycloalkenols and 1,2-benzocycloalken-3-ols, are being investigated.

In addition to enzymically mediated hydrolyses, we are studying the regio- and stereo-selectivity of a hydroxylating group of enzymes in Beauvaria sulfurescens. Although alcohols and amines have proved to be poor substrates, the N-phenyl carbamates of several alcohols are hydroxylated. However, for the reaction to be of synthetic utility the low yields of hydroxylated materials had to be increased. We have examined ways of elevating the level of the hydroxylating enzyme and developed a successful approach. This method will be used to study the regio- and stereo-chemical preferences of the enzyme.

The structure and stereochemistry of a photodimer obtained from the irradiation of methyl p-nitrocinnamate was assigned from a detailed nmr study of the material.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29006-17-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

The structure and dynamics properties of macromolecules

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

P.I. : Elliot Charney Research Chemist LCP-NIDDK

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COOPERATING UNITS *(if any)*

H-H. Chen, George Mason University, Fairfax, VA; E.D. Korn, LCB-NHLBI; Rodney Harrington, University of Nevada, Reno, Nevada; M.A.L. Atkinson, LCB-NHLBI; D.C. Rau, LCB-NIDDK

LAB/BRANCH

Laboratory of Chemical Physics

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Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Macromolecular structure, dynamics and polyelectrolyte properties of large biological polymers, in particular, polynucleotides and nucleic acids are being studied by electric-field induced dichroism and birefringence methods. Theoretical and computational methods supplement the experimental work.

The current research is a response to the fact that the knowledge of the structural effects of specific base-pair sequences on DNA translation and replication is still at a primitive stage. Only one or two biologically significant protein-DNA complexes from which such structural effects could be inferred have been crystallized and their structure determined. Using electro-optic birefringence and dichroism, it is now possible to quantitatively explore DNA structures in solution, albeit with less resolution than x-ray diffraction of crystals, but uninhibited by the problem of forming crystalline complexes. The two principal projects currently being pursued are the structural effects of the triplet sequence CAC/GTG and the flexibility of A form of DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-DK-29007-16-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Structure and interaction of biomolecules

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

P.I. :	Hideo Kon	Research Chemist	LCP-NIDDK
Others:	Yasunori Fukushima	Visiting Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

J.S. Vincent, UMBC; H.M. Fales, CH-NHLBI; J. Verma, Georgetown University; P.F. Kadar, LMOD-NEI

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In this project, we apply various techniques of electron paramagnetic resonance (EPR) to studying biological systems, and also attempt to develop a new mode of applications. Results of collaborative efforts in 1987 include: (a) EPR spectra of ferricytochrome c-cardiolipin complex were analyzed to confirm Fe(III) valence state of cytochrome c in the complex, and to derive the extent of crystal field distortion around iron owing to the perturbation by cardiolipin; (b) EPR studies of a vitamine E oxidative dimer, related to gamma tocopherol, demonstrated that the dimer -O- bond undergoes at room temperature a valence tautomerism with free radical fluxional structures; (c) Germination process of C. albicans was monitored by spin label method regarding changes in membrane fluidity. Solutions to several problems facing labeling of the microbe have been worked out such as, e.g., protoplasting prior to labeling; (d) spin label studies of blood cells from canine with galactosemic cataracts have shown that the generally held notion of a decreased red cell deformability accompanying galactosemia is not supported by the EPR measurements of intracellular viscosity and flow characteristics. In our own project, a new flow EPR technique developed in this project for assessing the degree of cell deformation and orientation in flow was applied to examine the effect of hypotonic treatment of resealed ghost, which is known to cause a shift of spectrin tetramer-dimer equilibrium toward the dimers. The deformability and orientability was found dramatically decreased by such a treatment when compared with those isotonically resealed. Investigation of the motional state in cytoskeletal network by maleimide spin labeling showed an increased degree of segmental freedom in the hypotinc preparations having an altered spectrin association state, as proven also by PAGE of spectrin extracts. The results can be interpreted as evidence for a crucial role played by the spectrin network in determining red cell rheological properties.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29008-16-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Electronic and molecular structural investigations

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

P.I. :	Ruth McDiarmid	Research Chemist	LCP-NIDDK
Others:	Abdol-Hakim Sheybani-Nezhad	Visiting Fellow	LCP-NIDDK
	Andrea Adams	Summer Student	LCP-NIDDK

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Preliminary experiments carried out the previous year suggest that 1,1,4,4-tetramethyl butadiene emits light at two different wavelengths when excited in the spectral region of the pi-pi* absorption. The excitation spectrum for each emission region is the same, but the excitation spectrum differs from the directly measured absorption spectrum. This is the first diene that has been observed to emit.

To characterize (1) the symmetry of, (2) the lifetime of and (3) the wavelength of the emitting state two different types of experiments were either implemented or initiated. The 2 photon resonant multiphoton (RMPI) ionization spectrum was measured over the region of pi-pi* absorption and the adjacent 3p-Rydberg transition. The molecule was observed not to have a strict center of symmetry. The RMPI signal was observed not to correspond to the absorption spectrum. The latter suggests that two different states with significantly different physical properties are contained within the nominal pi-pi* absorption envelope.

Instrumentation for time resolved emission measurements is being developed to enable the two emission wavelength regions to be identified, but these measurements have insufficiently progressed to be reported at this time.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29009-14-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies on sickle cell disease

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

P.I. :	William A. Eaton	Medical Officer	LCP-NIDDK
Others:	James Hofrichter	Research Chemist	LCP-NIDDK
	Pier Luigi San Biagio	Visiting Associate	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A quantitative description of the role of gelation in the pathophysiology of sickle cell disease is being formulated to aid in the development of agents that can be used in the treatment of patients. A new laser photolysis technique has been developed to assess the quantitative significance of the delay time of hemoglobin S gelation to the pathophysiology. The saturation at which polymers first form in individual sickle erythrocytes upon deoxygenation is much lower than the saturation at which polymers disappear upon reoxygenation. The results indicate that at physiological saturations with oxygen, gelation takes place in the large majority of cells at equilibrium, but is prevented from occurring in vivo because the delay times are sufficiently long that most cells return to the lungs and are reoxygenated before polymerization has begun.

These techniques are being extended to measure the delay time as a function of saturation on physiological times scales over a wide range of hemoglobin S concentrations and saturations. With this data it will be possible to provide a more accurate description of gelation in vivo. The measurement of the delay time on single cells in these experiments can also be used as very sensitive method to assess the potential efficacy of agents that are potential drugs for the treatment of sickle cell disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29010-15-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Conformation and electronic structure of biological molecules

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

P.I. :	William A. Eaton	Medical Officer	LCP-NIDDK
Others:	James Hofrichter	Research Chemist	LCP-NIDDK
	Eric R. Henry	Research Physicist	LCP-NIDDK
	Lionel P. Murray	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

Takashi Yonetani and Masao-Ikeda-Saito, University of Pennsylvania School of Medicine; Bernard Brooks, DCRT; Robin M. Hochstrasser, University of Pennsylvania; Maurizio Brunori and Massimo Coletta, University of Rome

LAB/BRANCH

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SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Time resolved optical spectroscopy with nanosecond lasers and molecular dynamics calculations have been employed to investigate ligand rebinding and conformational changes in hemoglobin subsequent to photodissociation of the carbon monoxide complex. In order to precisely measure the time course of the changes in the conformation of the deoxy photoproduct, which produce small spectral changes, as well as to determine the kinetics of ligand rebinding, an automated, sensitive nanosecond spectrometer has been developed to measure time-resolved spectra. The spectra have been analyzed using singular value decomposition to produce a set of orthonormal basis spectra and the time course of their amplitudes. With these techniques the kinetics of ligand rebinding and conformational changes have been studied with hemoglobins initially in the R and T quaternary structure. The R to T quaternary transition is observed for the completely unliganded R state molecule to occur at about 0.02 ms, while both R and T state molecules show tertiary conformational relaxations at about 50 ns and 500 ns. The 50 ns relaxation is simultaneous with geminate rebinding, suggesting that it is caused by motion of the ligand out of the heme pocket. Using the simplest kinetic model, a comparison of the geminate kinetics for R and T state molecules indicate that the difference in the factor of about 50 in the overall rate of ligand binding to the R and T states can be explained by differences in binding rates to the heme from within the heme pocket. Changes in the barriers to motion of the ligand inside the protein or between the protein and the solvent appear to play a minor role in determining the difference in overall rates.

The photodissociation process is being simulated using the technique of molecular dynamics, which describes the motion of the individual atoms. Calculations on a complete tetramer in vacuo show that the heme conformation change is a sub-picosecond process and that the excess vibrational energy of the heme is deposited in the surrounding protein in about 20 ps. The trajectories are being analyzed to determine the response of the globin conformation to the change in heme conformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29011-16-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

The physics and chemistry of photoreception

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

P.I. :	William A. Hagins	Medical Officer	LCP-NIDDK
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	F.M. Hagins	Guest Worker	LCP-NIDDK
	M.C. Foster	Research Physicist	LCP-NIDDK
	P. Ross	Research Chemist	LMB-NIDDK
	K. Spring	Research Med. Officer	LKM-NHI
	R. Tate	Computer Systems Analyst	CSL-DCRT

COOPERATING UNITS *(if any)*

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Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

An investigation of the mechanism of phototransduction in vertebrate photoreceptor cells.

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29012-17-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

The influence of molecular structure on chemical and biological properties

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

P.I. :	Norman E. Sharpless	Research Chemist	LCP-NIDDK
Others:	Ralph G. Adams	Research Physicist	LCP-NIDDK
	William H. Jennings	Research Physicist	LCP-NIDDK

COOPERATING UNITS *(if any)*

Peter F. Kador, EI, LMOP, NIH
Frank Quinn, NCI
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LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

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

Energy minimization calculations and quantum mechanical calculations on compounds of biological and pharmacological interest continue to give insights into and explanations of their modes of behavior, resulting in clues to their pharmacophores.

The inhibition of the enzyme aldose reductase by a wide variety of compounds continues under investigation by QSAR techniques, as well as by energy minimization computations, quantum mechanics and stereochemical considerations. The pertinent factors have now been shown to include also bulk terms.

Energy minimization and quantum calculations have been carried out on the various conformations and colchicine and isocolchicine to correlate binding properties with the energies and structures of their conformations.

Various compounds showing promise against the AIDS virus are being systematically investigated to obtain structural and electronic properties which may help elucidate the mechanism of their action, and thus lead to improved forms. Energetic, structural and electronic properties of AZT have been obtained, as well as rotational barriers due to the base group.

The binding of analogs of colchicine has been investigated further by QSAR methods. In addition to partition coefficients, binding efficiency depends on free energy and molecular volumes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29015-16-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Digital computer facilities for LCP and LMB

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

P.I.: W.H. Jennings, Jr. Research Physicist LCP-NIDDK

COOPERATING UNITS *(if any)*

Computer Systems Laboratory, DCRT: A.R. Schultz, Jr., J.I. Powell, D.C. Carpenter

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The laboratory computer facility serving LCP and LMB was in routine operation during the reporting period. A change to an alternate configuration has begun with the upgrading of the host 11/70 CPU to an 11/84 with 2Mb of memory. The magnetic tape drive was also replaced. Implementation of a workstation network in cooperation with CSL, DCRT has begun with the acquisition of a micro VAX and several SUN machines. This workstation network which uses Ethernet, UNIX and a network file server, will be developed as a parallel system and will not immediately impact operation of the existing facility. Closely related to the workstation network is a project to interconnect all terminals, personal computers and shareable peripherals. This system uses terminal servers on the Ethernet and will provide a bridge between the existing facility and the workstations.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29016-12-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

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

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

P.I. :	James Hofrichter	Research Chemist	LCP-NIDDK
Others:	William A. Eaton	Medical Officer	LCP-NIDDK
	Eric Henry	Research Physicist	LCP-NIDDK
	Pier Luigi San Biagio	Guest Researcher	LCP-NIDDK
	Lionel Murray	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Spectroscopy and Structure

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	

CHECK APPROPRIATE BOX(ES)

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

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

Transient spectroscopy is used to study the kinetics of conformational changes in macromolecules subsequent to excitation with a pulsed laser. Changes in both the tertiary and quaternary structure of hemoglobin have been observed following the photodissociation of carbon monoxide from the hemes.

Steady state photodissociation of carbon monoxide from hemoglobin S is used to study the thermodynamics and nucleation-controlled kinetics of the assembly of deoxyhemoglobin S into polymers. This technique has been used to study hemoglobin S in partially saturated solutions and to obtain delay times for solutions under physiological buffer conditions. Moreover, the kinetics of polymer formation can be monitored as the cell is being desaturated, permitting, for the first time, determination of the distribution of times required for cells to sickle at the saturations comparable to those of venous blood.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29017-08-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Spectroscopic investigation of membrane lipids and models

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

P.I.:	Ralph G. Adams	Research Physicist	LCP-NIDDK
Other:	Ira W. Levin	Research Chemist	LCP-NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

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

Integrated intensity analysis of spectra obtained by temperature programmed Raman spectroscopy of artificial phospholipid membranes shows that thermal history (prior to spectroscopy) of the specimens determines the course, rate and intensity of configurational alterations associated with the subtransition (crystal to gel state) of 12-18 carbon chain preparations. The more subtle spectral changes within the 2800-3100 cm^{-1} region (CH stretch), indicative of packing characteristics, we feel demonstrate that reorganization of packing occurs by domains rather than randomly.

Beginning efforts to understand the mechanics of lung surfactants in Adult Respiratory Distress Syndrome (ARDS), using the techniques above, show that a variety of surfactants, all effective, behave surprisingly differently considering that (theoretically) dipalmitoylphosphatidylcholine is the active ingredient common to all.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29019-07-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Theoretical studies on the dynamic aspects of macromolecular function

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

P.I. :	A. Szabo	Research Chemist	LCP-NIDDK
Other:	G. Lamm	Staff Fellow	LCP-NIDDK

COOPERATING UNITS *(if any)*
D. Eden, Dept. of Chemistry, San Francisco State University
R.M. Wightman, Dept. of Chemistry, Indiana University
D.E. Tallman, Dept. of Chemistry, South Dakota State University

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Laboratory of Chemical Physics

SECTION
Section on Macromolecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

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

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

Gas-phase normal mode analyses, that have been used to study the frequencies and amplitudes of collective motions in macromolecules, have been generalized to the liquid phase where frictional forces play a important role. Within the framework of the Langevin equation, the problem has been reduced to solving an eigenvalue equation involving supermatrix constructed from the force constant and friction matrices and computationally convenient expressions have been obtained for the relevant experimentally accessible correlation functions. Preliminary calculations indicate that this approach provides a viable means of determining the influence of solvent on the dynamics of collective motions in macromolecules. The transient electric birefringence (TEB) of polyelectrolytes such as DNA, reflects not only the rotational motion of the macroions but also the dynamics of the surrounding ion atmosphere. By correctly treating the coupling between the rotational and counterion dynamics, rigorous expressions for the TEB when an external electric field is turned on, reversed or oscillates were obtained and used to successfully analyze recent experimental data on short DNA restriction fragments. The theory of the current to microelectrodes with band and ring geometries has been developed and applied to the analysis of electrochemical measurements using such devices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK-29020-03-LCP

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Nuclear magnetic resonance: new methods and molecular structure determination

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

P.I. : Ad Bax, Visiting Scientist, LCP-NIDDK

Others: Edwin D. Becker, Research Chemist, LCP-NIDDK; Rolf Tschudin, Electronics Engineer, LCP-NIDDK; Laura Lerner, Arthritis Foundation Fellow, LCP-NIDDK; Sankaran Subramanian, Visiting Scientist, LCP-NIDDK; Vladimir Sklenar, Visiting Fellow, LCP-NIDDK; Hong The Ha, Biological Laboratory Aid, LCP-NIDDK; Lou Hughes, Guest Worker, LCP-NIDDK; Daniel Williamson, Summer Student, LCP-NIDDK

COOPERATING UNITS *(if any)*

M.F. Summers, NCDB/FDA; B. Brooks, DCRT

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Laboratory of Chemical Physics

SECTION

Section on Nuclear Magnetic Resonance

INSTITUTE AND LOCATION

NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.9	2.1	2.8

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Earlier developments of new methods for correlating proton chemical shifts with shifts of low-gamma nuclei have been continued. For the first time, it has been shown possible to record proton-carbon and proton-nitrogen shift correlation of small proteins (<15 kD) at natural isotopic abundance. A quite different approach has been developed for correlating proton and phosphorous chemical shifts and applied to the study of oligonucleotides.

New methods have been developed for recording phase-sensitive two-dimensional proton NMR spectra in water solution without the need for presaturation. In contrast to existing techniques, the new methods accomplish the water suppression in two stages: in the first stage a relatively low suppression is obtained, sufficient to overcome dynamic range problems in the receiver, in the second stage phase cycling removes the water signal from the spectrum almost completely. The new methods have been demonstrated for the important NOE, spin-locked NOE and homonuclear Hartmann-Hahn experiments.

A new procedure has been developed for measurement of previously unresolvable coupling constants. By suppressing the effect of all scalar couplings apart from the interaction of interest in a two-dimensional experiment it becomes possible to extract the coupling constants of interest. The procedure has been applied to measurement of J(C3'H-O-P) couplings in the oligonucleotide d(CGCGAATTCGCG)2. The corresponding dihedral epsilon angles show significant differences with X-ray crystallographic work.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-DK-29021-02-LCP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conformation and dynamics of biological macromolecules

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

P.I. : Eric R. Henry Research Physicist LCP-NIDDK

Other: William A. Eaton Medical Officer LCP-NIDDK

COOPERATING UNITS *(if any)*
Robin Hochstrasser, University of Pennsylvania
Bernard Brooks, DCRT

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Laboratory of Chemical Physics

SECTION
Section on Macromolecular Biophysics

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

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

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

We have performed transient spectroscopic studies on component I of trout hemoglobin over a wide range of temperatures. We have found that the rates and amplitudes of the spectral changes in the photolyzed deoxy hemes attributed to tertiary and quaternary changes in the protein are all strongly temperature dependent. We have also found that the amplitude of geminate rebinding of carbon monoxide to trout I hemoglobin decreases with increasing temperature, whereas the rate of this process is temperature independent. A simple analysis of these results suggests that the temperature dependence of the ligand rebinding properties of this protein is associated primarily with the entry of the ligand into the protein. We have simulated the photodissociation of bound ligands from the hemoglobin tetramer using molecular dynamics in order to probe the structural responses of the system to ligand dissociation. The heme conformational change, with the iron atom moving out of the mean plane of the heme atoms, appears to take place on a sub-picosecond time scale, consistent with our earlier simulations of isolated subunits of the protein. Preliminary analysis of the tetramer simulation shows no evidence of conformational responses of the protein to ligand dissociation which might be related to the known quaternary structural change. We have also performed molecular dynamics simulations of atomic motions in sperm whale myoglobin. The simulations predict the existence of multiple distinct conformations accessible to each tryptophan sidechain in the protein. Further analysis has shown that this structural heterogeneity can account for the fluorescence intensity and anisotropy decays observed for the tryptophans in myoglobins from sperm whale and other species. Our final molecular dynamics study has addressed the dissipation into the protein matrix of excess vibrational energy deposited in the heme by photo-excitation. This study predicts that the excess heme energy passes into the protein on a time scale of tens of picoseconds, via channels that appear to increase the temperature of all parts of the protein simultaneously.

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

ANNUAL REPORT OF THE LABORATORY OF BIOORGANIC CHEMISTRY

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION ON PHARMACODYNAMICS

Pharmacologically Active Compounds from Amphibians and Other Natural Sources

Alkaloids from Amphibians. Cutaneous granular glands are a shared character of adult amphibians, including caecilians, and are thought to be the source of most biologically active compounds in amphibian skin. Many species contain unidentified substances judged to be noxious based on predator aversion or human taste. Additionally, there is a great diversity of known compounds, some highly toxic as well as noxious, which can be tabulated under four broad categories: biogenic amines, peptides, bufodienolides (bufogenins) and alkaloids. The last category includes alkaloids derived from biogenic amines, water-soluble alkaloids (tetrodotoxins) and lipophilic alkaloids. Most compounds are known only from skin of adult amphibians, but the toxic and noxious properties of eggs and larvae of certain salamanders and toads can be attributed to tetrodotoxins and bufodienolides, which occur also in adult tissues other than skin. Predator aversion and various antipredator behaviors and aposematic colorations clearly prove the defensive value of these diverse metabolites, whether or not they are elaborated primarily (e.g. alkaloids) or secondarily (e.g. some peptides and biogenic amines) for this function. Lipophilic alkaloids include the samandarine alkaloids, known definitely only from an Old World genus of salamanders, and the more than 200 dendrobatid alkaloids. Nearly all the latter are unique to neotropical poison frogs of the genera Dendrobates and Phyllobates (Dendrobatidae), except for seemingly homoplastic occurrences of a few such alkaloids in small brightly colored anurans of several other families. Owing to recent discoveries and new structural information, the dendrobatid alkaloids are here partitioned among the following major and minor classes: batrachotoxins, histrionicotoxins, indolizidines, pumiliotoxin-A class and its allopumiliotoxn and homopumiliotoxin subclasses, decahydroquinolines, gephyrotoxins, 2,6-disubstituted piperidines, 2,5-disubstitued pyrrolidines, pyridyl-piperidines, indole alkaloids, azatricyclododecenes and amidine alkaloids, all the above contain a piperidine ring. A large number of piperidine-based alkaloids occur mainly as trace compounds in Dendrobates and remain unclassified; the only water-soluble toxin so far discovered in a dendrobatid (Colostethus) is structurally unknown, but conceivably an alkaloid.

Effect of Local Anesthetics on the Dissociation Rate of [^{3}H]Batrachotoxin-B Benzoate from Binding Sites on Sodium Channels. The steroidal alkaloid, batrachotoxin (BTX), and an equipotent benzoate analog (BTX-B) modify the properties of voltage-dependent sodium channels by binding specifically with high affinity to a single site associated with the channel. High affinity binding of [^{3}H]BTX-B is optimally achieved in the presence of a scorpion toxin-induced shift of the channel to an open or conducting configuration. Other sodium channel specific toxins, such as veratridine, aconitine, and grayanotoxin, competitively inhibit the specific binding of [^{3}H]BTX-B, as do a large number of local

anesthetics. The inhibition by local anesthetics is, however, not simple competition for the BTX-binding site, but is due to an allosteric change in the site. The consequence of this change is a reduction in the affinity of the site for BTX-B as evidenced by a marked increase in the dissociation rate of [^{3}H]BTX-B in the presence of local anesthetics. The dissociation rate for [^{3}H]BTX from sodium channels in synaptoneurosomes from guinea pig cerebral cortex has a $T_{1/2}$ = 31.2 min. measured in the presence of excess unlabeled BTX-B. Unlike the action of local anesthetics, true competitive ligands, such as veratridine or aconitine, do not increase the $T_{1/2}$ for [^{3}H]BTX-B beyond the rate achieved with BTX-B alone. By this criteria, reserpine is a true competitive ligand for the [^{3}H]-BTX-B binding site (K_i = 1.2 μM). A structure-activity study of a series of reserpines defines some structural features essential to binding to the [^{3}H]BTX-B site. Analysis of favored conformations of BTX-B, veratridine, aconitine, and reserpine suggest that these competitive ligands have certain structural elements in common, including a triad of oxygens, which when matched for these four compounds, produce similar allignments of the phenyl ring of the aryl ester function in each compound.

Putative Finding Site for Local Anesthetics on the Sodium Channel: In a attempt to characterize the putative binding site(s) for local anesthetics the isothiocyanate derivatives of procaine and proparacaine have been prepared. The isothiocyanate function was chosen as the alkylating moiety for procaine and proparacaine since this group reacts rapidly with amines and thiols, but is essentially inert with respect to water. Conversion of procaine and proparacaine to the corresponding isothiocyanate was easily accomplished by reaction with thiophosgene in a biphasic chloroform-aqueous sodium bicarbonate system. The isothiocyanate derivatives were found to irreversibly inhibit the specific binding of [^{3}H]BTX-B and to markedly accelerate the dissociation rate of [^{3}H]BTX-B from sodium channels in a synaptoneurosome preparation from guinea pig cerebral cortex. Scatchard analysis of the specific binding of [^{3}H]BTX-B in the presence of the isothiocyanate derivatives yielded classical noncompetitive kinetics with no change in the K_d value for BTX-B (.35 nM) and a progressive decrease in the BTX-B bound per mg protein. Dixon plots of [^{3}H]BTX-B bound (picomole/mg protein)$^{-1}$ versus inhibitor concentration yielded classical noncompetitive kinetics the isothiocyanate derivatives of procaine and proparacaine yielded apparent K_i values were 13.5 and 0.08 μM respectively. Similar studies with the parent compounds procaine and proparacaine yielded competitive kinetics with K_i values of 40 and 1.4 μM.

Mechanism of Cardiac Stimulation by Pumiliotoxins: The cardiotonic activities of pumiliotoxins, pyrethroids and sodium and calcium channel activators were assessed *in vitro* with spontaneously beating guinea pig atria. The ability of these compounds to stimulate phosphoinositide turnover was assessed in guinea pig cerebral cortical synaptoneurosomes. The activity of pumiliotoxins for both cardiotonic activity and phosphoinositide breakdown was strongly dependent on te structure and configuration of the side chain and there was a correlation between structure and activity in the two systems. Pyrethroids that had cardiotonic activity also induced phosphoinositide breakdown. Other sodium channel and calcium channel activators that induced phosphoinositide breakdown also were cardiotonic. It is suggested that phosphoinositide breakdown leading to inositio phosphates and diacylglycerides may represent a mechanism underlying the cardiotonic effects of certain agents. A phorbol ester, phorbol 12-myristate-13-acetat that mimics the activation of protein kinase C elicited by diacylglycerides, had cardiotonic activity.

Piperidines as Noncompetitive Blockers of the Nicotonic Acetyl Choline Receptor Channel Complex. The interactions of eight piperidine derivatives with nicotinic receptor complexes from Torpedo californica electric organ were studied using [^{125}I]alpha-bungarotoxin as a probe for the acetylcholine binding site and [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) as a probe for a site associated with the receptor-gated ion channel. Cis- and trans-2-methyl-6-n-undecanyl piperidines (MUP), major consituents of fire ant venom, had a high affinity for [^{3}H]H_{12}-HTX binding sites (Ki 0.1 - 0.24 μM) but had no effect on receptor binding. Affintiy of MUP isomers for [^{3}H]H_{12}-HTX binding sites was approximately doubled in the presence of 1 μM carbamylcholine. Introduction of a 2'-hydroxyl group to the undecanyl side channel had little effect on activity. The 2,6-dimethylpiperidine but not the 3,5-dimethylpiperidine was a moderately active inhibitor of [^{3}H]H_{12}-HTX binding (Ki = 9 μM). 2-Methylpiperidine was considerably less active (Ki = 600 μM), although it was more potent than either 3- or 4-methylpiperidine. The affinities of 2,6-dimethylpiperdine and 2-methylpiperdine for [^{3}H]H_{12}-HTX binding sites were decreased in the presence of 1 μM carbamylcholine. Carbamylcholine affinity for the receptor was increased by up to 7 fold in the presence of 10 and 32 μM MUP, but was decreased in the presence of 2,6-dimethylpiperidine and 2-methylpiperidine. These studies establish the importance of alkyl substitutions in the ortho position of the piperidine ring in conferring ion channel specificity, and the importance of at least one long alkyl side chain in conferring the ability of channel blockers to stabilize the nicotinic receptor complex in high affinity "desensitized" conformations.

Marine Natural Products. Bioassay-directed analysis of a New Zealand sponge of the genus Mycale (family Mycalidae, order Poecilosclerida) led to the isolation of mycalamide a compound with potent in vivo antitumor and antiviral properties. The structure solved by a combination of one- and two-dimensional NMR techniques, and by comparison with the insect toxin pederin.

Mechanism of Myotonic Activity of Pumiliotoxin B: The mechanism of the twitch potentiating action pumiliotoxin-B (PTX-B), an indolizidine alkaloid from the skin of the frog Dendrobates pumilio, was delineated with frog skeletal muscles. PTX-B potentiates and prolongs the muscle twitch by actions on both the nerve terminal and on muscle fibers. In the presence of PTX-B, a single stimulus to the muscle produced either a burst of repetitive action potentials superimposed on a depolarizing after potential or a single potential with a prolonged after-potential at junctional as well as extrajunctional regions of the frog skeletal muscle fibers. The alkaloid did not cause repetitive activity in quiescent cells nor did it cause spontaneous contractions. The typical pattern of repetitive action potentials and post-burst depolarization induced by PTX-B could be mimicked by depolarizing the muscle membrane with current pulses of long duration. Lowering the external calcium or sodium concentration reduced the ability of PTX-B to initiate repetitive action potentials, while a low external chloride concentration had no effect. The frequency of miniature endplate potentials (MEPPs) evoked by potassium, but not the spontaneous MEPP frequency, was increased by PTX-B, suggesting a selective effect on evoked transmitter release. PTX-B evoked repetitive endplate potentials in response to a single stimulus applied to the nerve, which were dependent upon the external calcium ion concentration. PTX-B enhances depolarization-evoked release of transmitter from nerve terminals even in the presence of tetrodotoxin. The effects of PTX-B are

likely to be the result of both a reduction in sodium conductance inactivation, which in turn affects both calcium influx and subsequent intracellular mobilization of calicium, and a direct activation of calcium channels.

Amphibian Alkaloids from Australian Frogs. The structures of three tricyclic tryptamine monoterpene alkaloids from the Australian burrowing frog Pseudophryne coriacea have been elucidated. Additional alkaloids of this class have been detected. The structures are reminiscent of physostigmine. In addition, extracts contain isomers of pumiliotoxin B and related alkaloids, one of which is many fold more potent in cardiac and other systems than pumiliotoxin B.

SECTION ON PHARMACODNAMICS

Nicotinic and Muscarinic Acetylcholine Receptor Agonists.

Isoarecolone methiodide has been shown to be a highly potent agonist in all of the assay systems described above (usually 10-50 times more potent than carbamylcholine and ca. 2 times more potent than ACh itself, depending on the assay). Interestingly, this ligand causes much less neuromuscular blockade, primarily due to desensitization, than do other known nicotinic agonists. Isoarecolone hydrochloride produced nicotine-like behavioral effects in rats. Structural analogs of isoarecolone are also potent nicotinic agonists, but not of the magnitude of the parent isoarecolone. Isoarecolone and related compounds showed moderate to weak muscarinic activity in comparison to ACh, carbamylcholine and arecoline methiodide.

SECTION ON PHARMACODYNAMICS

Pharmacology and Metabolism of Biogenic Amines and Related Compounds

Localization of COMT and Catecholestrogens in the parotid and pituitary gland of rat. Light and electron microscopic immunocytochemical observation of catechol estrogen localization in the posterior lobe of the rat pituitary gland was undertaken with a specific antibody to 2-hydroxyestrone coupled to bovine serum albumin and the peroxidase-antiperoxidase technique. Immunoreactive deposits were found in the pituicytes mainly in the peripheral part. The extended catechol estrogen-positive processes of the pituicytes enclosed or made contact with adjacent axon terminals and free extended catechol estrogen-positive processes were often found in the perivascular space. These results suggest that catechol estrogen may be involved in the regulation of neuroendocrine functions in the posterior lobe.

Catechol-O-methyltransferase (COMT) (ECT 2.1.1.6) and catechol-estrogen were localized in the parotid gland of rat using immunocytochemical methods. Specific immunoreactive deposits for COMT and catecholestrogen were found in the cytoplasm duct cells, but only those of COMT in myoepithelial cells. The pattern of localization of COMT and catecholestrogen in the parotid gland suggests a functional relationship between COMT and catechol-estrogen.

Localization and enzymatic activity of COMT in the endometrium of the Golden Hamster during implantation. The temporal changes in the endometrium of the Gold

Hamster during implantation are well characterized. This species was chosen to examine the time course and localization of COMT and catechol estrogen during implantation. Micromethods for measuring catechol estrogens and COMT activity based upon electrometric detector systems coupled to HPLC were developed with the required sensitivity for small tissue specimens (1-3 mgs). Post fertilization samples were collected at 61, 79, 85, 91 and 109 hours to correspond to the known changes in implantation status. Endometrial samples were taken at implantation sites and intersites. Pontine blue injections were utilized to positively identify implantation sites. Samples of uterus (myometrium), cervix, fallopian tubes, ovary, kidney and liver were also obtained. Preimplantation blastocysts were also isolated. Preliminary results indicate that cells at implantation sites by 85 hours contained elevated COMT activity while the activity in interimplantation endometria was neglible. Catecholestrogen concentrations were highest in the blastocysts themselves.

Adrenergic Properties of 2- and 6-fluoroepinephrines: We have extended our studies on the effects of aromatic fluroine substitution on the chemical and biological properties of catecholamines to epinephrine. 2- and 6-fluoroepinephrine (2-FEpi, 6-FEpi) were synthesized by a sequence of N-formylation, hydride reduction and hydrogenolysis of previously synthesized fluorinated dibenzyloxy-phenethanolamines. Similar to the dramatic change in adrenergic selectivity seen with norepinephrine (Science, 204, 1217, 1979; J. Med. Chem. 22, 1493, 1979), fluorine substitution on the 2- or 6- carbon of the aromatic ring alters the selectivity of epinephrine towards alpha- and beta-adrenergic receptors. Thus, 2-FEpi is a relatively specific beta-adrenergic ligand while 6-FEpi is a relatively specific alpha-adrenergic ligand. However, unlike the effect on norepinephrine but similar to the effect on phenylephrine (J. Med. Chem. 29, 1982, 1986) fluorine substitution can markedly increase the potency of epinephrine as well as induce selectivity. Thus 2-FEpi shows a 3-fold increase in affinity relative to epinephrine for beta-receptors as well as a greatly reduced affinity towards alpha receptors. 6-FEpi, on the other hand, not only has a greatly reduced affinity for beta-receptors, but shows a 3-fold increase in affinity towards alpha-1 receptors. While selective for alpha-2 receptors, 6-FEpi is equipotent with epinephrine, suggesting that the increase in potency observed with 6-FEpi is specific for alpha-1 receptors. We previously reported that the alpha selective agonist 6-fluorophenylephrine showed a 2-fold increase in potency relative to phenylephrine for both alpha-1 and alpha-2 receptors.

DISPLACEMENT OF ALPHA- AND BETA-SPECIFIC LIGANDS
FROM RECEPTORS IN MEMBRANES FROM RAT CEREBRAL CORTEX.

Agonist	Alpha-1 [^{3}]WB4101	Alpha-2 [^{3}H]Clonidine	Beta [^{3}H]Dihydroalprenolol
(-)Epi	4.8uM	9.1nM	6.0uM
(±)2-FEpi	76.	110.	3.5
(±)6-FEpi	3.2	14.	130.

The effect of fluorine substitution on the anodic oxidation of catecholamines and amino acids. The electronchemical behavior of the 2-, 5-, and 6-fluoro analogs of dopamine, norepinephrine, and 3,4-dihydroxyphenylalanine have been determined by cyclic voltammetry and by measuring fluoride release during bulk oxidations. At pH 7.4, the order of increasing redox potentials (E 1/2) for the DA series is 6-FDA < DA < 5-FDA < 2-FDA; for the NE series, 6-FNE < 5-FNE < 2-FNE < NE; and for the DOPA series, 6-FDOPA < 5-FDOPA =

2-FDOPA < DOPA. The 6-fluoro analog in each series of compounds is the most easily oxidized and appears to result from a two electron process rather than the 4-electron process (the ECE pathway) for the parent catecholamines or catecholamino acid. Potentiometric measurement with a fluoride ion specific electrode confirms that oxidation of the 6-fluoro analogs in each series results in a substantial release of fluoride ion. Molecular schemes for the rationalization of the unique behavior of the 6-fluoro analogs are presented.

The uptake, metabolism and cytotoxicity of Fluorine substituted DOPA's and Tyrosines in cultured PC12 cells. Cell cultures of PC12 cells derived from a pheochromocytoma were shown to be sensitive to increasing concentrations of Fluoride i Concentrations of Fluoride ion in the media of 10^{-4}M proved to be cytotoxic. Both and 2,6-difluorodopa were also cytotoxic at concentrations of 10^{-5} to 10^{-4}M and bo were shown to give rise to fluoride ion in the media. Uptake studies were perform by measuring the inhibition of ^{4}C-tyrosine uptake (Km = .03 μM, V_{max} = 0.36 nmol/m While the 2- and 2,6-fluoro DOPA's inhibited tyrosine uptake, melanin-like polymerization interfered with the measurements. Similar studies with 2- and 2,6-difluorotyrosine were carried out. The tyrosine derivatives both competitively inhibited tyrosine uptake yielding Ki values of 13 and 36 μM. HPLC analysis of PC12 cell cultures after 3 hr exposure to the fluorotyrosines indicated the presence of compounds which were tentatively identified as 2- and 2,6-difluorodopa. Fluoride ion began to appear in the media within 30 min following incubations with the fluorotyrosines. Clear evidence for cytotoxicity, presumably from fluoride release from fluorodopa's, was apparent after 24 hours.

SECTION ON PHARMACODYNAMICS

Cyclic Nucleotides and Other Second Messengers in the Nervous System

Phorbol Esters and Cyclic AMP-generation: Activation of protein kinase C by phorbol esters, such as phorbol-12-myristate-13-acetate (PMA), modulates responsiveness of the cyclase system in many cell types. In the neuroblastoma-hybrid cell line NCB-20 PMA causes a reduction in receptor-mediated acumulation of cyclic AMP. The reduction in receptor responses by PMA occurs within 3 min and is still apparent at 40 min. This occurs in a concentration dependent manner with an EC_{50} for PMA of approximately 30 nM. Accumulations of cyclic AMP that are elicited by prostaglandin E_2, vasoactive intestinal peptide or 2-chloroadenosine are decreased in the presence of PMA. Accumulations of cyclic AMP that are elicited by forskolin in the absence of a receptor agonist are unaffected by the presence of PMA. Inhibition of cyclic AMP generation by dopamine is not diminished by PMA suggesting the receptor-input through the inhibitory N_i-guanyl nucleotide binding protein is still functional after PMA treatment. The generalized inhibition of receptor-mediated responses by PMA could be due to a protein kinase C-mediated phosphorylation of the stimulatory N_s-guanyl nucleotide binding protein, but other mechanisms are possible.

Effects of Receptors Agonists, Sodium-channel Agents, and Ionophores on Phosphoinositide Breakdown in Synaptoneurosomes: Carbamylcholine, norepinephrine, histamine and glutamate stimulate the formation of [^{3}H]inositol phosphates in [^{3}H]inositol-labelled guinea pig synaptoneurosomes obtained from cor-

tex, striatum and hippocampus. Synaptoneurosomes prepared from cerebellum do not respond to receptor agonists. Agents that would enhance the influx of sodium ions through voltage-sensitive channels, such as batrachotoxin, scorpion venom and pumiliotoxin B, or a sodium ionophore, monensin, stimulate the formation of [^{3}H]inositol phosphates in synaptoneurosomes from all four brain regions. Neither calcium channel blockers nor receptor antagonists reduce the responses to batrachotoxin. Ionomycin, a calcium ionophore, also stimulates the formation of [^{3}H]inositol phosphates in synaptoneurosomes from all four brain regions. A phorbol ester inhibits formation of [^{3}H]inositol phosphates elicited by either receptor agonists or by sodium channel agents. The major [^{3}H]inositol-labelled lipid in synaptoneurosomes is phosphatidylinositol as analyzed by thin layer chromatography. While the carbamylcholine-elicited hydrolysis of phosphatidylinositol results in an increase of lipid labelling with [^{3}H]inositol, sodium channel agents cause a decrease in incorporation of [^{3}H]inositol. The results indicate that intracellular sodium may have a regulatory role in phosphatidylinositol turnover, and that unlike the receptor-mediated responses this regulation is present in all brain regions.

Sodium Influx and Phosphoinositide Breakdown in Synaptoneurosomes: Agents that increase intracellular concentrations of Na^+ stimulate phosphoinositide breakdown in guinea pig cerebral cortical synaptoneurosomes. When combined, these agents did not have additive effects on phosphoinositide breakdown, but did have additive or greater than additive effects with carbamylcholine. Scorpion venom (Leiurus quinquestriatus) and pumiliotoxin B, which induce small increases in influx of $^{22}Na^+$ in synaptoneurosomes, stimulate phosphoinositide breakdown by about 3- and 6-fold respectively; both effects are inhibited by tetrodotoxin (TTX). Batrachotoxin (BTX) and veratridine (VT), which cause a large increase in influx of $^{22}Na^+$ through activation of voltage-dependent sodium channels, induce a 5- to 6-fold, dose-dependent increase in phosphoinositide breakdown, which appears competitively inhibited by 5 μM TTX. BTX- and VT-elicited influx of $^{22}Na^+$ into synaptoneurosomes is virtually completely blocked by 5 μM TTX. Agents that block voltage-dependent calcium channels such as D-600, nifedipine and Co^{2+}, do not inhibit either influx of $^{22}Na^+$ or stimulation of phosphoinositide breakdown elicited by scorpion venom, pumiliotoxin B or BTX. Cadmium ions (200 μM), which are known to block TTX-resistant sodium channels, block phosphoinositide breakdown induced by agents that activate sodium influx through sodium channels. Cadmium blocks BTX-induced phosphoinositide breakdown with an IC_{50} value of 48 μM, while blocking BTX-induced $^{22}Na^+$ influx in synaptoneurosomes with a 13-fold lower potency (IC_{50}: 610 μM). In the presence of 0.5 μM TTX, the IC_{50} for inhibition of BTX-induced influx of $^{22}Na^+$ by cadmium is now 430 μM. Neither TTX- nor cadmium antagonize neurotransmitter- or monensin-indued phosphoinositide breakdown. It appears that BTX-induced phosphoinositide breakdown in guinea pig synaptoneurosomes is dependent primarily on activation of TTX-resistant, cadmium-sensitive sensitive sodium channels that account for only a small fraction of the total sodium influx induced by BTX in synaptoneurosomes. However, cadmium may also in some way inhibit phosphoinositide breakdown elicited by sodium channel agents at a point subsequent to sodium influx.

Adenosine Receptors: Structural Analysis of Agonist Activity: A series of 63 adenosine analogs were investigated as agonists for the A_1 adenosine receptors that mediate inhibition of adenylate cyclase activity in rat fat cells and for the A_2 adenosine receptors that mediate stimulation of adenylate cyclase in rat pheochromocytoma PC12 cells and human platelets. The lack of correspondence between the

structure-activity relationships of these analogs at the A_1 and A_2 receptors appear definitive in terms of establishing the existence of A_1 and A_2 subclasses of adenosine receptors. However, there are also significant differences in the agonist profiles at A_2 adenosine receptor subclass. Whether such differences are due to different species or different cell types is not known. A set of adenosine analogs, such as N^6-cyclo-hexyl-, N^6-R-, and N^6-S-(1-phenyl-2-propyl) adenosines, 5'-N-ethyl-carboxamidoadenosine and its N^6-cyclohexyl derivative, 2-chloroadenosine, and 2-phenylaminoadenosine, appear to represent a series of analogs useful for pharmacological characterization of A_1 and A_2 classes of adenosine receptors.

Adenosine Receptors: Structural Analysis of Non-xanthine heterocycles as antagonists: A variety of non-xanthine heterocycles are antagonists of binding of [^{3}H]phenylisopropyladenosine to rat brain A_1-adenosine receptors and of activation of adenylate cyclase via interaction of N-ethylcarboxamidoadenosine with A_2-adenosine receptors in human platelet and rat pheochromocytoma cell membranes. The pyrazolopyridines tracazolate, cartazolate and etazolate are several fold more potent than theophylline at both A_1 and A_2-adenosine receptors. The pyrazolopyridines are, however, still many fold less potent than 8-phenyltheophylline and other 8-phenyl-1,3-dialkyl-xanthines. A structurally related N^6-substituted 9-methyladenine is also a potent adenosine antagonist with selectivity for A_1 receptors. None of several aryl-substituted heterocycles, including a thiazolopyrimidine, imidazopyridines, benzimidazoles, a pyrazoloquinoline, a mesoionic xanthine analog and a triazolopyridazine exhibit the high potency typical of 8-phenyl-1,3-dialkylxanthines. A furyl-substituted triazoloquinazoline is very potent at both A_1 and A_2 receptors. A pteridin-2,4-dione, 1,3-dipropyllumazine, is somewhat less potent than theophylline at A_1 and A_2-adenosine receptors, while 1,3-dimethyllumazine is much less potent. A benzopteridin-2,4-dione, alloxazine, is somewhat more potent than theophylline. Other heterocycles with antagonist activity are the dibenzazepine carbamazepine and beta-carboline-3-ethyl carboxylate. The phenylimidazoline clonidine has no activity, while a related dihydroxyphenylimidazoline is a weak non-competitive adenosine antagonist.

N^6-substituted 9-methyladenines: a new class of adenosine receptor antagonist: A series of 15 N^6-substituted 9-methyladenines have been asessed as antagonists of A_2-adenosine receptor-mediated stimulation of adenylate cyclase in membranes of human platelets and rat PC12 cells and of A_1-adenosine receptor-mediated inhibition of adenylate cyclases in membranes of rat fat cells and as inhibitors of binding of N^6-R-[^{3}H]phenylisopropyladenosine to A_1-adenosine receptors in rat brain membranes. N^6 substitution can markedly increase the potency of 9-methyladenine at A_1 receptors, while having lesser effects or even decreasing potency at A_2 receptors. Effects of N^6-substituents on adenosine receptor activity of the 9-methyladenines are reminiscent of effects of N^6-substituents on activity of adenosine, suggesting that N^6-substituted 9-methyladenines bind to adenosine receptors in the same orientation as do N^6-substituted adenosines. N^6-Cyclopentyl-9-methyladenine with values at the A_1 receptors of 1.3 μM (fat cells) and 0.5 μM (brain) is at least 100-fold more potent than 9-methyladenine (K_i 100 μM, both receptors), while at the A_2 receptors K_B values of 5 μM (platelets) and 25 μM (PC12 cells) make it 5-fold more potent and equipotent, respectively, compared to 9-methyladenine (K_B 24 μM, both receptors). N^6-Cyclopentyl and several other N^6-alkyl and N^6-cycloalkyl analogs are selective for A_1 receptors, while 9-methyladenine is the most A_2 receptor selective antagonist. The N^6-R- and N^6-S-(1-phenyl-2-propyl)-9-methyladenines, analogous to N^6-R- and N^6-S-phenylisopropyladenosines, exhibit

stereoselectivity at both A_1 and A_2 receptors. Marked differences in potency of certain N^6-substituted 9-methyladenines at the A_2 receptors of human platelets and rat PC12 cells provide evidence that these are not identical receptors.

Cardiovascular Effects of Adenosine Antagonists: Caffeine, which is a nonselective adenosine receptor antagonist and 7-methyl-1,3-dipropylxanthine (MDPX), which is an A_2-selective antagonist, and a 1,3-dipropyl-8-phenylxanthine amine congener (XAC), which is an A_1 selective antagonist, were evaluated for in vivo selectivity at A_1 vs. A_2 adenosine receptors. Blockade of the negative chronotropic effect of bolus intravenous injections of 2-chloroadenosine, R-phenylisopropyladenosine and N-ethylcarboxamidoadenosine was utilized as an index of antagonism at A_1 receptors; blockade of the hypotensive effect of the same series of adenosine agonists was used as an index of activity at A_2 receptors. In addition, blockade of the potentiating effect of adenosine on the hypertensive and chronotropic effects of nicotine was studied to assess further the role of A_1 and A_2 adenosine receptors in this response. The potent antagonist XAC displayed considerable A_1 selectivity as demonstrated by blockade of adenosine receptor-mediated bradycardia at doses 5 to 10-fold lower than those antagonizing adenosine receptor-mediated hypotension. XAC also selectively blocked potentiation by adenosine of the positive chronotropic effect of nicotine, at doses which had minimal effects on the enhancement of the hypertensive effect of nicotine. The caffeine analog MDPX exhibited A_2 selectivity as demonstrated by prevention of adenosine receptor-mediated hypotension at doses that only minimally attenuated the bradycardiac effect of adenosine analogs. Caffeine displayed no selectivity for A_1 vs. A_2-adenosine receptors. The results indicate that MPDX and XAC will be useful for in vivo delineation of the receptors involved in physiological functions of adenosine.

SECTION ON OXIDATION MECHANISMS

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

Previous annual reports from this Section have described a systematic approach to the study of the cytotoxicity, mutagenicity and carcinogenicity of several polycyclic aromatic hydrocarbons. Briefly, these studies have consisted of i) synthesis of as many known and potential oxidative metabolites as was possible, ii) study of the metabolism of the hydrocarbons with these authentic standards in hand, iii) testing these compounds for cytotoxic and mutagenic activity with bacterial and mammalian cells both in the presence and in the absence of added drug metabolizing systems such as cytochrome P-450 and epoxide hydrolase, iv) identification of products formed by covalent addition of these reactive metabolites to biological macromolecules such as DNA and v) evaluation of the carcinogenicity of the synthesized metabolites in several animal models. These studies provided evidence which indicated that bay-region diol epoxides, formed by enzymatic epoxidation of trans-dihydrodiols, are the most potent carcinogenic metabolites of these hydrocarbons. We formulated the "bay-region" theory which predicts that diol epoxides that have the epoxide group in the bay region of the hydrocarbon will be the most chemically reactive and presumably biologically active diol epoxides from hydrocarbons that are tumorigenic. To date studies from our laboratory as well as several other laboratories around the world have either proved or implicated bay-region diol epoxides as ultimate carcinogens formed from benzo(a)pyrene, benz(a)anthracene, benz(c)acridine, 7-methylbenz(a)anthracene, 7-methylbenz(c)acridine, benzo(b)fluoranthene,

7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, dibenz(a,h)anthracene, two dibenzpyrenes, chrysene, 5-methylchrysene, benzo(c)phenanthrene, and certain methylated cyclopentaphenanthrenes. The theory has stimulated considerable researc in the field, all of which has supported our initial concepts. To date, there are significant known exceptions.

Aspects of hydrocarbon-induced carcinogenesis which the bay-region theory made attempt to take into account include effects of the relative and absolute stereochemistry of ultimate carcinogens, as well as the conformation of the hydroxyl grou on the benzo ring of the diol epoxides. Many of our current efforts address these questions. Studies of the dihydrodiols and resultant bay-region diol epoxides form from benz(a)anthracene as well as phenanthrene and chrysene by liver microsomes hav shown that these molecules are all superimposable with the corresponding benzo(a)pyrene metabolites when their bay regions are aligned. In the benzo(a)pyrene case, only one of four stereoisomeric bay-region 7,8-diol-9,10-epoxides exhibits strong tumorigenic activity, namely the predominant metabolically formed isomer. Tumor studies have shown that the related stereoisomer (R,S-diol-S,R-epoxide) is also the most active form from chrysene, benz(a)anthracene and benzo(c)phenanthrene. Result of the present studies are suggestive that there is a highly enantioselective site with which these carcinogens interact within the cell. Studies are in progress tha will further define the steric constraints of the active site of cytochrome P-450c, the principal oxidative enzyme responsible for the conversion of polycyclic aromat hydrocarbons to ultimate carcinogens.

We had previously observed that diol epoxide-1 isomers (in which the benzylic hydroxyl group is cis to the epoxide oxygen) normally exhibit little or no tumorigenic activity. In the absence of unusual steric or electronic factors, these isomers prefer the conformation in which the hydroxyl groups are pseudoaxial, whereas the diol epoxide-2 isomers (with the benzylic hydroxyl group trans to the epoxide oxygen) normally prefer the conformation with pseudoequatorial hydroxyl groups. The carcinogenic (R,S)-diol (S,R)-epoxides are of the latter type (diol epoxide-2). We proposed that pseudoaxial orientation of the hydroxyl groups (as in diol epoxides-1) might inhibit tumorigenic activity. This suggestion was supported by the observation that diol epoxide-2 from benzo(e)pyrene, whose conformation is unusual in that the hydroxyl groups are pseudoaxial, has extremely low tumorigenic activity. Futhermore, a diol epoxide-1 isomer from benzo(c)phenanthrene, whose hydroxyl grou prefer the pseudoequatorial conformation, "abnormal" for diol epoxide-1, exhibited substantial tumorigenic activity on mouse skin. A further test of the hypothesis pseudoaxial hydroxyl groups inhibit tumorigenic activity was designed using 6-fluorobenzo(a)pyrene (6-FBP) diol epoxides. 6-FBP diol epoxide-2 was expected to prefer the unusual conformation with pseudoaxial hydroxyl groups, although closely resembling the carcinogenic unfluorinated analogue in overall molecular dimensions. This prediction regarding conformation has now been shown to be the case. Studies of the effects of the fluorine substituent on the solution chemistry of the 6-FBP diol epoxides have been completed, and tumor studies are in progress.

Chemistry and Metabolic Formation of Arene Oxides and Their Derivatives. Be of our interest in the stereoselectivity of metabolism and tumorigenic activity o polycyclic aromatic hydrocarbon derivatives, the Section has a strong commitment the development of methods for the determination of absolute configuration of met bolites from these hydrocarbons, as well as the synthesis of these metabolites in optically pure form.

A previous annual report documented our observation of consistent patterns of physical properties for the enantiomeric K-region trans-dihydrodiols derived from a number of polycyclic aromatic hydrocarbons, such that the absolute configurations of these types of dihydrodiols could be predicted from selected physical properties. A new method has now been developed which permits the direct prediction of the absolute configurations of benzo-ring tetrahydroepoxides and K-region arene oxides of polycyclic aromatic hydrocarbons, based on the relative magnitude of the chemical shifts induced for the two oxirane protons in the presence of the chiral shift reagent, tris-[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III). The use of this shift reagent and of tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) also provides a convenient method for determination of the enantiomeric composition of a range of cyclic and acyclic epoxides as well as arene oxides. Thus, both enantiomeric excess and absolute configuration can be deduced from a single NMR experiment.

Triphenylene 1,2-oxide (1,2-epoxy-1,2-dihydrotriphenylene) has been synthesized from both racemic and optically pure precursors. Racemic triphenylene 1,2-oxide was obtained in each case, in accord with PMO calculations which predict rapid racemization. Liver microsomal metabolism of both triphenylene and racemic triphenylene 1,2-oxide yielded predominately the (-)-enantiomer (92 and 85% respectively) of the trans-1,2-dihydrodiol. The 1R,2R configuration was established for this enantiomer by preparative HPLC separation of the dimenthyloxyacetate diastereoisomers of the dihydrodiol and stereochemical correlation to (-)-(1R,2R)-trans-2-bromo-1-menthyloxyacetoxy-1,2,3,4-tetrahydrotriphenylene whose absolute configuration has been assigned by X-ray crystallographic analysis. The preferred formation of the (R,R)-dihydrodiol from triphenylene by the combined action of cytochrome P-450 and epoxide hydrolase in liver microsomes from 3-methylcholanthrene treated rats is consistent with studies on several other hydrocarbons, where the trans-dihydrodiol metabolites were in all cases enriched in the (R,R)-enantiomer.

K-region arene 5,6-oxides of chrysene, benzo(c)phenanthrene [B(c)Ph], and 7,12-dimethylbenz(a)anthracene (DMBA) have been synthesized from resolved cis-5,6-dihydrodiols by the ortho ester route as well as from separated bromo(menthyloxy)acetate precursors in the cases of chrysene and B(c)Ph. Absolute configuration of the 5,6-oxides and their precursors from chrysene and DMBA have been determined by nucleophilic trans addition of methanol to the oxirane ring and correlation by circular dichroism of the adducts with trans dihydrodiols of known configurations. Confirmation of the configurational assignments to the enantiomeric chrysene cis-5,6-dihydrodiols was achieved by reduction to cis-5,6-dihydroxy-1,2,3,4,5,6-hexahydrochrysene and determination of the skew sense of the resulting biphenyl chromophore through CD measurements. B(c)Ph 5,6-oxide enantiomers were assigned by direct comparison with a sample of known configuration on a chiral column.

The principal oxidative metabolites formed from B(c)Ph by the cytochromes P-450 in liver microsomes from control and treated rats were found to be the 3,4- and 5,6-arene oxides. A procedure was developed which allowed determination of the enantiomer composition and absolute configuration of these arene oxides based on HPLC separation of isomeric thiolate adducts formed with N-acetyl-L-cysteine in base. Incubation of [^{3}H]-B(c)Ph with highly purified cytochrome P-450c in a reconstituted monooxygenase system followed by trapping of the metabolically formed arene oxids as avove indicated that the 3,4-oxide was predominantly the (+)-(3S, 4R)-enantiomer (90%)

and that the 5,6-oxide consisted mainly of the (+)-(5S, 6R)-enantiomer (76%). Thes observations are consistent with the steric model which we have previously propose for the binding site of cytochrome P-450c, the most efficient cytochrome P-450 isoz known for metabolism of the polycyclic aromatic hydrocarbons.

Because of our interest in the comparative chemistry and metabolism of carbo- cyclic and aza polycyclic aromatic hydrocarbons, we have synthesized arene oxides a trans-dihydrodiols at the 5,6- and 7,8-positions of quinoline. High stability of b arene oxides allowed identification of the 5,6-oxide as a liver microsomal metaboli of quinoline. Both arene oxides were converted to trans-dihydrodiols by microsomal epoxide hydrolase.

Kinetics and Mechanisms of Reactions of Diol Epoxides in Aqueous Solution. 6-Fluorinated analogues of the mutagenic and carcinogenic benzo(a)pyrene 7,8-diol- 9,10-epoxides are of interest because the presence of the 6-fluoro group was expect to alter the conformation of the benzo-ring hydroxyl groups relative to the analog unfluorinated diol epoxides. Since fluorine substitution should have a negligible effect on the overall molecular dimensions, comparison of the fluorinated and unflu rinated diol epoxides should provide insights into the specific effect of conformat on the chemical properties and biological activity of these molecules. These 6-flu rinated benzo(a)pyrene 7,8-diol 9,10-epoxides were synthesized by epoxidation of (-)-trans-(7R,8R)-7,8-dihydroxy-7,8-dihydro-6-fluoro-benzo(a)pyrene, whose metabol formation from 6-fluorobenzo(a)pyrene we had previously reported. The products of epoxidation were (7R,8S,9R,10S)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-6-flu benzo(a)pyrene (6-FBP DE-1) and (7R,8S,9S,10R)-7,8-dihydroxy-9,10-epoxy-7,8,9, 10-tetrahydro-6-fluorobenzo(a)pyrene (6-FBP DE-2). NMR spectra indicate that the 7,8-diol group of 6-FBP DE-1 is almost exclusively pseudoaxial whereas the diol group in 6-FBP DE-2 also prefers the pseudoaxial orientation to a lesser extent. In both cases the preference for the pseudoaial conformation of the diol group is much stronger in the fluorinated diol epoxides than in the corresponding benzo(a)pyrene derivatives. Like the benzo(a)pyrene diol epoxides, these fluorinated analogues undergo hydrolysis and rearrangement in aqueous solutions to give tetraols and a 9-keto 7,8-diol, according to the rate law $k_{obsd} = k_H a_{H^+} + k_o$. Studies with 9,10-epoxy-7,8,9,10-tetrahydro-6-fluoro- benzo(a)pyrene and its corresponding benzo(a)pyrene derivative indicated that the electronic effect of the 6-fluoro group decreases k_H by ~7-fold and k_o by ~11-fold. Relative magnitudes of k_H for the fluorinated and unfluorinated diol epoxides can be accounted for solely by this electronic effect. On the other hand, k_o for 6-FBP DE-1 is much smaller and k_o for 6-FBP DE-2 is much larger than predicted when only the electronic substituent effect of fluorine is considered. The pH-independent rates for solvolysis of the fluorinated diol epoxides are thus markedly affected by their altered conformational equilibria due to the presence of fluorine. The observed differences in conformation of the fluorinated diol epoxides may account for the reduced mutagenicity of the 6-FBP diol epoxides, as well as for preliminary evidence that indicates a lack of high tumorigenicity for 6-FBP DE-2, relative to the corresponding benzo- (a)pyrene derivative, since bay-region diol epoxides in which the hydroxyl groups prefer the pseudoaxial conformation are known not to be highly carcinogenic.

We had previously reported that the hydrolysis of (±)-7β,8α-dihydroxy-9α,10α epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-2, in which the 7-hydroxyl group an epoxide oxygen are trans) is markedly catalyzed by DNA or poly(A). Analogous stu

have now been completed with (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-1, in which the 7-hydroxyl group and epoxide oxygen are cis). The rates of reaction of BPDE-1 in solutions of varied DNA, Poly(A), and Poly(G) concentrations were determined as a function of pH. The rate data were consistent with a mechanism in which BPDE-1 forms a physical complex with the polynucleotide, and this physical complex then reacts both by a pathway whose rate is first-order in respect to hydronium ion concentration (k^{H}_{cat} route) and by a second pathway whose rate is independent of hydronium ion concentration (k^{o}_{cat} route). Product studies showed that >95% of the products formed from both the k^{H}_{cat} and k^{o}_{cat} reactions were tetraols resulting from cis and trans hydration of the epoxide, and <5% of covalent binding of the diol epoxide to the polynucleotides occurred. The DNA- and Poly(A)-catalyzed hydrolyses of BPDE-1 are similar to those of BPDE-2 in that the physically bound diol epoxide reacts significantly faster (>50 fold) than free diol epoxide by the acid-catalyzed routes ($k^{H}_{cat} >> k_{H^+}$) and moderately faster (<5 fold) by the spontaneous pathway ($k^{o}_{cat} > k_{H_2O}$). Poly(G) is a significantly better catalyst than either DNA or Poly(A) for both k^{H}_{cat} and k^{o}_{cat} reactions. At pH ca. 7, however, the physical BPDE-1-DNA complex reacts mainly by the k^{o}_{cat} reaction, whereas the physical BPDE-2-DNA complex reacts mostly by the k^{H}_{cat} reaction.

Biological Activity of Oxygenated Metabolites. Benz(c)acridine is an aza analogue of benz(a)anthracene in which the bay-region C-12 has been replaced by nitrogen. Since 7-methyl substitution enhances the tumorigenicity of benz(a)anthracene, 7-methylbenz(c)acridine (7MB(c)AcR) and its potential metabolites were of considerable interest as possible tumorigens. Thus, the tumorigenicities of 7MB(c)ACR and its five metabolically possible trans-dihydrodiols were determined in two mouse tumor models. In initiation-promotion studies on mouse skin, a single topical application of 0.15 to 0.75 μmol of compound was followed 9 days later by twice weekly applications of 12-O-tetradecanoylphorbol-13-acetate for 20 weeks. Comparison of the average number of skin tumors per mouse indicated that 7MB(c)ACR 3,4-dihydrodiol, the metabolic precursor of a bay-region diol epoxide, was 4- to 6-fold more active than the parent compound as a tumor initiator. The 1,2-, 5,6-, 8,9- and 10,11-dihydrodiols of 7MB(c)ACR had no significant tumor-initiating activity at the doses tested. In newborn mice, a total dose of 0.35 μmol of compound was administered i.p. during the first 15 days of life, and tumorigenic activity was determined when the mice were 32 to 36 weeks old. 7MB(c)ACR 3,4-dihydrodiol induced about 8-fold more pulmonary tumors per mouse and 9-fold more hepatic tumors per male mouse than the parent aza-substituted hydrocarbon. The other four dihydrodiols of 7MB(c)ACR had no significant tumorigenic activity. The high tumorigenic activity of 7MB(c)ACR 3,4-dihydrodiol in both tumor models suggests that a bay-region 3,4-diol 1,2-epoxide may be an ultimate carcinogenic metabolite of 7MB(c)ACR. 7MB(c)ACR was at least 5-fold more active as a tumor initiator on mouse skin than was the unsubstituted aza-aromatic compound, benz(c)acridine. The latter result indicates that substitution of a methyl group at position 7 of benz(c)acridine leads to enhanced tumor-initiating activity, as has been previously demonstrated for benz(a)anthracene and its 7-methyl derivative.

We had previously reported that precocene I (7-methoxy-2,2-dimethyl-2H-benzo(b)-pyran), an antiallatotropic agent isolated from Ageratum species, is metabolized in vitro by rat liver enzymes to cis- and trans-3,4-diols, suggesting the formation of a reactive epoxide, analogous to those formed from polycyclic aromatic hydrocarbons, by mammalian bioactivation of this compound. We also reported that precocene I caused hepatic centrilobular necrosis in rats. In view of the potential use of precocene I as an insecticide, the mechanism for this hepatotoxicity was investigated.

Administration of a single dose of precocene I to male Sprague-Dawley rats caused a large depletion of liver glutathione (GSH) levels that was both time and dose dependent. Concomitant with the decrease of liver GSH, there was an increase in se glutamic pyruvic transaminase (GPT) levels which was also time and dose dependent. Administration of a single dose of [4-^{3}H]precocene I resulted in extensive covalent binding of the radiolabel to liver proteins and DNA in the liver; the extent of binding increased with increasing dose. Treatment of the rats with the mixed-funct oxidase inhibitor piperonyl butoxide, before administration of precocene I, significantly decreased the proportion of the radiolabel bound covalently to protei and DNA, although the total radioactivity (bound and unbound) in the liver remained the same. Piperonyl butoxide pretreatment limited both the liver GSH depletion and the hepatic necrosis normally caused by precocene I. These results are consistent with the view that the hepatotoxicity of precocene I is due to reactive metabolites formed through cytochrome P-450 mediated metabolism.

Potential Antitumorigenic Agents. A recent goal of research in the Section ha been the identification of compounds capable of blocking the biological activity of bay-region diol epoxides through their chemical inactivation. Several years ago, ellagic acid, a naturally occurring plant phenol, was identified by us as a potent inhibitor of the mutagenic action of benzo(a)pyrene diol epoxide-2, and was shown t inactivate the diol epoxide in aqueous solution by forming covalent ether adducts. Subsequent studies indicated that ellagic acid had a moderate inhibitory effect on the tumorigenicity of this diol epoxide on mouse skin, but was inactive against the parent hydrocarbon. The inability of ellagic acid to inhibit significantly the tumorigenicity of benzo(a)pyrene may possibly result from its poor availability in target cells. Since little information was available on the disposition of ellagic acid in animals, we investigated the elimination and tissue distribution of ellagic acid in mice. More lipophilic derivatives of ellagic acid were also investigated.

Ellagic acid, 3,3'-di-O-methylellagic acid, 4,4'-di-O-methylellagic acid and 3-O-decylellagic acid were found to have approximately equal antimutagenic activity against benzo(a)pyrene diol epoxide-2 in Salmonella typhimurium TA100. The tissue distribution and elimination of ellagic acid, 3,3'-di-O-methylellagic acid and 3-O-decylellagic acid were examined in CD-1 mice. Little or no ellagic acid (<1 nmol/g) was found in blood, lung or liver after the oral administration by gavage of 300 µmol of ellagic acid per kg body weight or after feeding 1% of ellagic acid in diet for 1 week. Following the i.p. administration of 120 µmol/kg of ellagic acid the blood and lung levels of ellagic acid were 15-20 nmol/g at 30 min after the do and the concentration of ellagic acid decreased to 1-3 nmol/g at 6-8 h after the d A portion of the administered i.p. dose precipitated in the abdominal cavity. Aft i.v. administration, ellagic acid was eliminated very rapidly from blood, lung and liver, and ~70% of the administered dose was recovered in the urine and feces as f ellagic acid and its conjugates. At 2 h after an i.v. injection of 60 µmol/kg of ellagic acid, 46% of the dose was recovered in the urine as ellagic acid and its conjugates. Of this amount, about half was excreted as free ellagic acid and half excreted as conjugates. An additional 25% of the dose was recovered in the feces (mostly as free ellagic acid) after 7 h. The disposition of 3,3'-di-O-methyl-ellagic acid or 3-O-decylellagic acid after i.v. administration (32 µmol/kg) was examined and compared to the disposition of the same i.v. dose of ellagic acid. T concentrations of ellagic acid, 3,3'di-O-methylellagic acid and 3-O-decylellagic a decreased rapidly in the blood, liver and lung, but the concentrations of 3-O-decy ellagic acid in the lung throughout the experimental period (2-360 min) was on the

average 20- to 40-fold higher than the corresponding average concentrations of ellagic acid or 3,3'-di-O-methylellagic acid.

The effect of ellagic acid and its more lipophilic derivative, 3-O-decylellagic acid, on the amount of DNA-bound adducts in the epidermis or lung of CD-1 mice treated with [^{3}H]benzo(a)pyrene ([^{3}H]B(a)P) was evaluated using several different treatment protocols. The i.v. administration of 50 μmol/kg of ellagic acid or 3-O-decylellagic acid either together with or 5 min before a 0.2 μmol/kg i.v. dose of [^{3}H]B(a)P did not inhibit the formation of pulmonary DNA-bound adducts. Feeding mice a diet that contained 1% ellagic acid for 10 days or the i.p. administration of 120 μmol/kg of ellagic acid 30 min before the i.v. adminstration of 0.2 μmol/kg of [^{3}H]B(a)P did not inhibit the formation of DNA-bound adducts in the lung. The application of 2500 nmol of ellagic acid or 3-O-decylellagic acid to mouse skin 5 min before the application of 2, 10 or 50 nmol of [^{3}H]B(a)P had little or no effect on the covalent binding of [^{3}H]B(a)P metabolites to epidermal DNA. Feeding mice a diet containing 1% ellagic acid for 10 days did not inhibit the formation of epidermal DNA-bound adducts after a topical dose of 2 nmol of [^{3}H]B(a)P. Similarly, the topical application of 2500 nmol of ellagic acid at 2 h, 1 h and 5 min before and at 10 min after the application of 2 nmol of [^{3}H]B(a)P did not inhibit the formation of DNA-bound adducts, but the same dosing regimen of 3-O-decylellagic acid (total dose of 10,000 nmol) resulted in a modest inhibition in the formation of DNA-bound adducts. The topical application of 1500 nmol of ellagic acid 1 h before the application of 1500 nmol of 3-methylcholanthrene (3-MC) to CD-1 or BALB/c mice twice weekly did not inhibit the development of skin tumors. Our results indicate that ellagic acid and 3-O-decylellagic acid are not effective in inhibiting [^{3}H]B(a)P DNA adduct formation in mouse skin and lung and that ellagic acid does not inhibit 3-MC-induced skin tumorigenesis in BALB/c or CD-1 mice.

Specific Inhibition of Cytochrome P-450c. Over the past several years, extensive research efforts in the Section have been directed toward the mapping of the substrate-binding site of cytochrome P-450c. This isozyme is the predominant form of cytochrome P-450 in the livers of 3-methylcholanthrene-treated rats, and it generally exhibits the highest turnover of any of the cytochrome P-450 isozymes for polycyclic aromatic hydrocarbon substrates. As described in this and previous annual reports, the stereoselectivity of cytochrome P-450c on metabolism of a large number of polycyclic aromatic hydrocarbons has provided valuable insights into the spatial tolerance of the substrate-binding site. Although the amino acid sequence of cytochrome P-450c is known, those amino acids that comprise the catalytic site and define the boundary of the substrate-binding site have not been established. We have now undertaken chemical modification studies to identify functionally important amino acid residues in cytochrome P-450 as an approach to gain further understanding of the mechanism of catalysis by this class of enzymes.

The alkylating agent 2-bromo-4'-nitroacetophenone (BrNAP) binds covalently to each of 10 isozymes of purified rat liver microsomal cytochrome P-450 (P-450a-P-450j) but substantially inhibits the catalytic activity of only cytochrome P-450c. Regardless of pH, incubation time, presence of detergents, or concentration of BrNAP, treatment of cytochrome P-450c with BrNAP resulted in no more than 90% inhibition of catalytic activity. Alkylation with BrNAP did not cause the release of heme from the holoenzyme or alter the spectral properties of cytochrome P-450c, data that exclude the putative heme-binding cysteine, Cys-460, as the major site of alkylation. Two residues in cytochrome P-450c reacted rapidly with BrNAP, for which reason maximal loss of catalytic activity was invariably associated with the incorporation of ~1.5

mol of BrNAP/mol of cytochrome P-450c. Two major radiolabeled peptides were isolat from a tryptic digest of [^{14}C]BrNAP-treated cytochrome P-450c by reverse-phase hig performance liquid chromatography. THe amino acid sequence of each peptide was determined by microsequence analysis, but the identification of the residues alkyla by BrNAP was complicated by the tendency of the adducts to decompose when subjected automated Edman degradation. However, results of competitive binding experiments w the sulfhydryl reagent 4,4'-dithiodipyridine identified Cys-292 as the major site o alkylation and Cys-160 as the minor site of alkylation by BrNAP in cytochrome P-450

The mechanism by which BrNAP inactivates cytochrome P-450c was shown to involv an uncoupling of NADPH utilization and oxygen consumption from product formation. Alkylation of cytochrome P-450c with BrNAP markedly stimulated (~30-fold) its rate anaerobic reduction by NADPH-cytochrome P-450 reductase, as determined by stopped f spectroscopy. This marked stimulation in reduction rate is highly unusual in that Cys-292 is apparently not part of the heme- or substrate-binding site, and its alkylation by BrNAP does not cause a low spin to high spin state transition in cytochrome P-450c. Under aerobic conditions the rapid oxidation of NADPH catalyzed by alkylated cytochrome P-450c was associated with rapid reduction of molecular oxygen to hydrogen peroxide via superoxide anion. The intermediacy of superoxide anion, formed by the one-electron reduction of molecular oxygen, established that alkylation of cytochrome P-450c with BrNAP uncouples the catalytic cycle prior to introduction of the second electron. The generation of superoxide anion by decomposition of the $Fe^{2+}.O_2$ complex was consistent with the observation that, in contrast to native cytochrome P-450c, alkylated cytochrome P-450c failed to form a 430 nm absorbing chromophore during the metabolism of 7-ethoxycoumarin. Alkylation of cytochrome P-450c with BrNAP did not completely uncouple the catalytic cycle such that 5-20% of the catalytic activity remained for the alkylated cytochrome compared to the native protein depending on the substrate assayed. The uncoupling effect was, however, highly specific for cytochr P-450c. Alkylation of nine other rat liver microsomal cytochrome P-450 isozymes w BrNAP caused little or no increase in hydrogen peroxide formation in the presence NADPH-cytochrome P-450 reductase and NADPH.

Covalent Modification of DNA by Diol Epoxides. In previous years we describe studies on the metabolic formation, tumorigenicity and mutagenicity of the optical active 3,4-diol 1,2-epoxides derived from benzo(c)phenanthrene trans-3,4-dihydrodi Two of these diol epoxides are the most active diol epoxide tumor initiators teste date on mouse skin. Because of the exceptionally high tumorigenic activity of the compounds, we anticipated that they would be highly interesting candidates for stu of their interactions with DNA. Covalent binding of these diol epoxides to calf - thymus DNA in vitro (~1 mg/ml) has now been shown to occur with unusually high efficiency (60-75% of total diol epoxide) relative to epoxide hydrolysis. Upon treatment of DNA with each configurationally isomeric diol epoxide, followed by enzymatic hydrolysis, mononucleoside adducts were obtained and separated by HPLC. Identification of the bases involved indicated extensive reaction of the benzo(c)- phenanthrene diol epoxides with deoxyadenosine as well as deoxyguanosine residues. This finding is in contrast to observations with benzo(a)pyrene diol epoxides, whi react predominantly with deoxyguanosine residues.

Structural characterization of the 16 principal adducts formed from the deoxyadenosine and deoxyguanosine residues of DNA upon reaction with the four con- figurationally isomeric benzo(c)phenanthrene 3,4-diol 1,2-epoxides has been accom- plished by chemical and spectroscopic means. The adducts (one cis and one trans

addition product derived from each of the four configurationally isomeric diol epoxides and either deoxyguanosine or deoxyadenosine) were prepared in quantities sufficient for structural studies via the reactions of the diol epoxides with deoxyguanylic and deoxyadenylic acids, followed by enzymatic removal of the phosphate group. The site of covalent attachment of the diol epoxide moiety to the nucleoside in these adducts is at the exocyclic amino group. For most of the deoxyguanosine adducts, the linkage between this nitrogen and C_1 of the tetrahydrobenzo(c)-phenanthrene system was established directly by NMR decoupling experiments using the pentaacetate esters of the adducts. Since the lack of observable NMR signals for the exocyclic N-H of adenine in the pentaacetates of the deoxyadenosine adducts made such decoupling experiments impossible, the site of attack of deoxyadenosine was deduced to be at the exocyclic N^6 by a combination of chemical stability considerations and pH titration (pK = 3.8 for a representative unacetylated adduct). The stereochemistry (cis or trans opening of the epoxide) of each adduct was assigned on the basis of the ^1H NMR spectrum of the corresponding pentaacetate ester. An empirical correlation has been found between R-absolute configuration at the benzylic carbon of the tetrahydroaromatic moiety and negative ellipticity of the major CD band for these benzo(c)phenanthrene diol epoxide adducts of deoxyguanosine and deoxyadenosine, as well as for analogous adducts derived from purine nucleosides and benzo(a)pyrene 7,8-diol 9,10-epoxides. Correlations of this type may prove useful in predicting the structures of deoxyribonucleoside-diol epoxide adducts that are formed in quantities too small for identification by NMR spectroscopy.

To determine whether benzo(c)phenanthrene (BcPh) is metabolically activated to bay-region diol epoxides that bind to DNA in cells, Sencar mouse, Syrian hamster, and Wistar rat embryo cell cultures were exposed to [5-^3H]-BcPh, and the BcPh-deoxyribonucleoside adducts formed were analyzed by immobilized boronate chromatography. Greater than 74% of the BcPh-deoxyribonucleoside adducts formed in all three species resulted from reaction of (4R,3S)-dihydroxy-(2S,1R)-epoxy- 1,2,3,4-tetrahydro-BcPh [(-)-BcPhDE-2] with DNA to yield deoxyadenosine and deoxyguanosine adducts in a ratio of 3:1. A much smaller proportion of BcPh-deoxyribonucleoside adducts was formed by reaction of (4S,3R)-dihydroxy-(2S,1R)-epoxy-1,2,3,4-tetrahydro-BcPh [(+)-BcPhDE-1] with deoxyadenosine. No BcPh-deoxyribo- nucleoside adducts arising from either (+)-BcPhDE-2 or (-)-BcPhDE-1 were detected. The absence of adducts from these isomers of BcPhDE was not due to failure of these isomers to react with DNA in cells, for reaction of (±)-BcPhDE-1 or (±)-BcPhDE-2 with DNA in solution or in hamster embryo cell cultures resulted in the formation of DNA adducts from both the (+)- and (-)-enantiomers of each BcPhDE. These results indicate that both the (+)- and (-)-3,4-dihydrodiols of BcPh are formed and that their metabolic activation to diol epoxides occurs with high stereospecificity in cells from all three species of rodents. The finding that the major DNA-binding metabolite is (-)-BcPhDE-2, the diol epoxide with the (R,S)-diol (S,R)-epoxide absolute configuration that is associated with high carcinogenic activity of diol epoxides of other hydrocarbons, demonstrates that these cells are able to activate BcPh to an ultimate carcinogenic metabolite. The fact that a high proportion of the BcPh-DNA adducts are deoxyadenosine adducts suggests that BcPh has DNA-binding properties similar to those of the potent carcinogen 7,12-dimethylbenz(a)anthracene. The stereospecificity observed in the metabolic activation of BcPh to DNA-binding metabolites and the reaction of these metabolites with both deoxyguanosine and deoxyadenosine suggest that studies of the interactions of BcPh with DNA <u>in vivo</u> may be a valuable approach for establishing the role of specific activation pathways and DNA adducts in tumor induction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31100-22 LBC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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	T. Spande	Research Chemist	LBC, NIDDK
	M. Edwards	Chemist	LBC, NIDDK
	F. Gusovsky	Visiting Fellow	LBC, NIDDK
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SECTION

Section on Pharmacodynamics

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☐ (a2) Interviews

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

Natural products have provided a wide range of biologically active agents many of which have unique profiles of pharmacological activity and therapeutic potential. Over two hundred alkaloids have been identified in extracts from amphibian skins. These include batrachotoxins, which are potent activators of sodium channels, histrionicotoxins, which are noncompetitive blocks of nicotinic receptor channel complexes and of potassium channels and pumiliotoxins, which have myotonic and cardiotonic activity due to inhibitory effects on closing of sodium channels. The effects of pumiliotoxin B on sodium channels results in repetitive activations of such channels, enhancement of neurotransmitter release, and potentiation of contraction in striated and cardiac muscle. The stimulatory effects of pumiliotoxins, pyrethroids and other sodium channel agents on cardiac function correlates with stimulation of phosphoinositide turnover leading to both mobilization of internal calcium and to activation of protein kinase C. The effects of pumiliotoxin B on sodium flux in synaptoneurosomes are markedly potentiation by certain polypeptide toxins, such as α-scorpion toxin. A variety of 2,6-disubstituted piperidines are potent noncompetitive blockers of the nicotinic receptor-channel complex. Those with one long side chain stabilize high affinity desensitized states of the receptor. Local anesthetics inhibit binding of a tritiated batrachotoxin analog either allosterically by enhancing dissociation or through direct competition. Reserpines appear to be true competitors. New structural classes of amphibian alkaloids have been delineated and include 2,5-disubstituted pyrrolidines, a 4-hydroxy-2,6-dialkylpiperidine, 2,5-disubstituted-trans-decahydroquinolines, a quinolizidine related in structure to the pumiliotoxin-A class of alkaloids, various 5-substituted-8-methyl indolizidines a hydroxypumiliotoxin C, an azatricyclododecene, several tricyclic amidines, and an unusual prenyl pyrrole-[2,3-b]indole ester. The biological activity of these new alkaloids is unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 31101-19 LBC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Pharmacology and Metabolism of Biogenic Amines and Related Compounds

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

P.I.:	C.R. Creveling	Research Chemist	LBC, NIDDK
Others:	J.W. Daly	Chief	LBC, NIDDK
	F. Gusovsky	Visiting Fellow	LBC, NIDDK

COOPERATING UNITS *(if any)* K.L. Kirk, LC, NIDDK; K. Inoue, Okayama U., Okayama, Japan; X. Breakfield, Harvard U., Boston, MA; M. Grossman, Children's Hospital, Phil. PA.; L.I. Goldberg, U. Chicago, IL; D. Thakker, DBB, FDA.; M. Orcini, U. Wisc., Madison, WI; D. Rossignol, Dupont, Wilmington, Del.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.3	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.)*

The chemistry, biochemistry, physiology, and pharmacology of biogenic amines, their amino acid precursors and metabolic products, and various synthetic derivatives thereof have been investigated. The areas of specific interest are: 1) Elucidation of the primary sequence of areas catechol-O-methyltransferase(COMT) and construction of a COMT-specific cDNA probe, 2) The immunohistochemical localization of COMT in malignant, physiologically and hormonally modified, and normal tissues from rodent, and human at the light and electron microscopic level including the following: Examination of the temporal and hormonal relationship between uterine epithelial COMT during the course of pregnancy in golden hamster; study of the relationship between breast adenocarcinoma in woman; COMT levels in gynomasty in man; and an examination of the distribution of catechol estrogens and COMT in parotid gland and anterior pituitary of rat, 3) A study of the chemical and biological properties of various fluoro derivatives of biogenic amines, amino acids, and related compounds including studies of the following: The electrochemical, redox properties and electron densities of fluorocatechols; the interaction of fluorophenylephrines and fluoroepinephrines with both alpha and beta receptor systems; the interaction at the active site of COMT; the uptake and metabolism of 6-fluorodopa and 6-fluorodihydroxyphenylserine in vitro and in vivo; the application of fluorine-18 analogs of dopa and dihydroxypehnylserine as PETT scanning agents for dopamine and norepinephrine neurons in the intact animal; and the uptake and mechanism of toxicity of 6-fluorodopa, 2,6-fluorodopa, 6-fluorotyrosine and 2,6-difluorotyrosine in cultured pheochromocytoma and melanoma cell lines.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 31102-16 LBC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Cyclic Nucleotides and Other Second Messengers in the Nervous System

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

P.I.	J.W. Daly	Chief	LBC, NIDDK
Others:	W. Padgett	Biologist	LBC, NIDDK
	F. Gusovsky	Visiting Fellow	LBC, NIDDK
	M. Shamim	Guest Worker	LBC, NIDDK

COOPERATING UNITS *(if any)* B. Fredholm, L. Gustafsson, Karolinska Inst., Stockholm; C. Post, Astra R. & D., Sodertalje, Sweden; J. Carney, U. Oklahoma; R. Weir, Howard U., Wash., D.C.; R. Olsson, U. So. Florida, Tampa, FL; K. Seamon, FDA, D.A. Bergstrom, U. No. Dakota; K. Kirk, K. Jacobson, LC, NIDDK; R.J. Wurtman, MIT, Boston, MA.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.1	1.8	1.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Physiological functions are mediated in different cells by a variety second messengers including cyclic AMP and cyclic GMP. Ions such as calcium, sodium and magnesium can serve after translocation through ion channels or by transport proteins as second messengers to activate release of ions, and activation of contratile proteins, protein kinases, phospholipases, ATPases and other enzymes. Enzymatic hydrolysis of phospholipids can also generate second messengers such as arachidonate, which serves as a precursor of prostanoids; diacylglycerides, which serve as activators of protein kinase C; and inositol phosphates, which serve as mobilizers of internal calcium ions. Receptors of various types serve to modulate generation of second messengers. The interrelationships of second messengers and receptors and the delineation of selective agents for such systems have been investigated. Phorbol esters, which like the diacylglycerides activate protein kinase C, inhibit receptor-mediated formation of cyclic AMP in a neuroblastoma-hybrid cell line. A variety of receptor agonists and agents that activate voltage-dependent sodium or calcium channels cause activation of phospholipase C and breakdown of phosphoinositides in synaptoneurosomes. Phorbol esters inhibit these responses. A small subpopulation of sodium channels with high sensitivity to cadmium ions and low sensitivity to tetrodotoxin appear losely associated with phosphoinositide metabolism. Adenosine stimulates cyclic AMP formation through an A_2 receptor and inhibits cyclic AMP formation through an A_1 receptor. A series of adenosine analogs have been delinated for pharmacological characterization of receptors involved in physiological functions of adenosine. A variety of non-xanthine heterocycles, including a series of N^6-substituted-9-methyladenines have been characterized as adenosine receptor antagonists. Certain xanthine analogs have been shown to have selective effects as antagonists of the hypotensive effects of adenosine, the cardiac depressant effects of adenosine and the synergism between adenosine and nicotine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 31103-10 LBC

PERIOD COVERED

October 1, 1986 - September 30, 1987

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

Receptors for Neurotransmitters and Drugs in Brain and Peripheral Tissues

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

P.I.: P. Skolnick Section Chief LBC, NIDDK

COOPERATING UNITS *(if any)* E. Kempner, LPB, NIDDK; E.A. Jones, S. Gammal, DDB, NIDDK, S. Paul, J. Crawley, R. Drugan, P. Sudzak, CNB, NIMH, N. Ostrowski, CPB, NIMH, E. Hanna, LMG, NICHD; D. Klein, LDN, NICHD; J. Cook, M. Trudell, T. Hagen, M. Allen, Univ. WI; J. Barrett, USUHS; B.A. Weissman, Israel Inst. of Biolog. Res.

LAB/BRANCH

Laboratory of Neuroscience

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	8	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been transferred to Laboratory of Neuroscience, NIDDK. The new project number is Z01 DK 58,501-01 LNS.

GPO 914-919

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 31104-19 LBC

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

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

P.I.:	D.M. Jerina	Section Chief	LBC, NIDDK
Others:	J. Sayer	Research Chemist	LBC, NIDDK
	H. Yagi	Visiting Scientist	LBC, NIDDK
	L. Pannell	Expert	LBC, NIDDK
	A. Cheh	Research Chemist	LBC, NIDDK
	S.G. Grossman	Staff Fellow	LBC, NIDDK
	N.T. Nashed	Staff Fellow	LBC, NIDDK
	A. Chadha	Visiting Fellow	LBC, NIDDK

COOPERATING UNITS (if any) Pharmacy Dept., U. of Sydney (Australia); Chem. Dept., The Queen's U. of Belfast (N. Ireland), and LAC, NIDDK; Lab. Expt. Carcinogenesis & Metabolism, Roche Inst. (Nutley, NJ); Chem. Dept., U. MD (Catonsville); Chem. Dept., U. OK (Norman); Med. Chem. & Pharmacognosy Dept., Purdue U. (W. Lafeyette, IN); Basic Res.

LAB/BRANCH: Laboratory of Bioorganic Chemistry

SECTION: Section on Oxidation Mechanisms

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	7.5	1

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The primary goal has been the elucidation of the structures of reactive metabolites which are responsible for the carcinogenic, cytotoxic and mutagenic activity of polycyclic aromatic hydrocarbons. The approach taken consists of: i) synthesis of primary and secondary oxidative metabolites, ii) study of the metabolism of the hydrocarbons with liver microsomes, as well as with purified and reconstituted cytochrome P-450 systems with and without epoxide hydrolase, iii) tests for mutagenicity of the synthetic metabolites, iv) elucidation of the roles of the cytochrome P-450 system and epoxide hydrolase in potentiating or obliterating the mutagenicity of these metabolites, v) determination of the carcinogenic activity of these compounds, vi) determination of the reaction rates and nature of the products formed by arene oxides and diol epoxides upon reaction with biopolymers and model compounds, and vii) search for agents capable of preventing the tumorigenic action of active metabolites. Current chemical studies have included the synthesis and assignment of absolute configuration of the optically active 5,6-oxides derived from chrysene, 7,12-dimethylbenz(a)anthracene and benzo(c)phenanthrene as well as assignment of absolute configuration of the predominant 1,2-dihydrodiol metabolite from triphenylene. The absolute configurations of the principal 3,4- and 5,6-oxides formed from benzo(c)phenanthrene by cytochrome P-450c have been determined. An NMR method for determining the enantiomeric composition of arene oxides as well as for predicting their absolute configuration by the use of chiral lanthanide shift reagents has been developed. Diastereomeric 6-fluorobenzo(a)pyrene 7,8-diol 9,10-epoxides, which differ in conformation from the unfluorinated analogues, have been synthesized, and a marked effect of conformation on their rates of solvolysis demonstrated. The mechanism of specific inhibition of cytochrome P450c by 2-bromo-4'-nitroacetophenone has been elucidated. The deoxyguanosine and deoxyadenosine adducts formed by alkylation of DNA by 4 optically active benzo(c)phenanthrene 3,4-diol 1,2-epoxides have been characterized, and several of these adducts have been identified upon treatment of rodent embryonic cells in culture with the parent hydrocarbon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 31105-02 LBC

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nicotinic and Muscarinic Acetylcholine Receptor Agonists.

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

P.I.:	J.A. Waters	Research Chemist	LBC, NIDDK
Others:	J.W. Daly	Chief	LBC, NIDDK
	E.B. Hollingsworth	Staff Fellow	LBC, NIDDK

COOPERATING UNITS *(if any)* A. Aronstom, Med. Coll., Georgia; R. Barlow, U. of Bristol, Engl; T. Gund, Newark C. of Eng. & Chem., N.J.; R. Lukas, Barrow Neurol. Instit., Phoenix, AZ; D. Marsh, U. of New Foundland, Canada; C. Spivak, NIDA, Baltimore, MD; J. Sagen, U. of Illinois, Chicago, ILL; I. Stolerman, U. of London, England.

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The main objective has been to synthesize potent, semirigid nicotinic and muscarinic acetylcholine receptor agonists in order to further understand the receptor recognition sites. Numerous compounds, generally of the acetyl substituted piperidine, piperazine and bicyclic amine-type, have been prepared for structure-activity correlations. Computer assisted modeling studies have given minimum energy conformations, hydrogen bonding to cationic distances/ superpositions and electrostatic potentials, providing additional information for a rational approach to the design of new, potent agonists. Isoarecolone (1-methyl-4-acetyl-1,2,3,6-tetrahydropyridine) methiodide is a very potent nicotinic agonist as shown in various assays: (i) Torpedo electric tissue, (ii) TE671 human medulloblastoma cell line, frog rectus abdominus muscle and rat esophageal striated muscle (neuromuscular receptors), (iii) rat PC12 cells and guinea pig ileum (ganglionic receptors), (iv) rat brain membranes (central receptors). Also, isoarecolone hydrochloride produced nicotine-like discriminative effects in rats. Isoarecolone methiodide is only moderately potent at muscarinic M_1 receptors (brain) and exhibits weak activity at M_2 receptors (heart).

Nicotinic agonists and muscarinic agonists/antagonists may be useful in the treatment of cholinergic deficient diseases such as Alzheimer's disease, where reduced levels of ACh, AChR's and cholineacetyltransferase are found, and myasthenia gravis, where autoantibodies are directed to the main immunogenic region (MIR) of the α-subunit of the nicotinic AChR. We plan to study the effectiveness of the isoarecolone salts in animal models of Alzheimer's disease (systemically and intracerebroventricularly).

Annual Report of the Laboratory of Molecular Biology

National Institute of Diabetes and Digestive and Kidney Diseases

The Laboratory of Molecular Biology has as its principal goal the understanding of biological processes at the molecular level. The research program involves application of both theoretical and experimental methods to a wide variety of problems in molecular genetics, regulation of gene expression in eukaryotes, mechanisms of DNA replication, nucleic acid and protein structure, bioenergetics and transport properties. Specific areas under investigation include the structures and chemical properties of biologically important materials. These involve studies of the organization of DNA and proteins within the eukaryotic nucleus, investigations of enzyme and immunoglobulin structures by X-ray diffraction, investigation of polynucleotide chemistry and structure by synthetic and spectroscopic methods, and of protein interactions by calorimetric methods, studies of the conformations of supercoiled DNA and their effect on biological properties, and theoretical studies of the mechanism of energy conversion in biology, muscle contraction, microtubule formation, ion transport and biochemical kinetics. Other investigations are concerned directly with biological processes. These include studies of the process of transformation by the tumor virus SV40, of immunoglobulin gene rearrangement, of nonheritable antibiotic resistance, of the effects of macromolecular crowding, and of the mechanisms of genetic recombination and DNA replication. Important advances have been made in these areas this year.

Chromatin Structure and Function

We have continued our studies of chromatin structure in the neighborhood of expressed genes, making use of the globin gene family in chicken erythrocytes as a model system. We have examined in detail the structure of the nuclease hypersensitive domain in the 5' flanking region of the adult β globin gene isolated from cells in which that gene is expressed. We have shown that multiple sequence-specific factors are bound in this region, and we have partially purified and characterized some of them. Deletion analysis shows that at least one of these factors is a negative regulatory element that depresses β globin gene transcription, while another is stimulatory. We have also analyzed the enhancer domain at the 3' side of this gene, which we had discovered earlier. The DNA positive regulatory elements of the enhancer are contained in a region 136 base pairs long and four discrete sets of cellular factors have been shown by footprint analysis to bind there. Using the primary erythrocyte transfection techniques which we developed, we have shown that the enhancer, in combination with the β promoter, functions less effectively in 4 day embryonic erythrocytes than in 9 day erythrocytes, strongly suggesting that the enhancer/promoter combination is developmentally specific. We have continued the refinement of the transfection techniques, and determined details of the transfection mechanism. We have also continued physicochemical studies of chromatin structure. We have shown that when an alternating G-C DNA segment contained in a plasmid is converted to the left-handed Z form by supercoiling, there is a shift in the placement of nucleosome core particles assembled on the plasmid, in such a way that the Z DNA segment is excluded from the central portion of the nucleosome. The result suggests that this may be a general property of non-B DNA structures.

Effects of DNA Supercoiling on the Topological Properties of Nucleosomes

In the nucleosome core particle, at least 145 base pairs of DNA are bound to the histone octamer in a superhelical conformation. We have asked what effect the presence of these particles has on the ability of DNA gyrase to supercoil DNA. Synthetic minichromosomes, constructed by reconstituting complexes of core histones with the closed circular plasmid pBR322, were treated with various amounts of DNA gyrase. We have found that the maximum level of supercoiling that is attainable is nearly identical for protein-free plasmids and for plasmids half-saturated with core histones, even though supercoiling does not result in a loss of histones from the complex. It appears that, at sufficiently high levels of supercoiling, the core particle is disrupted in such a way that the DNA bound to histones is no longer constrained.

Enzyme Structure

New inhibitor X-ray data have been measured for the aspartyl proteinase from Rhizopus chinensis. A mechanism of action has been proposed.

The crystal structure of tryptophan synthase from Salmonella typhimurium has been determined at 2.3Å resolution and is currently being refined. Preliminary analysis of the structure accounts for the behavior of many mutants. It also shows how such a double enzyme system can be catalytically advantageous.

Three-Dimensional Structure of Proteins of the Immune System

The high resolution structure of the J539 immunoglobulin molecule has been refined. All the amino acid residues have been located with the exception of three that appear to be disordered.

The three-dimensional structures of two crystal forms of a complex between lysozyme and the Fab fragment of a monoclonal antibody to lysozyme have been determined. The method used was molecular replacement in which the known structures of the Fab and of lysozyme were used as probes to determine their orientations and positions in the new crystal.

Chemical and Structural Investigation of Nucleic Acids and Related Molecules

This project has the objective of understanding the chemistry and structure of nucleic acids and relating this knowledge to the biological functions of these molecules. Methods used include chemical synthesis of defined sequence DNA fragments and of enzyme substrates, enzymatic synthesis of polynucleotides, study of nucleic acids by circular dichroism, ultraviolet, infrared, and nuclear magnetic resonance spectroscopy, study of thermal transitions and dependence of physical properties on solution conditions. Subjects of investigation include factors which determine the stability of helical complexes, specificity of nucleic acid interactions, location and affinity of binding sites.

We have continued our collaborations on 2D NMR of DNA fragments containing restriction endonuclease recognition sequences. Resonance assignments have been obtained for all the nonexchangeable protons in the dodecanucleotide d(GAATTC-GAATTC) and approximate sugar conformations and glycosidic dihedral angles determined. The molecule has a B-DNA conformation with both strands identical.

A new heteronuclear ^{1}H-^{31}P shift correlation method was used to assign all of the ^{31}P resonances in the oligonucleotide d(CATGCATm^{5}CCATG).

Information on nucleic acid mispairing is relevant to such important biological functions as mutation, gene expression and control, splicing, and feedback control. We have begun examination of a series of oligonucleotides small enough to permit analysis of structural and energetic changes caused by introduction of selected mispairings. An initial finding of importance is that the position of AG mispairing is crucial for its effect on helix stability. Surprisingly, two AG's replacing two CG's in the center of a dodecamer have little effect on T_m, whereas the same substitutions two positions removed from the center have a large effect. A similar result is observed with AI pairing. Preliminary work with AC mispairing shows that it is strongly destabilizing.

Thermal Measurements of Biomolecular Systems

Emphasis has been directed toward extracting information from the shape of differential scanning calorimetry (DSC) curves. Theoretical thermograms have been constructed for discrete polymerization and depolymerization reactions so that one may, for example, by comparison with experiment determine the number of subunits in an oligomeric protein. This analysis has been implemented in a computer program which is available for other researchers.

In addition to DSC measurements on well defined polyribonucleotide and protein (HSA) systems, work has been carried out on more complex biological membranes and viral capsid particles.

Influences of Macromolecular Crowding on Biochemical Systems

A patent covering the polymer-stimulated ligation of DNA has been issued.

Macromolecular crowding has been used to obtain efficient phosphorylation by T4 polynucleotide kinase of types of termini in duplex DNA -- such as termini at "nicks" or recessed termini -- which are otherwise only very slowly or incompletely labeled under conventional procedures.

Macromolecular crowding was found to extend the range of ionic conditions which support high DNA polymerase reaction rates. High concentrations of non-specific polymers increased polymerase activity under otherwise inhibitory conditions resulting from relatively high ionic strength. The primary mechanism of the polymer effect seems to be to increase the binding of polymerase to DNA. We have suggested that this effect on protein-DNA complexes is only one example of a general "metabolic buffering" action of crowded solutions on a variety of macromolecular interactions.

Origins of Mammalian DNA Replication in Normal and SV40 Transformed Cells

The DNA sequence $(GA)_n \cdot (CT)_n$ has been found to slow replication fork progression in monkey cells. It is suggested that this sequence may therefore play an important role in gene amplification.

Several of the sequences we previously isolated as potential origins of replication have been shown to stimulate plasmid replication in transfection experiments. We have been investigating one of these sequences intensively

because it is a member of a moderately reiterated sequence, often found in tandem in an arrangement reminiscent of bacterial transposons. We have identified a portion of this "O-family" sequence which is site-specifically protected by a protein present in cell extracts. We are currently attempting to demonstrate transposon-like activity for this sequence.

Nonheritable Antibiotic Resistance

We previously described the induction by salicylates and other weak acids of nonheritable antibiotic-resistance in Escherichia coli and other bacteria. Two mechanisms appear to underlie these effects. First, uptake of cephalosporins through the outer membrane into the periplasmic space is reduced 4- to 5-fold in E. coli treated with salicylate. Thus, salicylate affects the permeability of the outer membrane. Second, since some Gram positive bacteria (without an outer membrane) also show increased antibiotic-resistance in the presence of salicylate, another mechanism must be involved, possibly one that affects inner membrane function.

Additional effects of salicylate on E. coli have been demonstrated: decreased uptake of β-galactosides; altered susceptibility to kanamycin; interference with isoleucine-valine metabolism.

Replication of ColE1 DNA

Studies on the mechanism of ColE1 DNA replication and its regulation have been continued. A nascent transcript (RNA II) made by RNA polymerase which starts 555 nucleotides upstream of the replication origin, forms a persistent hybrid with the template DNA near the origin. The hybridized transcript is cleaved by RNase H and used as the primer of DNA synthesis by DNA polymerase I. Functional RNA II has a unique secondary structure that folds in a specific tertiary conformation.

ColE1 DNA can also be maintained in bacteria lacking RNase H, indicating the presence of multiple modes of ColE1 DNA replication. Hybridization of RNA II with the template DNA is always required for initiation of DNA synthesis and the difference in the mode of replication lies in the way the hybridized RNA II is used. Hybridized RNA II is cleaved by RNase H to form a primer or used as a primer without cleavage by RNase H. Hybridization also creates a single-stranded region on the nontranscribed strand that can serve as the template for synthesis of the lagging strand. The latter mode of replication does not require DNA polymerase I and is inhibited by RNase H.

For synthesis of the lagging strand DNA of plasmid ColE1, hybridization of the primer transcript (RNA II) with the template DNA is necessary. The hybridization creates a single-stranded region on the nontranscribed strand starting at 17 nucleotides upstream of the normal replication origin. When a stretch of 20 deoxyadenosine residues is inserted into the template strand, the hybridized transcript terminates in the stretch and determines the downstream limit of the displaced region. For efficient plasmid replication in bacteria lacking both RNase H and DNA polymerase I and for efficient synthesis of lagging strand in extracts from these bacteria, the single-stranded region should be at least 45 nucleotides long. No specific nucleotide sequence is required in the region downstream of the replication origin, but a functional region of a minimum

length cannot accommodate a long palindrome. The single-stranded region probably provides a site for initial binding of a helicase for further unwinding.

Termination of Transcription

Most primer transcripts (RNA II) elongating on plasmid ColE1 form persistent hybrids with the template DNA near the replication origin. When a stretch of 20 deoxyadenosine residues is inserted into the template strand downstream of the origin, about 95% of the hybridized transcripts and about 10% of unhybridized transcripts terminate at the insert. Most transcripts terminate within the first 10 deoxyadenosine residues. Nonetheless, when the number of inserted residues is 10, the efficiencies of termination of hybridized and unhybridized transcripts are reduced to about 60% and 3%, respectively. We conclude that both hybridized and unhybridized transcripts terminate at similar positions in the dA inserts although the efficiency of termination is much higher for hybridized transcripts, that the DNA sequence beyond the termination site is involved in termination, and that transcription terminates by detachment of the RNA polymerase but not of the RNA product from the template DNA.

Energy Conversion in Biology

A large number of different topics have been studied in the general field of free energy transduction. The most important areas in which progress has been made are the study of oscillations in microtubule growth dynamics, the role of kinesin in fast axonal transport, free energy transduction by random fluctuations, proton pumping in oxidative phosphorylation, kinetic schemes for DNA gyrase, and cooperativity in cytochrome oxidase.

Statistical Thermodynamics of Protein and Polynucleotide Systems

Statistical mechanics was used to derive the binding isotherm for a ligand on a one-dimensional polymer when ligand-ligand interactions extend over an arbitrary number of sites. The main application is to large proteins binding on DNA.

Studies of Immunoglobulin Gene Rearrangement

The level of immunoglobulin gene V-J recombination activity in cell lines derived from lymphoid or nonlymphoid lineages was examined. The assay uses an extrachromosomal DNA substrate, and thus avoids difficulties associated with the use of chromosomally integrated DNA. The recombination activity is found to vary progressively during B lymphoid development. It is low at a very early stage (pro-GMB), much higher in pro-B cells, but then declines through later stages, reaching an undetectable level in mature B cells. The activity is also present in multi-potential progenitors of myeloid cells, and in pre-T but not mature T cells. No activity was found in several nonhematopoietic cell lines.

Studies of Complexes Between DNA Gyrase and DNA

Complexes of DNA gyrase with defined DNA fragments, previously studied by electro-dichroism, have been further investigated by neutron scattering and dynamic light scattering. The results are compatible with the previously

proposed model of a single loop of DNA of 110 base pairs bound to the enzyme, with tails of DNA emerging which become folded back onto the protein core when ATP or one of its nonhydrolyzable analogs is added.

Nucleotide processing by DNA gyrase has also been studied. We found that under certain conditions the supercoiling reaction displays a great deal of "slip", in that the limiting supercoiling is well below the level which would fully utilize the free energy of hydrolysis of ATP. A kinetic analysis of this situation has been devised.

Studies on the Mechanism of Genetic Recombination

The major objective of this project is to uncover the enzymatic steps involved in various genetic rearrangement reactions and to study the mechanism of action of the enzymes involved. We are currently concentrating our efforts on the mechanism of the transposition-replication reaction of bacteriophage Mu.

By making use of a cell-free reaction system we developed several years ago, we have been able to divide the transposition reaction into two separate steps:

(1) The first step involves a pair of DNA strand transfers which generate an intermediate DNA molecule with a branched structure. The formation of the intermediate can be carried out by three purified protein factors; Mu A, Mu B and E. coli HU proteins. The Mu A protein binds to the Mu end DNA sequence specifically, and carries out the phosphodiester bond cleavage and joining steps. The Mu B protein possesses an ATPase activity which is stimulated by Mu A protein and DNA, and selectively stimulates the utilization of intermolecular target DNA molecules which do not carry Mu end sequences. The reaction requires a transposon donor molecule which has two Mu end sequences in their proper relative orientation and is negatively supercoiled, while the transposition target DNA can be in relaxed form. Evidence was obtained which indicates that recognition of the relative orientation of the two Mu end DNA sequences makes use of the energy of DNA supercoiling and requires a specific geometry of the Mu end DNA segments within the synaptic complex.

(2) Intermediate DNA molecules can be converted into cointegrates by DNA replication or into simple inserts by nucleolytic cleavages and gap repair. This second reaction is supported by an E. coli cell extract and does not require Mu proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 33000-21 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies of Functions Involved in Genetic Recombination

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

PI: Martin Gellert, Chief, Section on Metabolic Enzymes LMB/NIDDK

Others: James Tamura, Guest Worker LMB/NIDDK
Mary H. O'Dea, Research Chemist LMB/NIDDK
Hans Westerhoff, Guest Worker LMB/NIDDK

COOPERATING UNITS *(if any)*

Dr. G. Zaccai, Institut Max Von Laue-Paul Langevin, Grenoble, France
Dr. A. Maxwell, University of Leicester, Leicester, U.K.
Ms. S. Krueger, University of Maryland, College Park, MD

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Metabolic Enzymes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Complexes of DNA gyrase with defined DNA fragments, previously studied by electro-dichroism, have been further investigated by neutron scattering and dynamic light scattering. The results are compatible with the previously proposed model of a single loop of DNA of 110 base pairs bound to the enzyme, with tails of DNA emerging which become folded back onto the protein core when ATP or one of its nonhydrolyzable analogs is added.

Nucleotide processing by DNA gyrase has also been studied. We found that under certain conditions the supercoiling reaction displays a great deal of "slip", in that the limiting supercoiling is well below the level which would fully utilize the free energy of hydrolysis of ATP. A kinetic analysis of this situation has been devised.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 33001-03 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies of Immunoglobulin Gene Rearrangement

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

PI:	Martin Gellert	Chief, Section on Metabolic Enzymes	LMB/NIDDK
	Kiyoshi Mizuuchi	Visiting Scientist	LMB/NIDDK
Others:	Joanne Hesse	Research Chemist	LMB/NIDDK
	Michael Lieber	Guest Worker	LMB/NIDDK
	Tommie McCarthy	Guest Worker	LMB/NIDDK
	Susanna Lewis	Guest Worker	LMB/NIDDK
	Tamio Fujiwara	Guest Worker	LMB/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION

Section on Metabolic Enzymes

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	6	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The level of immunoglobulin gene V-J recombination activity in cell lines derived from lymphoid or nonlymphoid lineages was examined. The assay uses an extrachromosomal DNA substrate, and thus avoids difficulties associated with the use of chromosomally integrated DNA. The recombination activity is found to vary progressively during B lymphoid development. It is low at a very early stage (pro-GMB), much higher in pro-B cells, but then declines through later stages, reaching an undetectable level in mature B cells. The activity is also present in multi potential progenitors of myeloid cells, and in pre-T but not mature T cells. No activity was found in several nonhematopoietic cell lines.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 33002-01 LMB

PERIOD COVERED: October 1, 1986 to September 30, 1987

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

Effects of DNA Supercoiling on the Topological Properties of Nucleosomes

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

PI:	Martin Gellert	Chief, Section on Metabolic Enzymes	LMB/NIDDK
	Gary Felsenfeld	Chief, Section on Physical Chemistry	LMB/NIDDK
Others:	Mark M. Garner	Guest Worker	LMB/NIDDK
	Mary H. O'Dea	Research Chemist	LMB/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Molecular Biology

SECTION: Section on Metabolic Enzymes

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In the nucleosome core particle, at least 145 base pairs of DNA are bound to the histone octamer in a superhelical conformation. We have asked what effect the presence of these particles has on the ability of DNA gyrase to supercoil DNA. Synthetic minichromosomes, constructed by reconstituting complexes of core histones with the closed circular plasmid pBR322, were treated with various amounts of DNA gyrase. We have found that the maximum level of supercoiling that is attainable is nearly identical for protein-free plasmids and for plasmids half-saturated with core histones, even though supercoiling does not result in a loss of histones from the complex. It appears that, at sufficiently high levels of supercoiling, the core particle is disrupted in such a way that the DNA bound to histones is no longer constrained.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 33006-09 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	Kiyoshi Mizuuchi,	Visiting Scientist	LMB/NIDDK
Others:	K. Adzuma	Visiting Fellow	LMB/NIDDK
	R. Craigie	Visiting Associate	LMB/NIDDK
	M. Mizuuchi	Visiting Fellow	LMB/NIDDK
	T. Fujiwara	Guest Worker	LMB/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

CHECK APPROPRIATE BOX(ES)

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

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

The major objective of this project is to uncover the enzymatic steps involved in various genetic rearrangement reactions and to study the mechanism of action of the enzymes involved. We are currently concentrating our efforts on the mechanism of the transposition-replication reaction of bacteriophage Mu.

By making use of a cell-free reaction system we developed several years ago, we have been able to divide the transposition reaction into two separate steps:

(1) The first step involves a pair of DNA strand transfers which generate an intermediate DNA molecule with a branched structure. The formation of the intermediate can be carried out by three purified protein factors; Mu A, Mu B and E. coli HU proteins. The Mu A protein binds to the Mu end DNA sequence specifically, and carries out the phosphodiester bond cleavage and joining steps. The Mu B protein possesses an ATPase activity which is stimulated by Mu A protein and DNA, and selectively stimulates the utilization of intermolecular target DNA molecules which do not carry Mu end sequences. The reaction requires a transposon donor molecule which has two Mu end sequences in their proper relative orientation and is negatively supercoiled, while the transposition target DNA can be in relaxed form. Evidence was obtained which indicates that recognition of the relative orientation of the two Mu end DNA sequences makes use of the energy of DNA supercoiling and requires a specific geometry of the Mu end DNA segments within the synaptic complex.

(2) Intermediate DNA molecules can be converted into cointegrates by DNA replication or into simple inserts by nucleolytic cleavages and gap repair. This second reaction is supported by an E. coli cell extract and does not require Mu proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34001-22 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Gary Felsenfeld, Chief, Section on Physical Chemistry LMB/NIDDK

OTHERS:

Stephen Clark, Visiting Fellow	LMB/NIDDK	Catherine Lewis, Staff Fellow	LMB/NIDDK
Mark Garner, Guest Worker	LMB/NIDDK	Mark Minie, Staff Fellow	LMB/NIDDK
Hannah Gould, Expert	LMB/NIDDK	Joanne Nickol, Research Chemist	LMB/NIDDK
P. David Jackson, Chemist	LMB/NIDDK	Marc Reitman, Research Chemist	LMB/NIDDK
Takeshi Kimura, Visiting Fellow	LMB/NIDDK		

COOPERATING UNITS *(if any)*

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NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

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

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

We have continued our studies of chromatin structure in the neighborhood of expressed genes, making use of the globin gene family in chicken erythrocytes as a model system. We have examined in detail the structure of the nuclease hypersensitive domain in the 5' flanking region of the adult β globin gene isolated from cells in which that gene is expressed. We have shown that multiple sequence-specific factors are bound in this region, and we have partially purified and characterized some of them. Deletion analysis shows that at least one of these factors is a negative regulatory element that depresses β globin gene transcription, while another is stimulatory. We have also analyzed the enhancer domain at the 3' side of this gene, which we had discovered earlier. The DNA positive regulatory elements of the enhancer are contained in a region 136 base pairs long, and four discrete sets of cellular factors have been shown by footprint analysis to bind there. Using the primary erythrocyte transfection techniques which we developed, we have shown that the enhancer, in combination with the β promoter, functions less effectively in 4 day embryonic erythrocytes than in 9 day erythrocytes, strongly suggesting that the enhancer/promoter combination is developmentally specific. We have continued the refinement of the transfection techniques, and determined details of the transfection mechanism. We have also continued physicochemical studies of chromatin structure. We have shown that when an alternating G-C DNA segment contained in a plasmid is converted to the left-handed Z form by supercoiling, there is a shift in the placement of nucleosome core particles assembled on the plasmid, in such a way that the Z DNA segment is excluded from the central portion of the nucleosome. The result suggests that this may be a general property of non-B DNA structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34002-23 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	David R. Davies, Chief, Section on Molecular Structure		LMB/NIDDK
Others:	Gerson H. Cohen	Research Chemist	LMB/NIDDK
	Craig Hyde	Staff Fellow	LMB/NIDDK
	Kaza Suguna	Visiting Fellow	LMB/NIDDK
	Eduardo Padlan	Expert	LMB/NIDDK

COOPERATING UNITS *(if any)*
Edith Miles, LBP, NIDDK
W. Carlson, Harvard University, Cambridge, MA
Clark Smith, Upjohn Co. Research, Kalamazoo, MI

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Section on Molecular Structure

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NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

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

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

1) New inhibitor X-ray data have been measured for the aspartyl proteinase from Rhizopus chinensis. A mechanism of action has been proposed.

2) The crystal structure of tryptophan synthase from Salmonella typhimurium has been determined at 2.3A resolution and is currently being refined. Preliminary analysis of the structure accounts for the behavior of many mutants. It also shows how such a double enzyme system can be catalytically advantageous.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 34003-19 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Three-Dimensional Structure of Proteins of the Immune System

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

PI:	David R. Davies,	Chief, Section on Molecular Structure	LMB/NIDDK
Others:	T. N. Bhat	Visiting Scientist	LMB/NIDDK
	Gerson H. Cohen	Research Chemist	LMB/NIDDK
	Enid W. Silverton	Research Chemist	LMB/NIDDK
	Eduardo A. Padlan	Special Expert	LMB/NIDDK
	Steven Sheriff	Staff Fellow	LMB/NIDDK
	Christina John	Special Volunteer	LMB/NIDDK

COOPERATING UNITS *(if any)*

Sandra Smith-Gill, National Cancer Institute, NIH

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Section on Molecular Structure

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

1) The high resolution structure of J539 has been refined. All the amino acid residues have been located with the exception of three that appear to be disordered.

2) The three-dimensional structure of two crystal forms of a complex between lysozyme and the Fab of a monoclonal antibody to lysozyme have been determined. The method used was molecular replacement in which the known structures of the Fab and of lysozyme were used as probes to determine their orientations and positions in the new crystal.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 35000-23 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Chemical and Structural Investigations of Nucleic Acids and Related Molecules

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

PI: H. Todd Miles, Chief, Section on Organic Chemistry LMB/NIDDK

Others: F. B. Howard Research Chemist LMB/NIDDK
J. Frazier Research Chemist LMB/NIDDK
H. Miyashiro Visiting Fellow LMB/NIDDK

COOPERATING UNITS *(if any)*

Girjesh Govil, Tata Institute Fundamental Research, Bombay, India

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Laboratory of Molecular Biology

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Section on Organic Chemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

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

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

This project has the objective of understanding the chemistry and structure of nucleic acids and relating this knowledge to the biological functions of these molecules. Methods used include chemical synthesis of defined sequence DNA fragments and of enzyme substrates, enzymatic synthesis of polynucleotides, study of nucleic acids by circular dichroism, ultraviolet, infrared, and nuclear magnetic resonance spectroscopy, study of thermal transitions and dependence of physical properties on solution conditions. Subjects of investigation include factors which determine the stability of helical complexes, specificity of nucleic acid interactions, location and affinity of binding sites.

We have continued our collaborations on 2D NMR of DNA fragments containing restriction endonuclease recognition sequences. Resonance assignments have been obtained for all the nonexchangeable protons in the dodecanucleotide d(GAATTCGAATTC) and approximate sugar conformations and glycosidic dihedral angles determined. The molecule has a B-DNA conformation with both strands identical. A new heteronuclear ^{1}H-^{31}P shift correlation method was used to assign all of the ^{31}P resonances in the oligonucleotide d(CATGCATm^{5}CCATG).

Information on nucleic acid mispairing is relevant to such important biological functions as mutation, gene expression and control, splicing, and feedback control. We have begun examination of a series of oligonucleotides small enough to permit analysis of structural and energetic changes caused by introduction of selected mispairings. An initial finding of importance is that the position of AG mispairing is crucial for its effect on helix stability. Surprisingly, two AG's replacing two CG's in the center of a dodecamer have little effect on T_m, whereas the same substitutions two positions removed from the center have a large effect. A similar result is observed with AI pairing. Preliminary work with AC mispairing shows that it is strongly destabilizing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 35050-16 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Replication, Recombination and Repair of Microbial DNA

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

PI:	J. Tomizawa	Chief, Section on Molecular Genetics	LMB/NIDDK
Others:	H. Masukata	Visiting Associate	LMB/NIDDK
	S. Nakasu	Visiting Fellow	LMB/NIDDK
	M. Brenner	Expert	LMB/NIDDK

COOPERATING UNITS *(if any)*

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SECTION
Section on Molecular Genetics

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NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

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

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

Studies on DNA replication of plasmid ColE1 have been continued. Transcripts (RNA II) that start 555 nucleotides upstream of the replication origin by RNA polymerase form a hybrid with the template DNA. The hybridized transcript is cleaved by ribonuclease H at the origin and used as the primer for leading strand synthesis by DNA polymerase I.

ColE1 DNA can replicate also in the absence of the RNase H and DNA polymerase I. RNA II hybridized with the template DNA displaces the nontranscribed strand. This allows synthesis of the lagging strand on the displaced single-strand. Because this mechanism involves formation of hybrid between RNA II and the template DNA, it is subjected to the negative regulation by RNA I, an antisense RNA, as DNA replication in the presence of RNAase H and DNA polymerase I.

The primer transcripts that had extended beyond the normal origin terminated at various positions. A correlation was found between spontaneous termination of RNA II at specific positions and hybrid formation between the transcripts and the template DNA. When we inserted a stretch of dA residues at various positions of the template strand downstream of the origin we found a large fraction of the transcripts hybridized with the template DNA terminated at the stretch while unhybridized transcripts terminated much less efficiently. Studies on termination of these transcripts give important information on ρ-independent termination: involvement of DNA sequences beyond the actual sites of termination separation of RNA polymerase as the mechanism of termination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 36002-13-LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Novel Recombination Systems

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

PI:	J.L. Rosner	Research Biologist	LMB/NIDDK
Others:	R. Khanna	Visiting Fellow	LMB/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

N DDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 36003-03 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Nonheritable Antibiotic Resistance

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

PI:	J.L. Rosner	Research Biologist	LMB/NIDDK
Others:	D.M. Murray	Biologist	LMB/NIDDK
	J.D. Foulds	Research Biochemist	LSB/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Molecular Biology

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.25	2.25	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We previously described the induction by salicylates and other weak acids of nonheritable antibiotic-resistance in Escherichia coli and other bacteria. Two mechanisms appear to underlie these effects. First, uptake of cephalosporins through the outer membrane into the periplasmic space is reduced 4-5 fold in E. coli treated with salicylate. Thus, salicylate affects the permeability of the outer membrane. Second, since some Gram positive bacteria (without an outer membrane) also show increased antibiotic-resistance in the presence of salicylate, another mechanism must be involved, possibly one that affects inner membrane function.

Additional effects of salicylate on E. coli have been demonstrated: decreased uptake of β-galactosides; altered susceptibility to kanamycin; interference with isoleucine-valine metabolism.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 36051-19 LMB

PERIOD COVERED
October 2, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Origins of Mammalian DNA Replication in Normal and SV40 Transformed Cells

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

PI:	Robert G. Martin,	Chief, Section on Microbial Genetics	LMB/NIDDK
Others:	R. L. Lechner	Senior Staff Fellow	LMB/NIDDK
	B. S. Rao	Visiting Fellow	LMB/NIDDK
	S. S. Wang	Research Chemist	LMB/NIDDK

COOPERATING UNITS *(if any)*
Foreign: M. Zannis-Hadjopoulos, McGill Cancer Center, Montreal, Canada
G. Kaufmann, Tel Aviv University, Tel Aviv, Israel
H. Manor, Technicon U. Haifa, Israel

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INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

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

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

The DNA sequence $(GA)_n.(CT)_n$ has been found to slow fork progression in monkey cells. It is suggested that this sequence may therefore play an important role in gene amplification.

Several of the sequences we previously isolated as potential origins of replication have been shown to stimulate plasmid replication in transfection experiments. We have been investigating one of these sequences intensively because it is a member of a moderately reiterated sequence, often found in tandem in an arrangement reminiscent of bacterial transposons. We have identified a portion of this "O-family" sequence which is site specifically protected by a protein present in cell extracts. We are currently attempting to demonstrate transposon-like activity for this sequence.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 36101-13 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Terrell L. Hill, Chief, Section on Theoretical Molecular Biology, LMB, NIDDK

Others:
Y. Chen, R.C., LMB/NIDDK
H.V. Westerhoff, G.W., LMB/NIDDK
F. Kamp, V.F., LMB/NIDDK
R.J. Rubin, Expert, LMB/NIDDK
R.D. Astumian, S.F., LB/NHLBI
R.W. Hendler, Sect. Chief, LCB/NHLBI
D. Juretic, S.F., LCB/NHLBI
A. Szabo, R.C., LCP/NIDDK

COOPERATING UNITS *(if any)*
M.F. Carlier, CNRS, France
K. van Dam, Univ. Amsterdam, Netherlands
A.K. Groen, Univ. Amsterdam, Netherlands
R. Wanders, Univ. Amsterdam, Netherlands
P. Plomp, Univ. Amsterdam, Netherlands
D.B. Kell, Univ. College Wales, Aberystwyth, UK
G.R. Welch, Univ. New Orleans
T.Y. Tsong, J. Hopkins Sch. Med., Balt.

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	4.7	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 large number of different topics have been studied in the general field of free energy transduction. The most important areas in which progress has been made are the study of oscillations in microtubule growth dynamics, the role of kinesin in fast axonal transport, free energy transduction by random fluctuations, proton pumping in oxidative phosphorylation, kinetic schemes for DNA gyrase, and cooperativity in cytochrome oxidase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36102-16 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Statistical Thermodynamics of Protein and Polynucleotide Systems

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

PI: Terrell L. Hill, Chief, Section on Theoretical Molecular Biology, LMB, NIDDK

OTHERS: Y. Chen Research Chemist LMB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Theoretical Molecular Biology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Statistical mechanics was used to derive the binding isotherm for a ligand on a one-dimensional polymer when ligand-ligand interactions extend over an arbitrary number of sites. The main application is to large proteins binding on DNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 36104-06 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	P.D. Ross	Research Chemist	LMB/NIDDK
Others:	A.C. Steven	Visiting Scientist	LPB/NIDDK
	W.A. Hagins	Research Chemist	LCP/NIDDK
	A. Shrake	Research Chemist	DBBP/CDB

COOPERATING UNITS *(if any)*
Lindsay W. Black, Univ. Maryland Medical School, Baltimore, MD
R. Burchard, Dept. Biological Sciences, Univ. Maryland, Catonsville, MD
W. Kirchoff, Chem. Thermodynamics Div., Natl. Bureau Standards, Gaithersburg, MD

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	4	0

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

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

Emphasis has been directed toward extracting information from the shape of differential scanning calorimetry (DSC) curves. Theoretical thermograms have been constructed for discrete polymerization and depolymerization reactions so that one may, for example, by comparison with experiment determine the number of subunits in an oligomeric protein. This analysis has been implemented in a computer program which is available for other researchers.

In addition to DSC measurements on well defined polyribonucleotide and protein (HSA) systems which are described in detail under Major Findings, work has been carried out on more complex biological membranes and viral capsid particles.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 36105-05 LMB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Influences of Macromolecular Crowding on Biochemical Systems

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

PI:	S.B. Zimmerman	Research Chemist	LMB, NIDDK
Others:	B. Harrison	research Chemist	LMB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

CHECK APPROPRIATE BOX(ES)

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

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

A patent covering the polymer-stimulated ligation of DNA has been issued.

Macromolecular crowding has been used to obtain efficient phosphorylation by T4 polynucleotide kinase of types of termini in duplex DNA -- such as termini at "nicks" or recessed termini -- which are otherwise only very slowly or incompletely labeled under conventional procedures.

Macromolecular crowding was found to extend the range of ionic conditions which support high DNA polymerase reaction rates. High concentrations of nonspecific polymers increased polymerase activity under otherwise inhibitory conditions resulting from relatively high ionic strength. The primary mechanism of the polymer effect seems to be to increase the binding of polymerase to DNA. We have suggested that this effect on protein-DNA complexes is only one example of a general "metabolic buffering" action of crowded solutions on a variety of macromolecular interactions.

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

ANNUAL REPORT OF THE METABOLIC DISEASES BRANCH
National Institute of Diabetes and
Digestive and Kidney Diseases

The general goals of the Branch are to investigate the mechanism of action of hormones controlling ion transport and mineral metabolism and to investigate the immunological and pathological factors mediating kidney disorders. The branch currently includes sections of Molecular Pathophysiology (Dr. Spiegel), Mineral Metabolism (Dr. Marx), Endocrine Regulation (Dr. Aurbach) and Kidney Disease (Dr. Balow). Integration of these sections is related to common interests in the pathophysiology of metabolic disorders which interface with the kidney. Systems under study include renal and skeletal tissue, transgenic mice, isolated cells (kidney and parathyroid) in culture, hormone receptors (beta adrenergic, parathyroid hormone, and 1,25 dihydroxy vitamin D), parathyroid cell growth factors, regulatory proteins of the adenylate cyclase complex, and T cell and B cell function in disorders of immunoregulation.

Analysis of Hormone Receptor

Interactions of catecholamines with adrenergic receptors and activation of adenylate cyclase are under study with the plasma membranes of several cell systems. Specific receptors have now been identified on turkey erythrocytes, parathyroid cells, pineal cells, rat, guinea pig and monkey lung membrane preparations, rat osteosarcoma cells and rat liver membranes. Control of receptor accumulation in isolated cell culture systems in vitro are being studied with a view toward gaining knowledge about the molecular biology of receptors and how they are linked to intracellular response systems.

Calcitonin has been shown to decrease intracellular cAMP at concentrations 300-fold lower than those that increase cAMP [Drs. Barsony, Marx].

Receptors for Parathyroid Hormone

Studies in collaboration with Dr. T. Murray (St. Michael's Hospital, Toronto) have led to development of radiolabeled intact bovine parathyroid hormone as a ligand. The radiolabeled agonist binds to receptors in canine renal

plasma membranes or in cultured osteosarcoma cells with kinetic properties distinct from those of radiolabeled synthetic amino terminal bioactive fragments.

The radioiodinated parathyroid hormone binds to specific receptors on cultured rat osteosarcoma cells and interaction with these receptors correlates well with stimulation of cyclic AMP production in this cell system.]

Signal Transduction by Guanine Nucleotide Binding Proteins (G-proteins)

A family of guanine nucleotide binding proteins (G-proteins) functions in transmembrane signalling as receptor-effector couplers. G proteins couple to a diverse array of receptors including those for hormones, neurotransmitters, light, odorants, and certain growth factors. Effector functions regulated (positively and, in some instances, negatively) by G-proteins include cAMP formation, phosphoinositide breakdown, potassium and calcium channels, and cGMP degradation. We have used a variety of techniques to study the expression, distribution, regulation, structure, and function of G-proteins. A brief summary of the major findings in each area follows:

A. Molecular Biologic Studies - We have isolated cDNAs for human G_s-alpha, and 1 form of G_i-alpha and determined their sequence. We have defined the existence of distinct forms of G_i-alpha as separate gene products with different distribution in brain and peripheral tissues. Evolutionary conservation of nucleotide sequence in untranslated portions of mRNA for each G-alpha subunit suggests a regulatory role for this portion of mRNA and provides a convenient marker for distinguishing G-alpha subtypes [Drs. Carter, Spiegel].

B. Immunochemical Studies - Antisera raised by immunization with synthetic peptides have proved useful in studying the distribution of different G-alpha subunits in various tissues. One such antiserum identifies a 40 kDa pertussis toxin substrate highly abundant in neutrophils as the protein encoded by a cDNA (G_i-alpha2) not previously linked to any purified G protein. Western blotting and immunohistochemical studies with specific antisera have allowed us to define developmental and differentiation dependent changes in G-protein expression [Drs. Goldsmith, Spiegel].

C. Protein Purification and Reconstitution - We have isolated a novel G protein from bovine brain and identified it with specific antisera and other methods as equivalent to the major pertussis toxin substrate purified from bovine neutrophils. Another novel G protein purified from bovine brain appears to represent a form of G_o differing in charge (perhaps due to differences in post translational

modifications). Studies in which these and other purified G proteins are reconstituted with purified receptors and effectors help define the specificity of G-protein interactions. Similar studies, also employing recombinant DNA techniques, with recently cloned muscarinic receptor cDNAs also should facilitate defining the basis for receptor G protein coupling [Drs. Goldsmith, Brann, Spiegel].

Our studies highlight the diversity within the G-protein family, provide the basis for understanding the role of G-proteins in normal signal transduction, and for elucidating possible defects in G-protein structure or function as the basis for abnormal signal transduction.

Pseudohypoparathyrodism (PHP)

Subjects with PHP and the phenotypic features of Albright's hereditary osteodystrophy (AHO) generally show resistance to multiple hormones. Previous studies identified the molecular defect in such subjects (PHP Ia) as a deficiency in the activity of the stimulatory guanine nucleotide binding protein (G_s) associated with adenylate cyclase. Using cloned cDNA probes for the alpha (guanine nucleotide binding) subunit of G_s, we now find reduction in steady-state mRNA levels in Northern blots of total fibroblast RNA from subjects with PHP Ia compared with normal subjects. These studies show that reduced synthesis of G_s-alpha is the likely basis for deficient G_s activity in PHP Ia. Studies in progress, including cloning of genomic DNA for G_s-alpha and analysis of Southern blots from subjects with PHP Ia, should elucidate the molecular basis for this inherited form of hormone resistance. [Drs. Spiegel and Carter, NIDDK].

Primary Hyperparathyroidism and Familial Hypercalcemia

Clinical studies are continuing on primary hyperparathyroidism and its familial variants. Detailed family screening and case findings has produced approximately 65 kindreds for analysis. These studies have allowed segregation of the commonest familial variants into two apparently distinct disease syndromes - familial multiple endocrine neoplasia type 1 (FMEN I) and familial hypocalciuric hypercalcemia (FHH). FHH was distinguished from FMEN I by 1) virtually a 100% penetrance for hypercalcemia before age 20, 2) milder clinical manifestations - low incidence of recurrent nephrolithiasis or recurrent peptic ulceration, 3) no hypercalciuria, 4) normal basal concentrations of gastrin, and 5) poor response to subtotal parathyroidectomy. Distinction between the two syndromes, both inherited as autosomal dominant traits, is important because in FHH the clinical course is generally milder and subtotal parathyroidectomy is less likely to be

beneficial. FHH accounts for approximately 10% of all unsuccessful parathyroidectomies in hypercalcemia. In FHH the ionized and ultrafiltrable calcium concentration in serum are elevated in proportion to the increase in total calcium. In these patients the filtrable load of calcium is high in association with a marked decrease in renal calcium clearance. Even when these patients become surgically hypoparathyroid, the low renal clearance of calcium is strikingly persistent during calcium infusion. The concentration of parathyroid hormone in plasma is lower in patients with FHH than in typical primary hyperparathyroid patients with similar degrees of hypercalcemia whether assessed by PTH radioimmunoassay or by renal clearance of cAMP or phosphate. The parathyroid glands show hyperplasia in most cases. In several kindreds one or more members have exhibited life-threatening primary hyperparathyroidism in the neonatal period. This may result sometimes from a double dose of the FHH gene. Dispersed parathyroid cells from one severely affected neonate showed a striking decrease in sensitivity of PTH secretion to extracellular calcium [Drs. Marx, Spiegel, Fitzpatrick, and Aurbach].

Familial multiple endocrine neoplasia type 1 (FMEN1) is an autosomal dominant disorder characterized by hyperfunction of parathyroids, pancreatic islets, and anterior pituitary. Affected organs show features suggestive of increased proliferation. Virtually all subjects expressing the gene show primary hyperparathyrodism. Primary hyperparathyroidism is usually first recognizable between ages 20-40, and it shows a high recurrence rate after subtotal parathyroidectomy (approximately 50% after 10 years). We have evaluated multiple indices for use in screening in a very large kindred. We tested 221 members and newly identified 16 as carriers. Albumin-adjusted calcium and PTH were most useful; gastrin and prolactin analyses were not useful for screening but showed promise in followup of known carriers. Analysis in this family has so far excluded close genetic linkage to 13 polymorphic biochemical markers [Drs. Marx, S. Bale, A. Bale, Mulvill, Sparkes].

With cultured bovine parathyroid cells, we found abnormally high mitogenic activity in plasma from 23 of 27 subjects with FMEN1. Well-characterized growth factors or known parathyroid secretagogues showed far less parathyroid mitogenic activity than these FMEN1 plasmas. The mitogenic factors(s) appear to be a protein of 50,000 mw. We have begun purifying this factor for further characterization. [Drs. Brandi, Sakaguchi, Fitzpatrick, Aurbach, Goldsmith, Spiegel, Bliziotes, Nanes, Marx].

Studies on noninvasive and invasive modes of localizing parathyroid tumors continue. Parathyroid adenoma localization has been evaluated using the new non-invasive magnetic resonance imaging technique (Drs. Aurbach, Marx,

Spiegel, Bliziotes, Nanes, Fitzpatrick, NIDDK; Dr. Miller, Dr. Doppman, Dr. Shawker Diagnostic Radiology). Initial results were disappointing but the acquisition of a specialized neck collar has led to better resolution in the paratracheal and mediastinal areas. Patients are currently under evaluation with this new technique. A high degree of success has been obtained in localizing tumors through vascular catheterization procedures. Parathyroid arteriography developed and performed by Dr. John Doppman afforded, in approximately 45% of cases tested, the identification of abnormal masses of tissue proven at surgery to be parathyroid. In the most difficult cases, localization of parathyroid tissue can be aided by identifying high concentrations of parathyroid hormone by radioimmunoassay in veins draining the lesion. Fine needle aspiration is another new method that can obviate other invasive localization procedures. We have aspirated with guidance by computerized tomography or ultrasound approximately 20 such lesions that were subsequently confirmed surgically as parathyroid. RIA of the aspirates showed high concentrations of PTH in all but one. Eight mediastinal adenomas have been treated nonsurgically by percutaneous injection via catheter of occlusive agents into the arterial blood supply with 7 complete and one partial remissions. [Drs. Aurbach, Marx, Spiegel, Fitzpatrick, Bliziotes, Nanes, Zimering, Weinstein, Streeten, NIDDK: Dr. Norton, NCI, Drs. Doppman and others, Diagnostic Radiology, CC].

Rapid determination of intraoperative UcAMP excretion (using the Gammaflo machine for rapid cAMP radioimmunoasay) has proven to be a valuable tool in guiding surgery for primary hyperparathyroidism, particularly in patients with multigland disease. Persistent elevation of UcAMP requires continued search for abnormal tissue even after 1 or more abnormal glands have been removed. A rapid (mean 1.5 hours) drop in UcAMP to the normal range obviates the need for continued exploration even in cases where histologic confirmation of parathyroidectomy is lacking. Spurts in UcAMP above baseline may provide a clue to the location of abnormal parathyroid tissue. [Drs. Spiegel, Marx, Fitzpatrick, Bliziotes, Nanes, Zimering, Weinstein, Streeten, and Aurbach, NIDDK: Dr. Norton, NCI Surgery].

Determination of urinary cAMP excretion postoperatively in patients undergoing neck exploration for primary hyperparathyroidism is a useful method for assessing postoperative parathyroid function. UcAMP excretion declines postoperatively in all patients in whom hypercalcemia is corrected but not in those with persistent hypercalcemia. In patients becoming severely hypocalcemic (and requiring vitamin D therapy) postoperatively, UcAMP measurement enables one to distinguish patients with decreased parathyroid reserve as the cause for hypocalcemia

(low UcAMP excretion) from patients with healing osteitis fibrosa ("hungry bones" with secondary hyperparathyroidism) as the basis for hypocalcemia. UcAMP in the latter group is often elevated but can be suppressed if serum calcium is normalized. Elevated UcAMP excretion postoperatively in the face of hypocalcemia enables one to predict that vitamin D therapy will be required temporarily (if at all) and precludes the need for parathyroid autografts. [Drs. Spiegel, Marx, Fitzpatrick, Bliziotes, Zimering, Weinstein, Streeten, and Aurbach, NIDDK].

Postoperative patients with surgically corrected hyperparathyroidism are being actively evaluated in a five year follow up study (Dr. Udelsman, Norton NCI, Drs. Marx, Fitzpatrick NIDDK). These patients are being studied for sequalae such as hypoparathyroidism, recurrent hyperparathyroidism, and complications such as vocal cord paralysis.

Secretion of Parathyroid Hormone

PTH secretion from parathyroid glands in vivo and cells in vitro is controlled by intracellular calcium and cyclic AMP. Control by calcium is altered in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Agents that alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP, but most of its effect to inhibit secretion is independent of changes in cellular cAMP.

Calcium inhibition of parathyroid hormone secretion was evaluated utilizing pertussis toxin as a probe. Pertussis toxin catalyzes ADP- ribosylation and inactivation of the inhibitory guanine nucleotide regulatory protein, N_i. Studies in dispersed bovine parathyroid cells indicates that calcium inhibition of parathyroid hormone secretion is mediated via N_i. Further studies with calcium channel agents show that calcium channels are involed in regulation of PTH secretion. Two enantiomers, (+)202-791 and (-) 202-791, were supplied by Sandoz, Ltd, Basle. The former is a calcium channel agonist and the latter, a calcium channel antagonist. The agonist (opens calcium channels, facilitating Ca entry) inhibits secretion. The antagonist stimulates secretion. Studies with pertussis toxin indicate that calcium channel regulation of secretion is linked through a guanine nucleotide regulatory protein [Drs. Fitzpatrick, Brandi, and Aurbach].

Further evidence that classical calcium channels of the "L" type are involved in regulating parathyroid hormone secretion has been obtained using antibodies against the skeletal muscle T-tubule calcium channel protein. One class of antibody acts as a calcium channel agonist; another type

inhibits the channel. Affinity purified antibodies of the agonist type open the channel and inhibit PTH secretion [Drs. Fitzpatrick, Chin, Nirenberg, Aurbach].

A bovine parathyroid cell culture line had been established earlier to study growth of cells and secretion therefrom. This system is proving to be a valuable in vitro model to study factors that stimulate or inhibit growth. Use of this cell system has facilitated identification of a parathyroid cell growth factor circulating in familial multiple endocrine neoplasia type I. In autoimmune hypoparathyroidism, an IGM has been found that causes complement- dependent cytotoxicity in parathyroid cells. [Drs. Aurbach, Brandi, Fitzpatrick, Sakaguchi, Zimering, Marx].

More recently we have obtained a cloned parathyroid cell line from rat parathyroid glands. These cells show many of the classical functions of the parathyroid in vivo: secretion of biologically active hormone; control by calcium; stimulation of cAMP and hormone release by secretin. A pituitary fraction was identified that stimulates growth of these cells [Drs. Sakaguchi, Brandi, Zimering, Aurbach].

Vitamin D Resistance and Related Disorders

The role of $1,25(OH)_2D_3$, the most potent natural metabolite of vitamin D, has been assessed in hypocalcemic states. This very rapidly acting drug has simplified the management of hypocalcemia following parathyroidectomy: during this time skeletal remineralization imposes large but rapidly diminishing requirements for calcium.

We have evaluated patients with extreme resistance to $1,25(OH)_2D$. This can be a transient state as following parathyroidectomy or a permanent state as in familial cases. We have evaluated 20 patients with familial resistance to $1,25(OH)_2D$. Most patients have hypocalcemic rickets, usually with associated total alopecia. The alopecia is associated with the highest grades of resistance to $1,25(OH)_2D$, implicating calcitriol in physiology of the hair follicle. Mineral homeostasis is usually improved by treatments that sustain $1,25(OH)_2D$ levels at 10-100 times normal. One patient had absent intestinal response to $1,25(OH)_2D$, documented with a new stable isotope technique (Drs. Yergey, Viera, Bliziotes, Nanes, Marx). Treatment with high doses of calcium intravenously each day for 4 months caused dramatic clinical improvement, showing that calcium could replace most functions of the 1,25(OH)2D receptor.

Specific intracellular defects have been evaluated using cultured skin fibroblasts from these patients. With

skin fibroblasts cultured from normals, a typical $1,25(OH)_2D$-receptor can be identified by binding in soluble extracts, by nuclear uptake of hormone with intact cells, or by elution of occupied receptor from DNA-cellulose. Fibroblasts from patients with familial resistance to $1,25(OH)_2D$ have shown a spectrum of defects including nonfunctional receptors, diminished numbers of receptors, and apparently normal receptors. Among cases with normal hormone binding sites on the receptors some show receptors with deficient binding to nucleus while others show normal binding to nucleus but abnormal interaction with nonspecific DNA (as DNA-cellulose). In one patient, osteoblast-like cells from bone biopsy exhibited a defect analogous to that in skin fibroblasts of the same patient. Even when receptors have unmeasurable hormone-binding activity, the receptor protein has been present in normal amounts according to immunoassay suggesting point mutations in the hormone-binding region. Cellular action of $1,25(OH)_2D_3$ can be analyzed by measuring its induction of the 25(OH)D 24-hydroxylase enzyme system. Cultured skin fibroblasts from all patients with hereditary resistance to $1,25(OH)_2D$ exhibit defects in this induction. [Drs. Marx, Bliziotes, Barsony, Brandi, Nanes, MDB, NIDDK; Dr. Liberman, Israel; Drs. Pike and Haussler, U. Arizona].

New world primates show resistance to many steroid hormones, including $1,25(OH)_2D$. EB virus transformed B lymphocytes from a new world primate showed receptors with lower affinity and capacity for $1,25(OH)_2D_3$ then in similar cells from old world primates (human or macaque) [Drs. Marx; Liberman, (Israel)].

The Kidney Disease Section research activities are focussed on the pathogenesis of immunologically mediated glomerular diseases. Lupus nephritis is the prototype of these diseases and is being intensively investigated from the perspective of the immunoregulatory abnormalities of systemic lupus erythematosus, the pathogenesis of the renal lesions in murine models and in human disease, as well as the therapeutic effect of immunosuppression. The clinical studies are being conducted in collaboration with the Arthritis and Rheumatism Branch of NIAMS.

I. Lupus Nephritis

A. Immunopathogenesis. Murine models are being utilized to investigate the different forms and components of lupus nephritis. The immunologic characteristics of the immune complex deposits and the lymphoid cell infiltration are being dissected by immunohistologic and electron microscopic techniques. The effects of various immunomodulating drugs on immunologic features and on the renal lesions are being investigated. Differences among the strains promise to enhance our understanding of the diverse manifestations and response to treatment of human lupus nephritis (Austin, Balow).

B. Immunoregulatory Studies. A multiplicity of T and B lymphocyte abnormalities have been found in patients with SLE. Heightened and poorly regulated B cell activity is characteristic of SLE. Defective T suppressor cell activity was found to be present in some but not all cases of active SLE. Moreover, T cytotoxic cell and natural killer cell activities are deficient and could permit the emergence of abnormal and unregulated autoantibody producing cells. An alternative immunoregulatory defect leading to excessive B cell activity has been noted in certain lupus mouse strains, namely, T helper cell hyperactivity. Our group has found increased numbers of circulating T cells bearing activation markers and proto-oncogene expression which function to increase immunoglobulin secretion by autologous B cells. It will be important to delineate whether different mechanisms underlie the heightened production of antibodies by B cells in different patients with lupus nephritis (Tsokos, Eleftheriades, Mitchell, Balow).

C. Proliferative lupus nephritis. Current protocols are designed to increase and refine the therapeutic index of different immunosuppressive drugs for lupus nephritis. Studies to date have shown that cytotoxic drugs are superior to conventional prednisone therapy and that intermittent high-dose therapy maintains efficacy while reducing toxicity. Patients with proliferative forms of lupus nephritis are being intensively treated with pulse methylprednisolone or pulse cyclophosphamide to compare these two types of drugs and also to assess whether intensity or duration of cyclophosphamide is more important in stabilizing the renal disease. Laboratory studies of lymphoid cell modulation by the different drug regimens are ongoing in order to improve monitoring, drug administration and efficacy (Balow, Austin, Webb and members of ARB, NIAMS).

D. Membranous lupus nephropathy. This form of lupus nephritis produces substantial morbidity from nephrotic syndrome and an insidious loss of renal function. Preliminary evidence indicates that the immunopathogenesis of membranous nephropathy is distinct from that of proliferative lupus nephritis. These studies will include examination of the pathophysiology and histopathology of membranous lupus nephropathy and evaluation of the comparative efficacy of corticosteroids, cyclosphosphamide and cyclosporin A in this disease (Balow, Webb, Austin).

II. Glomerulonephritis

A. Nephritic Factors. Patients with membranoproliferative glomerulonephritis and lupus nephritis develop autoantibodies to complement converting enzymes which cause abnormal consumption of complement components. These nephritic factors may participate in the pathogenesis of the renal diseases, but studies of their exact role has been hindered by lack of substantial quantities of pure preparations. Epstein-Barr virus transformed and sustained B cell lines which actively produce nephritic factors have been produced. One line from a patients with membranoproliferative glomerulonephritis secretes an IgG antibody which binds and stabilizes the alternate pathway C3 convertase enzyme. Another from a patient with lupus, binds the classical pathway C3 convertase. Nephritic factors with these functional activities correspond to known abnormalities of complement activation through the different pathways in these diseases. Studies of the binding sites, turnover and modulation of these autoantibodies are continuing (Tsokos, Thyphronitis, Balow).

B. Complement in Immune Regulation. Abnormal levels of complement components and deposition in sites of immunological reactions are characteristic of several forms of glomerulonephritis. The interactions of complement components and activation products with receptors on lymphoid cells are being studied to gain new insights into their potential role in lupus nephritis, membranoproliferative glomerulonephritis and other renal disorders. The precise role of complement receptors on B cell may be particularly relevant to the appearance of autoantibodies associated with these diseases. Studies are underway to determine the mechanism of the modulation of B cell responses through interaction of the complement receptor with natural complement ligands, Epstein-Barr virus and monoclonal antibodies (Tsokos, Thyphronitis, Pillemer, Balow).

C. Crescentic Glomerulonephritis. Rapidly progressive glomerulonephritis with severe crescent formation is being studied. Crescentic glomerulonephritis without significant antigen-antibody deposits is of unknown pathogenesis but may be caused by cell mediated immune injury. Abnormalities of systemic immune responses and characterization of local immune cell phenotypes within the renal lesions are being investigated. Therapeutic studies include a comparison of intensive pulse methylprednisolone versus cyclophosphamide in patients with idiopathic crescentic glomerulonephritis (Balow, Austin, Webb).

Studies of the mechanisms of glomerulosclerosis

This laboratory has been interested in the cellular mechanisms leading to the development of glomerular scarring. The hypothesis is that resident glomerular cells play a key role in glomerular sclerosis which is favored by an increase in transcapillary pressure. This is being studied using both in vivo and in vitro approaches. The work focuses on cell-cell interaction and their various responses to growth factors in health and disease, with a special emphasis on diabetes mellitus.

III. Glomerulosclerosis (L. Striker, G. Striker)

A. Transgenic mice. We have identified several lines of mice transgenic for the early region of simian virus 40 that develop glomerular abnormalities which resemble those seen in human focal glomerulosclerosis and are proteinuric. We have demonstrated that the renal lesions are due to the presence of T antigen in the kidney. Several lines of glomerular endothelial, mesangial and epithelial cells have been isolated and cloned. In addition glomerular cells from normal littermates have been isolated and are being characterized.

We are using these cells to understand some of the interaction of glomerular cells, and whether glomerulosclerosis results from an abnormal response to individual growth factors. (MacKay, Elliot, Striker, Striker)

B. Transfection of human glomerular cells. In order to study interaction between glomerular cells in vitro, we are developing lines of human glomerular cells. Primary outgrowth of human glomerular cells have been infected using a recombinant adeno-virus-SV40. We have obtained cells which are transfected as shown by positivity for T antigen. Epithelial and mesangial cells have been passaged multiple times. Establishment of stable human cell lines will allow study of glomerular functions in health and disease (Lange, Striker, Elliot, Striker, Doi, Bernstein).

C. Biology of insulin and IgFλ receptors in glomerular cells. We are currently study the binding of insulin and IgFλ on glomeruli and glomerular cells from normal mice. When these data are established, we will study the nature of the receptor and its possible modulation in mice who develop diabetes (N.O.D.) and glomerulosclerosis (transgenic mice). We are currently studing the binding of insulin and IgFλ on mesangial cells from normal mice. We have demonstrated that these cells have a surface receptor for IgF but not for insulin. In addition IgF has a mitogenic effect on these cells. (Conti, Striker, Elliot, Striker, MacKay).

D. Further characterization of mesangial cell biology. It has been claimed that mesangial cells have a receptor for Angiotensin II which is regulated by insulin. We are currently investigating this hypothesis using mouse mesangial cells. We have developed a line of smooth muscle cells derived from the aorta of mice transgenic for SV40 which retain their

Angiotensin II receptors and therefore constitute a good model for mesangial cells. These cells express a receptor for Angiotensin II in late passages. (Elliot, Striker, MacKay, Conti).

IV. Studies of the Regulation of Glomerular Pressure (K. Bernstein)

It has been suggested that elevated glomerular pressure leads to glomerulosclerosis. The regulation of angiotensin converting enzyme (ACE) production plays a central role in maintaining normal glomerular pressure. This project is designed to isolate the gene encoding the enzyme to further study its regulation and expression, using cultured glomerular endothelial cells as a model. RNA has been isolated from mouse kidneys by polysome precipitation. Active ACE was obtained from mouse kidneys and lungs. Using sepharose, we have bound various pharmacological inhibitors of ACE, and will use these columns to isolate the RNA coding for ACE from the polysomes (Bernstein).

V. Kidney Disease in the Pima Indians

The natual history of the glomerulosclerosis occuring in the Pima Indian is being studied in association with Dr. Bennett's branch in Phoenix. Physiological studies and histological assessment of the renal lesions will be performed. (Striker, Conti, Striker, Lange).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43002-22 MD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Secretion and Mechanism of Action of Parathyroid Hormone

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

PI:	G.D. Aurbach, M.D.	Chief, MDB, NIDDK	
OTHERS:	L.A. Fitzpatrick, M.D.	Senior Staff Fellow	MDB, NIDDK
	M.L. Brandi, M.D.	Visiting Associate	MDB, NIDDK
	K. Sakaguchi, M.D.	Visiting Fellow	MDB, NIDDK
	M. Zimering	Medical Staff Fellow	MDB, NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Biochemical Genetics, NHLI
Endocrine Unit, Massachusetts General Hospital
Gastroenterology Research Lab. - University of Western Ontario

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Endocrine Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4	2.25	1.75

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

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

There is still much to be learned about the nature and secretory control of circulating parathyroid hormone (PTH) in disease. It is the purpose of this project to study the secretion, function, and mechanism of action of parathyroid hormone, its relationship to human disease, and to develop clinically useful tests for circulating parathyroid hormone. From these studies it is expected that one can understand the pathophysiology of certain metabolic diseases of bone and endocrine disturbances. The entire structures of bovine, porcine, rat and human parathyroid hormone have been determined. Synthetic polypeptides representing bovine rat and human parathyroid hormone have been synthesized. These molecules show all the biological properties of the native hormonal polypeptides. Highly sensitive radioimmunoassays for the hormone have been developed and are being modified further for improved clinical diagnostic parameters. Studies show that the mechanism of action of the hormone is mediated through direct hormonal activation of adenylate cyclase in bone and kidney. Isolated parathyroid cells and culture systems have been develped that allow studies on secretory control of parathyroid hormone, and provide test systems to elucidate the pathophysiology of certain hypoparathyroid and hyperparathyroid. states.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43003-22 MD

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Studies on the Mode of Action of Thyrocalcitonin

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

PI:	S.J. Marx, M.D.	Chief, Min. Metab. Sec. MDB, NIDDK
Others:	J. Barsony, M.D.	Guest Researcher MDB, NIDDK
	K. Martin	Chemist, Endo. Reg. Sec. MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Mineral Metabolism Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The purpose is to study the interaction of calcitonin with its specific receptor target organs. The current investigations should provide further insight into the structure-function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful for characterizing hormone receptors in kidney, bone and other tissues. Studies are in progress to characterize further the interaction of calcitonin with tissue receptors. It wil also be of interest to solubilize the receptors and characterize them chemically. Calcitonin increases cAMP in MCF 7 breast cancer cells. At 300-fold lower concentration calcitonin decreases cAMP in these cells. The decrease in cAMP is prevented by preexposure of cells to agents that interfere with inhibitory guanyl regulatory proteins.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43004-22 MD

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Studies on pseudohypoparathyroidism and related disorders

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

PI:	A. Spiegel, M.D.	Chief, Molec. Patho. Sec.	MDB,NIDDK
Others:	A. Carter, Ph.D.	Senior Staff Fellow	MDB,NIDDK
	R. Collins, Ph.D.	Research Geneticist	MDB,NIDDK
	C. Bardin	Biological Lab Tech.	MDB,NIDDK

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Metabolic Diseases Branch

SECTION: Molecular Pathophysiology Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	1.50	

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism" (PHP). Patients with this disorder differ from those with idiopathic hypoparathyroidism: they show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and do not respond to exogenous parathyroid hormone (PTH). Subsequent to the original report, patients lacking the typical somatic features of AHO but resistant to endogenous and administered PTH have been described. In PHP, UcAMP (urinary cyclic AMP) does not increase normally in response to PTH administration. This indicated that there is a defective hormone receptor- adenylate cyclase complex in this disorder. We have now shown that many patients with PHP+AHO (PHP Ia) show an approximately 50% reduction in activity of Gs (the stimulatory guanine nucleotide binding protein associated with adenylate cyclase) in membranes from multiple tissues. Gs deficiency presumably accounts for resistance to multiple hormones in such patients. Patients with PHP without AHO show normal Gs activity (PHP Ib) and resistance only to PTH, and preliminary studies suggest a PTH-receptor defect in such patients. Rare patients with PHP and AHO and multiple hormone resistance show normal Gs activity.

Using cloned human cDNA probes for the alpha subunit of Gs, we now find that steady state mRNA levels from fibroblasts of subjects with PHP Ia are reduced by approximately 50% compared with normals. Genomic cloning and other molecular biologic approaches are being used to define the genetic abnormality responsible for Gs deficiency in PHP Ia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI DK 43005-22 MD

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Guanine nucleotide binding proteins as receptor-effector couplers

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

PI:	A. Spiegel, M.D.	Chief, Molec. Patho. Sec.	MDB, NIDDK
Others:	R. Collins, Ph.D.	Research Geneticist	MDB, NIDDK
	A. Carter, Ph.D.	Senior Staff Fellow	MDB, NIDDK
	P. Goldsmith, Ph.D.	Research Biologist	MDB, NIDDK
	C. Woodard	Biochemistry Lab Tech	MDB, NIDDK
	R. Vinitsky	Microbiologist	MDB, NIDDK
	L. Weinstein, M.D.	Medical Staff Fellow	MDB, NIDDK
	K. Rossiter, M.D.	NRSA	MDB, NIDDK
	M. Brann, M.D.	Senior Staff Fellow	MDB, NIDDK

COOPERATING UNITS *(if any)*

P.Bray, M.Nirenberg, (NHLBI); G.Milligan, Glasgow Univ., Scotland; H. Malech (NIAID); M.Caron, (Duke Univ., NC); Y.Zick, R. Sagi-Eisenberg, (Weizmann Institute, Israel); P.Gierschik, Heidelberg, Univ of Germany; R.Cerione, Cornell Univ., T.Bonner, N.Buckley (NIMH)

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6	6	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A family of guanine nucleotide binding proteins (G-proteins) functions in transmembrane signalling as receptor-effector couplers. G proteins couple to a diverse array of receptors including those for hormones, neurotransmitters, light, odorants, and certain growth factors. Effector functions regulated (positively and, in some instances, negatively) by G-proteins include cAMP formation, phosphoinositide breakdown, potassium an calcium channels, and cGMP degradation. We have used a variety of techniques to study the expression, distribution, regulation, structure and function of G-proteins. Our studies highlight the diversity within the G-protein family. We have purified novel G-proteins and using cloned cDNAs, defined their primary structure and distribution. We have demonstrated developmental and differentiation-dependent regulation of G protein synthesis. Using peptide specific antibodies, in situ hybridization and northern analyses, and protein reconstitution techniques, we have defined the specificity of G-proteins in coupling to receptors and effectors. These studies provide the basis for understanding the role of G-proteins in normal signal transduction and for elucidating possible defects in G-protein structure or function as the basis for abnormal signal transduction.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43006-12 MD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Hyperparathyroidism: Etiology, Diagnosis and Treatment

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

PI:	G.D. Aurbach, M.D.	Chief, MDB, NIDDK
OTHERS:	S.J. Marx, M.D.	Ch., Min. Met. Sec., MDB, NIDDK
	A.S. Spiegel, M.D.	Ch., Molec. Patho. Sec., MDB, NIDDK
	L.A. Fitzpatrick, M.D.	Senior Staff Fellow, MDB, NIDDK
	M.M. Bliziotes, M.D.	Medical Staff Fellow, MDB, NIDDK
	M.S. Nanes, M.D., Ph.D.	Medical Staff Fellow, MDB, NIDDK
	L. Weinstein, M.D.	Medical Staff Fellow, MDB, NIDDK
	M. Zimering, M.D.	Medical Staff Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*
Radiology Department, CC; Surgery Branch, NCI; Digestive Diseases Branch, NIDDK

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Endocrine Regulation Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.75	2.50	2.25

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

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

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent or recurrent hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, ultrasonography, radiothallium scanning, magnetic resonance imaging, CAT scanning, selective arteriograpy and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43007-07 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Study of Humoral Hypercalcemia of Malignancy

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

PI: A. M. Spiegel Section Chief MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43008-06 MD

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vitamin D Resistance and Related Disorders

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

PI:	S.J. Marx, M.D.	Chief, Min. Metab. Sec.	MDB, NIDDK
Others:	M. Nanes, M.D., Ph.D.	Medical Staff Fellow	MDB, NIDDK
	J. Barsony, M.D.	Guest Researcher	MDB, NIDDK
	M.L. Brandi, M.D.	Visiting Associate	MDB, NIDDK
	G.D. Aurbach, M.D.	Chief	MDB, NIDDK
	W. McCoy	Chemist	MDB, NIDDK

COOPERATING UNITS *(if any)*
Metabolism Unit, Beilinson Hospital, Petah Tikva, Israel
Biochemistry Department, University of Arizona, Tucson
Biochemistry Department, University of Wisconsin, Madison

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Mineral Metabolism Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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

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

The calciferols were the first class of hormonally active steroids to be discovered and also the first for which subjects with hormone resistance could be idetified. With recognition that vitamin D is the precursor for 1,25-dihydroxyvitamin D, it has become possible to characterize defects in the activation (1-hydroxylation) of vitamin D and defects in the target action of activated (1,25-dihydroxy)vitamin D. We have demonstrated a broad spectrum of manifestations of hereditary resistance to 1,25(OH)2D ranging from infantile rickets with alopecia and no intestinal response to calciferols to adult onset osteomalacia with satisfactory intestinal response to high doses of calciferols and with no epidermal abnormalities. Alopecia is found only in cases with the most severe grades of resistance to 1,25(OH)2D. This finding implicates the 1,25(OH)2D receptor, for the first time, in normal function of a tissue (hair follicle) outside the classical target in duodenal mucosa. A similar disorder has been recognized in new world monkeys. Cases with total lack of responses to calciferols have been treated with extroadinary doses of calcium administered intravenously. Thus, calcium alone can replace most functions of the 1,25(OH)2D receptor. Cultured skin fibroblasts display many components of the 1,25(OH)2D effector system. Skin fibroblasts from all subjects with hereditary resistance to 1,25(OH)2D display abnormalities in this effector system, and defects in many discrete steps of this pathway have been identified with these cells. Other cells, such as bone cells, lymphocytes, and parathyroid cells can also be used to evalute actions of 1,25(OH)2D in vitro. Cells with mutations in the 1,25(OH)2D effector pathway will be used to explore mechanisms of calciferol action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43009-02 MD

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Regulation of Mineral Metabolism

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

PI:	S.J. Marx, M.D.	Chief, Min. Metab. Sec.	MDB, NIDDK
Others:	M. Nanes, M.D., Ph.D.	Medical Staff Fellow	MDB, NIDDK
	M.L. Brandi, M.D.	Visiting Associate	MDB, NIDDK
	W. McCoy	Chemist	MDB, NIDDK
	G. Aurbach, M.D.	Chief	MDB, NIDDK
	E . Streeten, M.D.	Medical Staff Fellow	MDB, NIDDK

COOPERATING UNITS *(if any)*

EEB, CEB, LB, NCI
Belvedere Medical Center - Carlisle, PA.

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Mineral Metabolism Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

Disorders of mineral metabolism have been evaluated with methods extending from epidemiology to cellular biology. Two forms of familial hyperparathyroidism have been characterized in detail. Familial hypocalciuric hypercalcemia is an autosomal dominant trait associated with abnormal interactions with calcium in parathyroid and kidney. Familial multiple endocrine neoplasia type 1 is an autosomal dominant trait causing hyperfunction of parathyroids, pancreatic iseit and anterior pituitary. It is associated with gradual but abnormal proliferaltion of the tissues affected. Genetic linkage studies in a large kindred have so far excluded close linkage to 13 biochemical markers. Plasma from affected persons shows high mitogenic activity upon cultured bovine parathyroid cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43200-08 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Disorders of Immune Regulation in Patients with Systemic Lupus Erythematosus

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

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
Others: J. E. Balow, Senior Investigator, MDB, NIDDK
E. G. Eleftheriades, Visiting Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*

Clinical Center (C. Mitchell, Biologist).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.00	1.75	.25

CHECK APPROPRIATE BOX(ES)

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

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

Patients with systemic lupus erythematosus have been found to have various disturbances of the cell-mediated immune response. Cellular aberrations include enhanced spontaneous B cell activity with abnormal triggering in vitro, deficient immunoregulatory T cell circuits, deficient cytotoxic responses, including natural killer cell activity, alloantigen and viral cytotoxicity, and abnormal production of and response to different lymphokines as well as increased expression of proto-oncogenes in highly activated peripheral blood lymphocytes. The goal of these studies is to elucidate further the mechanisms of these alterations of the immune system which are apparently involved in the pathogenesis of this disease. The modulation of the above disturbances by immunosuppressive agents, i.e. corticosteroids and cyclophosphamide, is actively studied, aiming at the restoration of normal immune status in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43201-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Production and Characterization of Nephritic Factors

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

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
Others: G. Thyphronitis, Visiting Fellow, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)

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

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

The nephritic factor of the alternative pathway of complement (NeFa) has been found in the sera of patients with membranoproliferative glomerulonephritis (MPGN) and partial lipodystrophy (PLD) and has been described as a factor which is able to induce cleavage of the third component of complement (C3) in normal human serum through the alternative pathway. It has been demonstrated that NeFa binds to and stabilizes C3bBb (alternative C3 convertase). NeFa appears to be antigenically and structurally similar to IgG and therefore it might be an autoantibody directed against C3bBb complex. Sera from patients with systemic lupus erythematosus (SLE) contain autoantibodies which bind and stabilize the C3 convertase of the classical pathway. This is classical pathway nephritic factor (NeFc). The relation between the development of renal lesions and the NeFa mediated persistent hypocomplementemia remains unexplained. To study the production of nephritic factors, we isolated B lymphocytes from peripheral blood mononuclear cells from patients with MPGN, SLE and normal individuals and established B cell lines by infecting them with Epstein-Barr virus (EBV) containing supernatants. We found that EBV transformed B cell lines derived from patients with MPGN, but not from normal individuals, produce an IgG molecule which stabilizes that C3bBb convertase activity. Supernatants from EBV transformed B cell lines from patients with SLE contain IgG molecules which stabilize C4b2a convertase activity. Full chemical and functional characterization of these antibodies to convertases is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43202-04 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Regulation of Human Immune Response by Complement

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

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
Others: G. Thyphronitis, Visiting Fellow, MDB, NIDDK
S. R. Pillemer, Medical Staff Fellow, MDB, NIDDK
J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Complement factors and breakdown products acting through cell surface membrane receptors block the differentiation of human B lymphocytes into immunoglobulin secreting cells. Complement receptors are associated with B cell surface immunoglobulin under certain circumstances. Furthermore, complement receptor expression is cell cycle dependent and increased among the cells actively secreting immunoglobulin. Understanding of the mechanism of regulation of immune responses by complement and the role of complement receptors on human B cells is crucial for the understanding of the immunopathogenesis of autoimmune diseases since they are frequently associated with complement activation, depression of complement factor levels and changes in complement receptors.

PHS 6040 (Rev. 1/84) GPO 914-910

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43203-06 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Immunosuppression and Plasmapheresis in Goodpasture's Syndrome

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

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
Others: H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)*

Foreign: None Scripps Clinic and Research Foundation, La Jolla, CA
Walter Reed Army Medical Center, Washington, D. C.

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43204-07 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

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

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
Others: H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)*

NIAMS (Drs. J. Klippel, P. Plotz, A. Steinberg, R. Wilder).
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)

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

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

The efficacy of intensive, intermittent immunosuppressive drug therapy will be evaluated in patients with active lupus glomerulonephritis over a 30 month study period. Patients with renal biopsy documented active glomerulonephritis with or without renal functional deterioration will be treated with low dose corticosteroids and randomized to receive (a) intravenous pulse methylprednisolone monthly for 6 months or (b) intravenous pulse cyclophosphamide monthly for 6 months or (c) intravenous pulse cyclophosphamide monthly for 6 months and then every 3 months for the remaining 24 months of the study. During the final 24 months of the study, all patients will receive low dose prednisone. Active disease, as manifested by renal functional deterioration, increased proteinuria or worsened urinary sediment, will be treated by increased prednisone. Comparison will be made of the number of favorable outcomes of renal function, glomerular pathology and drug related toxicities achieved by each treatment group at the end of the 6th and 30th study months. Between April 1981 and July 1987 there have been more than 60 patients entered into this protocol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43205-10 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Renal Biopsy Pathology in Systemic Lupus Erythematosus

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

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
Others: H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)* Clinical Center (Dr. D. E. Webb)
Armed Forces Institute of Pathology, Washington, D. C.
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

The pathogenetic mechanisms underlying the different forms of lupus nephritis are being investigated. Detailed analysis of renal biopsy pathology is being conducted on specimens from patients with systemic lupus erythematosus. Biopsies are classified by major category of lupus nephritis, as well as scored on a semiquantitative scale for specific histologic changes indicating the degree of activity and of chronic sclerosing features. The patterns of immune complex deposition and lymphoid cell interaction with different segments of the nephron are being investigated by immunohistologic techniques and electron microscopy. These approaches have facilitated the analysis of the effects of various types of immunosuppressive agents used to halt the progression of lupus nephritis and they will enhance our understanding of the pathogenesis of this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43206-03 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunoregulatory Disorders in Patients With Juvenile Rheumatoid Arthritis

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

P. I.: G. C. Tsokos, Guest Researcher, MDB, NIDDK
Others: S. R. Pillemer, Medical Staff Fellow, MDB, NIDDK

COOPERATING UNITS *(if any)*
LaRabida Children's Hospital, Chicago, IL (Dr. D. Magilavy)
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

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

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

This project is inactive.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43207-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Viral Interferon and Localization of Pre-formed Complexes in Mice

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

P. I.: G. Striker, Director, DKUHD, NIDDK
Others: L. Striker, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Washington School of Medicine, University of Washington, Seattle, Washington (Dr. M. Mannick).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43210-03 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

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

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

P. I.: K. MacKay, Medical Staff Fellow, MDB, NIDDK
Others: G. Striker, Director, DKUHD, NIDDK
L. Striker, Expert, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*
School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (Drs. R. Brinster and C. Pinkert).
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

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

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

This project is inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43211-03 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histopathology of Renal Lesions in Pima Indians

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

P. I.: L. Striker, Expert, MDB, NIDDK
Others: G. Striker, Director, DKUHD, NIDDK
F. Conti, Visiting Fellow, MDB, NIDDK
M. Lange, Guest Researcher, MDB, NIDDK
L. Agodoa, Medical Officer, MDB, NIDDK

COOPERATING UNITS *(if any)* Epidemiology and Clinical Research Branch, NIDDK, Phoenix, Arizona (Dr. P. Bennett). Director of Nephrology at Stanford University, Stanford, California (Dr. B. Myers). Howard University, Washington, D.C. (Dr. B. Brenner) and Emory University, Atlanta, Georgia (Dr. W. Mitch). Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Renal Cell Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

Autopsies from diabetic and non-diabetic Pima Indians will be examined from a series drawn as a representative sample of the autopsy population by Dr. Peter Bennett. Routine light microscopic studies, and potentially electron microscopic studies, will be performed to assess the histopathologic lesions present in these autopsy specimens. Particular attention will be paid to epithelial basement membranes and vascular extracellular matrix areas.

In addition, the PI is chairman of the Natural History Committee of the Pima Indian Project funded by contracts NO1-DK-7-2291 and NO1-DK-6-2285. The Natural History portion will correlate the histologic, renal physiologic and cell biologic aspects of the kidney disease of diabetes mellitus of the Pima indians.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43212-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Coagulation Studies Using Human Glomerular Endothelial Cells

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

P. I.: M. Lange, Guest Researcher, MDB, NIDDK
Others: L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
T. Doi, Visiting Associate, MDB, NIDDK

COOPERATING UNITS *(if any)*

University of Michigan, Arnn Arbor, Michigan (Dr. R. Wiggins).

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Human glomerular endothelial cells are isolated and cloned from glomeruli obtained from nephrectomy specimens which have been removed for medical or surgical reasons. Some glomeruli will be obtained from specimens which were initially designated to be used as cadavor transplants but were not able to be utilized for technical or other reasons. The principal assays to be used will be to assess the procoagulant activity of supernatants and and cytoplasmic preparations from the cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43214-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Cell and Molecular Biology of Glomerular Cells Derived From Transgenic Mice

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

P.I.: K. MacKay, Medical Staff Fellow, MDB, NIDDK
Others: L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania (Drs. R. Brinster and C. Pinkert) and National Cancer Institute, Bethesda, Maryland (Dr. L. Wakefield). Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)

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

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

Current models of glomerulosclerosis (GS) have yielded little information about the cellular and molecular abnormalities that are critical in the initiation and progression of this disease. The complexity of the kidney and glomerulus make isolation and examination of pure cultured populations of glomerular cells an attractive method for beginning to answer these questions. Unfortunately other models of GS involve extrarenal causes of glomerular injury. Because of this it is quite likely that glomerular cells isolated from these models would not maintain the abnormal behavior in vitro which led to the development of GS in vivo.

We have identified several lines of mice transgenic for the early region of simian virus 40 (SV40) that develop progressive glomerulosclerosis. As there are no evident extrarenal sources of injury, and since expression of the foreign DNA has been documented to occur in whole kidney we suspect that the glomerular disease may be secondary to expression of the foreign DNA by glomerular cells in vivo.

We have isolated lines of glomerular endothelial, mesangial, and epithelial cells from transgenic mice and have isolated pure cultures of mesangial and epithelial cells from their normal litter mates. As preliminary data from the in vivo model indicates that proliferation of glomerular cells is an early event in the development of GS in transgenic mice we plan to begin the evaluation of several growth factors on the behavior of individual cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43215-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Effect of Endotoxin on Human Glomerular Endothelial Cells

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

P.I.: L. Striker, Expert, MDB, NIDDK
Others: G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Washington, Seattle, Washington (Drs. G. Raghu and J. Harlan).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43216-03 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Progression of Glomerulosclerosis in Experimental Membranous Glomerulonephritis

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

P. I.: L. Striker, Expert, MDB, NIDDK
Others: G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Washington, Seattle, Washington (Drs. S. Adler and W. Couser).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43217-03 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Renal Lesions in Leukemias, Lymphomas and Carcinomas

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

P. I.: L. Striker, Expert, MDB
Others: G. Striker, Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*
National Cancer Institute, Bethesda, Maryland (Drs. M. Linehan and M. Merino).

Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

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

We have been examining the glomerular lesions in kidneys from patients who undergo a nephrectomy for renal cancer. In areas non-invaded by the tumor there is in half the cases examined a marked mesangial proliferation and occasional synechiae suggesting a glomerular disease which could be mediated by growth factors released by the tumor.

PHS 6040 (Rev. 1/84) GPO 914-4

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 43218-02 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Development of Human Glomerular Cell Lines

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

P.I.: M. A. Lange, Guest Researcher, MDB, NIDDK
Others: G. Striker, Director, DKUHD, NIDDK
L. Striker, Expert, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

Synergen Colorado (K. Van Doren).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

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

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

Primary outgrowth of human glomeruli containing mixed populations of epithelial cells, mesangial cells, and endothelial cells were infected with a recombinant adenovirus 5-simian virus 40. Foci of transfected cells arose which are being isolated and characterized. These cell lines all exhibit nuclear staining for the SV40 large T antigen, and immunoprecipitation revealed bands characteristic for large T antigen. Epithelial cells have been obtained in large numbers, and can be passaged.

GPO 814-816

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43219-02 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Glomerular Endothelial Cells and Immune Complexes

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

P.I.: M. A. Lange, Guest Researcher, MDB, NIDDK
Others: L. Striker, Expert, MDB, NIDDK
G. Striker, Director, DKUHD, NIDDK
L. Agodoa, Medical Officer, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

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

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

Very little is known about the initial subendothelial localization of immune complexes in glomeruli. Using human glomerular endothelial cell lines established in this laboratory, cells will be evaluated for transcytosis of immune complexes of human serum albumin and anti-human serum albumin (HSA). In order to determine whether immune complex interaction with glomerular endothelial cells is an active or passive process, three methods will be employed. First, using radiolabeled immune complexes dose response curves will be obtained testing for the presence of saturation kinetics. Second, endothelial cell-immune complex interaction will be followed using video enhanced fluorescence microscopy. Third, complexes and endothelial cells will be evaluated by immunoelectron microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43320-02 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Regulation of Expression of Angiotensin Converting Enzyme in Renal Glomeruli

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

P. I.: K. Bernstein, Special Assistant to Associate Director, DKUHD, NIDDK

COOPERATING UNITS *(if any)*

National Institute of Mental Health, Bethesda, Maryland (Dr. B. Martin).

Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

It has been suggested on an experimental basis that elevated intraglomerular pressure leads to glomerulosclerosis. The regulation of angiotensin converting enzyme (ACE) production plays a central role in maintaining normal intraglomerular pressure. This project is designed to isolate the gene encoding ACE from mouse kidney to further study the regulation and expression of the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 43221-02 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Insulin Receptors in Glomerular Cells

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
P.I.: F. Conti, Visiting Fellow, MDB, NIDDK
Others: L. Striker, Expert, MDB, NIDDK
G. E. Striker, Director, DKUHD, NIDDK
K. MacKay, Medical Staff Fellow, MDB, NIDDK
S. Elliot, Bio. Lab. Tech., MDB, NIDDK

COOPERATING UNITS *(if any)*
Diabetes Branch, NIDDK (M. Lesniak)
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

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

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

We propose to study insulin specific binding on glomeruli from mice and humans. Binding of insulin to mesangial cells from normal transgenic mice, and human kidneys is being investigated. The nature of the receptor will be studied and elucidated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43222-02 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Pathogenesis of Murine Lupus Nephritis

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

P. I.: H. A. Austin, Expert, MDB, NIDDK
Others: J. E. Balow, Senior Investigator, MDB, NIDDK

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, D. C. (Drs. Antonovych and Sabnis).
Foreign: None

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

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

The pathogenetic mechanisms underlying the diverse forms of murine lupus nephritis are being investigated. Renal morphology is being studied by light, immunofluorescence, immunoperoxidase and electron microscopy to delineate the types of glomerular and tubulo-interstitial lesions, as well as the characteristics of the immune deposits and the lymphoid cell proliferation. The impact of provocative maneuvers on serologic and renal histologic features is being examined to develop a model of a flare of lupus nephritis. Innovative treatment strategies will be studied as part of an ongoing effort to refine our approach to this disease.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 43223-02 MD

PERIOD COVERED
October 1, 1986 through September 30, 1987

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

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

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK
Others: H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)*
Clinical Center (Dr. D. E. Webb).
Foreign: None

LAB/BRANCH
Metabolic Diseases Branch

SECTION
Kidney Disease Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

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.)*

Crescentic glomerulonephritis is a rapidly progressive renal disease with a high risk of development of end-stage renal failure within a few weeks or months of onset. The choice and effectiveness of therapy are controversial. High-dose pulse methylprednisolone is widely preferred for treatment of crescentic glomerulonephritis at the present time, but its efficacy is acknowledged to be less than ideal in preserving renal function. Our study is designed to test the efficacy of intensive, intermittent immunosuppressive drug therapy in patients with crescentic glomerulonephritis over a 6 month study period. Patients with renal biopsy documented active crescentic glomerulonephritis will be treated with a short course of oral corticosteroids and randomized to receive in addition: (a) intravenous methylprednisolone monthly for 6 months, or (b) intravenous cyclophosphamide monthly for 6 months. Comparisons will be made of the number of favorable outcomes of renal function and renal pathology, as well as drug-related toxicities for each treatment group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 43224-01 MD

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Membranous Lupus Nephropathy

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

P. I.: J. E. Balow, Senior Investigator, MDB, NIDDK

Others: H. A. Austin, Expert, MDB, NIDDK

COOPERATING UNITS *(if any)* Foreign: None

Stanford University, Stanford, CA (Dr. B. Myers). Clinical Center (Dr. D. Webb; K. Joyce, E. Vaughan, Nursing). NIAMS (Dr. J. Klippel).

LAB/BRANCH

Metabolic Diseases Branch

SECTION

Kidney Disease Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, MD 20892

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

CHECK APPROPRIATE BOX(ES)

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

☒ (a1) Minors

☐ (a2) Interviews

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

The efficacy and toxicity of three immunosuppressive drug regimens will be evaluated in patients with membranous lupus nephropathy over a 12 month study period. Detailed tests of renal function, glomerular permselectivity and kidney biopsy morphology will be conducted at the beginning and end of treatment. Patients with systemic lupus erythematosus, nephrotic range proteinuria and biopsy documented membranous nephropathy will be treated with alternate day prednisone and will be randomized to receive: a) no additional therapy (control group), b) intravenous pulse cyclophosphamide up to 1.0 gram per square meter body surface area every other month for 6 total doses, or c) oral cyclosporin A up to 200 mg per square meter body surface area daily for a total of 11 months. Lupus disease activity, renal function tests and drug toxicities will be monitored closely. Analysis will include comparison of the number of favorable outcomes of glomerular function and pathology as well as drug-related toxicities appearing in each treatment group at the end of 12 months of study.

PHS 6040 (Rev. 1/84)

GPO 914-918

As part of the observance of the Centennial year of the National Institutes of Health, the Branch sponsored one of the four symposia in honor of noted mentors. The symposium honored Dr. J.E. Rall, who was the first Chief of CEB, later became Scientific Director of NIADDK and currently is Associate Director for Intramural Research, NIH.

The Branch also sponsored the first Edelhoch Memorial Lecture, which was delivered by Dr. Arthur Schneider in the Endocrine Grand Rounds series. Dr. Schneider had been a fellow with Dr. Edelhoch and he spoke on his current studies on the biosynthesis and metabolism of thyroglobulin.

Research fellows from abroad contributed significantly to the research of the Branch. They included scientists from Greece, Italy, Brazil and Japan. Dr. Jamshed Tata from London and Dr. Teruo Matsuura from Kyoto returned to Bethesda to complete their terms as Fogarty Scholars.

I. Thyroid Biochemistry and Pathophysiology

The major thyroid hormone binding proteins in human plasma are thyroxine binding globulin (TBG), prealbumin (transthyretin), and albumin. They provide for peripheral storage of the hormones as well as a buffering capacity that maintains the free hormone concentration at an extremely low level, but they are not essential for the action of the hormones. We have initiated a study to determine whether other serum proteins capable of binding smaller quantities of the hormones may have specific functions in the passage of hormone into cells.

The lipoproteins are good candidates for such a role since they are involved in specific intracellular transport of another hydrophobic compound - cholesterol. In order to establish that lipoproteins do indeed interact with thyroid hormones, and to characterize this interaction, normal and abnormal plasmas and their d < 1.210 lipoprotein fractions were investigated. The overall binding to lipoproteins in normal plasma is greatest for T_3, the most active hormone (≈ 6% of total plasma T_3), intermediate for T_4 (≈ 3%) and much less for the inactive hormone analog, reverse T_3 (0.2%). Among the individual lipoproteins, HDL (high density lipoprotein) accounts for more than 90% of T_3 and T_4 binding but only 55% of reverse T_3 binding. Furthermore, the active hormones are bound to HDL subfractions having lower molecular weight. It was also found that apolipoprotein A_1, a major component of HDL, possesses specific affinity for the hormone. Plans are being made to evaluate a possible role for $apoA_1$ in the transport of thyroid hormone into cultured cells. (Robbins, Benvenga)

Thyroxine-binding globulin (TBG), the major thyroid hormone carrier in plasma, is a glycoprotein secreted by the liver. We have previously shown with the highly differentiated human hepatoma cell line, HepG2, that completely deglycosylated TBG is capable of being secreted. To explore further the role of glycosylation in TBG secretion, the effect of

swainsonine, an inhibitor of α-mannosidase that is responsible for trimming mannose residues from the oligosaccharide chains and permitting branching to take place was investigated. It was found that TBG containing high mannose oligosaccharide chains was secreted more rapidly than fully glycosylated TBG. In contrast to the findings of other workers, these results show that complete processing of oligosaccharide moieties is not required for TBG secretion. (Robbins, Bartalena)

B. Thyroid Hormone Metabolism

The ability of thyroid hormones to enter cells by mechanisms other than passive transport is still controversial. Our previous studies showed that a saturable, energy dependent transport system is present in L6E9 rat skeletal myoblasts as well as in the intact skeletal muscle. The work with cultured myoblasts was extended by comparing the intracellular transport of the active hormone, L-T_3, and its inactive enantiomer, D-T_3. As was shown by others for other tissues, isolated nuclei bound both compounds with equal affinity, but intact myoblasts showed a 50% lower total uptake and a 60% lower nuclear uptake of D-T_3 compared to L-T_3. Uptake of the dextroisomer was also less affected by inhibitors of ATP production or endocytosis. Thus, the mechanism responsible for the different uptake of L-T_3 and D-T_3 is located outside of the nucleus and probably at the plasma membrane. (Robbins, Pontecorvi, Lakshmanan)

Although saturable, energy dependent T_3 uptake has now been demonstrated in many types of cells, the presence of carrier mediated transport of T_4 is less certain. Because neurons obtain 80% of their T_3 from intracellular T_4 by deiodination, it is important to know whether these cells have a specific mechanism for T_4 uptake. Therefore, both T_3 and T_4 uptake were studied in a well differentiated mouse neuroblastoma cell line, NB41A3, as a model of neuronal transport. Neuroblasts were shown to possess a saturable, energy dependent and stereospecific mechanism for uptake of both T_3 and T_4. Using the initial rate of uptake to assess the kinetic parameters, K_m and V_{max} for T_4 were 2.44 nM and 6.74 fmol/min/10^6 cells at 37°C, compared to 2.15 nM and 18.03 fmol/min, respectively, for T_3. Thus, the availability of T_3 within nerve cells may be regulated at the plasma membrane by variation in the uptake of T_4. (Robbins, Goncalves, Lakshmanan, Foti)

Since neural differentiation appears to be associated with a decreased dependency on thyroid hormones, we investigated the changes in L-T_3 cell and nuclear uptake in neuroblastoma cells differentiated by exposure to 1 mM sodium butyrate. This agent causes an increased cytoplasm:nucleus ratio, neurite outgrowth, and induction of tyrosine hydroxylase. Cells exposed to butyrate had a transient increase in total cell uptake of T_3 at 4 days, and a decrease below the control at 7 days. Similar changes were found for saturable cell uptake after 60 min and for the initial rate of uptake (1 min). The affinity of isolated nuclei for T_3 ($5nM^{-1}$) was unaffected by butyrate but the apparent nuclear Ka measured in intact cells decreased from $10.6\ nM^{-1}$ to $4.3\ nM^{-1}$ after 7 days exposure to butyrate. Since the higher Ka in intact cells presumably reflects a higher intracellular than extracellular free T_4 concentration, these data indicate that differentiation of NB41A3 cells is associated with decreased transport of T_3 across the cell membrane.

Similar studies have been initiated in several different neural cell lines, including human medulloblastoma TE671, human neuroblastoma HTB11, human glioma Hs863, all of which show saturable T_3 uptake. We also have initiated studies with primary cultures of rat glioma cells in order to investigate apparent differences between glial and neuronal cells in their transport of thyroid hormone. (Robbins, Goncalves, Lakshmanan)

C. Thyroid Hormone Action

A major effect of the thyroid hormones is to increase the synthesis of certain proteins, one of which is malic enzyme in rat liver. Our previous work showed that this control is exerted at several levels, including the rate of gene transcription and stabilization of messenger RNA. Further studies have been carried out with this system with a view toward elucidating the mechanism of these hormone actions. Additional photoaffinity labeling experiments were done because of continuing controversy concerning the size of the nuclear receptor for T_3. The analysis after photoaffinity labeling of intact cells confirmed the presence of one predominant component of 60 kDa and a minor one of 57 kDa supporting the concept that smaller components are the result of breakdown during preparation. These results also are compatible with the recent work of Weinberger et al (Nature 324, 641, 1986) demonstrating a relationship between the T_3 receptor and the oncogene, cErb a. (Cahnmann, Nikodem)

The project to map the structure of the malic enzyme gene has been completed. The gene was shown it to be of rather large size, containing 115 kilobases and 14 exons. Heterogeneity in where transcription begins was demonstrated by primer extension and S1 nuclease analysis. Primer extension analysis identified two transcription start sites corresponding to 5' untranslated regions of 30 and 31 nucleotides in length. S1 nuclease protection experiments showed three additional sites of 81, 85 and 86 nucleotides. Unexpectedly, no TATA or CCAT-like boxes were found in the vicinity of any of the transcriptional start sites.

The heterogeneity of the 3'-untranslated region was determined by S1 nuclease mapping. Two protected fragments were in regions that contained sequences known to specify polyadenylation in other mRNAs. They correspond to the 3'-untranslated regions of 289 and 1303 nucleotides. Accordingly, the two malic enzyme mRNAs reported previously can result either from differential processing and polyadenylation of a common transcript or from two different sized transcripts. (Nikodem, Morioka)

The sequence of 914 base pairs 5' from the coding region of the malic enzyme gene has also been determined. A TATA box, often located 20 to 30 bp upstream from capsites, lies at -622, and the sequence CCGAT between -144 to -140 resemble the CCAAT consensus sequence often found 80 bp upstream from transcription start sites. Strikingly, there are nine GC boxes with sequence CCGCC. Six are found upstream from the major capsite, from -376 to -10, one is in an untranslated region (+10) and two are within the first intron. The 6 bp GC sequence is the same that is repeated six times within the SV40 promoter and is also a core element in the decanucleotide sequence that binds Sp1, a transcription factor in HeLa cells. Some of these GC boxes may be involved in regulation of ME gene expression. Other sequences in the ME gene similar to possible

regulatory regions of other genes have also been noted. (Nikodem, Morioka, Tennyson)

To determine the location of sequences required for maximal promoter activity, 5' deletional analysis was used. Chimeric genes containing various parts of the 5' flanking region of the malic enzyme gene were placed upstream from the coding sequence of the chloramphenicol acetyl transferase gene and were tested in transient transfection assays using several cell lines. It was found that sequences +1 to -41 are sufficient to initiate expression, but maximal promoter activity required inclusion of sequences up to -177. Interestingly, the mutant ending at -145 contains a CAT box-like sequence and the mutant ending at -177 contains the putative Sp1 binding sequence.

Inclusion of nucleotides from -177 to -882 had no further effect on CAT expression in Hepa I or HeLa cells. In CHO cells, however, nucleotide sequences -243 to -348 reduced CAT expression. Thus they appeared to contain a negative regulatory element acting in a tissue specific manner. It was further shown that the malic enzyme 5' flanking fragment -177 to -882 was able to down regulate, when placed in the opposite orientation, the promotion of the CAT gene by the thymidine kinase promoter. (Nikodem, Morioka, Tennyson)

The chromatin structure of the malic enzyme gene has been analyzed in different thyroidal states. DNAse-I-hypersensitive sites have been unambiguously localized to three positions in the 5'-flanking region (-50,-170,-310) and may bear a relation to the promoter elements described above. A fourth hypersensitive site is at approximately -4.1 kb. DNAse hypersensitive sites were also found in the 3' flanking region. Five were spaced approximately 150 bp apart between the two polyadenylation sites of the gene, and may be related to nucleosomal phasing. A sixth was 600 bp downstream from the second polyadenylation site and is possibly involved in transcription termination.

Triiodothyronine given to rats was found to increase the proportion of chromatin displaying malic enzyme gene hypersensitivity. By the employment of in situ hybridization to localize malic enzyme RNA in hepatocytes, it was shown that two populations of hepatocytes existed in hypothyroid and euthyroid states. About 70% were active and 30% inactive with respect to expression of the malic enzyme gene. After 10 days of T_3 administration, however, all hepatocytes were active. These results indicate that part of the thyroid hormone effect is through recruitment of hepatocytes to transcribe the gene. (Nikodem, Usala, Morioka)

Our previous studies showed that rat liver malic enzyme mRNA is regulated by thyroid hormone at two levels - the rate of transcription and apparent mRNA stabilization - the latter being the major effect. Analysis of nuclear and cytoplasmic malic enzyme mRNA indicates that the stabilization occurs mainly in the nucleus. In order to prove that this is so, it is necessary to use intronic DNA probes since these will react only with nuclear RNA. Highly repetitive DNA sequences were excluded in order to increase the specificity, and probes reacting with extronic DNA were also excluded to eliminate reaction with cytoplasmic contaminants. Six intronic probes were selected and subcloned. Among three probes tested so far, one which is located between exons 3 and 4 exclusively

hybridized with nuclear RNA. This nuclear RNA was increased 11-fold in T_3-treated rats, in agreement with the 11-fold stimulation of cytoplasmic mRNA. Although these findings support our earlier hypothesis, they must be considered preliminary since the other 2 probes generated hybridization signals with cytoplasmic preparations. (Nikodem, Song)

Studies carried out in collaboration with Dr. Leonard Kohn (LBP, NIDDK) have demonstrated that malic enzyme mRNA is present in the cultured rat thyroid cell line FRTL-5 and is increased by TSH or cyclic AMP. Further studies showed that TSH has no effect on the rate of transcription of the malic enzyme gene, indicating that the TSH effect is exerted entirely at the level of mRNA stabilization. This stabilization was shown to require ongoing protein synthesis. The mechanism of this effect of TSH, and its possible relation to the growth cycle of the cell are under investigation. (Nikodem, Greico)

D. Studies in Thyroid Disease

In the course of ongoing studies of patients with thyroid cancer, an extraordinary patient was encountered. This young woman had undergone total thyroidectomy for multifocal papillary carcinoma. Followup radioiodine scanning in preparation for ^{131}I ablation therapy revealed a focus of ^{131}I uptake in the pelvis, and differential scanning showed that this was in pelvic soft tissue rather than bone. To provide a positive diagnosis, as well as to avoid unnecessary ovarian radiation, pelvic exploration was performed, revealing the presence of a teratoma in the wall of the rectum. Histologically, the predominant cell type resembled gastric mucosa, which was the only detected site of ^{131}I trapping. This is the first known instance where gastric mucosa was responsible for ^{131}I uptake in a teratoma. (Robbins, Lakshmanan)

Studies that are in progress on patients with thyroid cancer include the following: 1) The effect of lithium carbonate on the secretory rate of radioiodine by thyroid cancer metastases. The purpose of this study is to improve the risk/benefit ratio of radioiodine therapy. 2) The effect of lithium carbonate on the secretion rate of radioiodine from thyroid remnants remaining after initial surgery for thyroid cancer. The purpose of this study is to improve the yield of complete ablation by low dose (30 mCi ^{131}I) ablation therapy. 3) The design of a simplified low iodine diet in preparing patients for radioiodine therapy. The purpose is to improve the detection of metastases by ^{131}I scanning and to increase the radiation to thyroid remnants and cancer metastases during therapy. 4) The management of patients on renal dialysis during radioiodine scanning and therapy. The purpose is to design safe and effective therapy in thyroid cancer patients who lack normal renal handling of radioiodine. 5) Effects of short term, profound hypothyroidism that occurs during radioiodine testing and/or therapy on a) postural hypotension and catecholamine responses and b) neuropsychiatric effects as determined by mood testing.

II. Hormones and Cell Differentiation

Growth and developmental hormones induce accumulation of specialized mRNAs in a tissue specific manner. A few studies (for example, estrogen effect on oviduct ovalbumin and on hepatic vitellogenin) have compared

the relative rates of synthesis and degradation, and showed a 2-5 fold increase in transcription but a 20-50 fold increase in stability of the induced mRNA. Differentiation of rat L6A1 myoblasts induced by insulin is accompanied by an 80-fold increase in creatine kinase (CK) activity, a similar increase in CK mRNA, and a coordinate rise in myosin heavy chain (MHC) mRNA. Removal of insulin was found to result in rapid degradation of CK mRNA but not MHC mRNA. Conditions were established whereby cycloheximide produced a reversible block of protein synthesis without injuring the cells. Under these conditions, cycloheximide prevented the selective destabilization of CK mRNA in a reversible manner. Actinomycin D had a similar effect. These findings strongly suggest the involvement of a short-lived protein(s) in regulating the stability of induced mRNAs, and that this protein is coded for by a short-lived mRNA.

Under the same conditions of mRNA stabilization as during de-induction, a superinduction of CK mRNA but not MHC mRNA was observed if the two inhibitors were added during induction in the continuous presence of insulin. Thus de-induction and superinduction appear to be mirror images of each other. It is concluded that one or more short-lived proteins selectively regulate inducible mRNAs and may be an important requirement for normal developmental processes. (Robbins, Pontecorvi, Tata)

III. Mechanisms of Cell Secretion

The role of tubulin and microtubules in cell secretion is a long range goal of this laboratory. During studies directed toward elucidation of tubulin-lipid interactions, it was discovered that Nile Red is a sensitive fluorescence indicator of non-polarity. This was verified by studies on a number of proteins with known hydrophobic surfaces under various conditions. The Nile Red reaction has been used to investigate changes in the physical state of tubulin. Increasing tubulin concentration in Mes assembly buffer causes a 6-fold enhancement of fluorescence of the dye and a shift of the emission peak from 665 to 618 nm, which parallels dimer dissociation. In 1M glutamate, emission remains between 620 and 630, and light scattering measurements indicate that the tubulin dimer is stable. Thus Nile Red behaves as a monitor of the formation of the subunit contact surface. (Wolff, Sackett, Knipling)

IV. Adenylate Cyclase of Bacterial Origin

Bordetella pertussis contains a highly potent, calmodulin-activated adenylate cyclase, most of which is found in the periplasmic space of the organism or is secreted into the medium. This cyclase may be a virulence factor that enters host cells, there producing large quantities of cAMP that can paralyze cell functions. The present investigation aims to elucidate the entry of the cyclase into the host cell. Because of the very high concentrations of the bacterial cyclase, special methods had to be developed to distinguish extra- from intra-cellular measured enzyme or cAMP. It was found that crude cyclase preparations were able to enter cells whereas partially purified cyclase could not enter. With crude cyclase, there was a more than 1000-fold increase in host cell cAMP that occurred rapidly after exposure. Endocytosis inhibitors had little effect, but anticalmodulin drugs decreased cAMP accumulation. Whether this is an effect of intracellular production or not is not yet known.

Antimicrotubule drugs inhibit entry of cAMP but not entry of enzyme. Inhibitors of energy production inhibit cAMP accumulation only if intracellular ATP levels drop substantially. Studies are now in progress to identify the form of the enzyme - or the helper substances - that are required for penetration of the cyclase into the host cell. (Wolff, Gentile, Raptis, Knipling)

V. Interaction of Proteins with Cell Membranes

The formation of coated pits on the surface of cells, and their transition to coated vesicles, are key elements in the uptake of many biological substances. The mechanism of their formation has been investigated by detailed examination of the requirements for polymerization and depolymerization of the major protein constituent, clathrin. It was previously reported that 8S clathrin trimers, or triskelions, form larger 27S oligomers upon dialysis into low ionic strength buffer, 2 mM Mes pH 5.9. In collaboration with J.E. Heuser of Washington University, St. Louis, it was shown by electron microscopy that the 27S species are closed tetrahedra composed of four clathrin triskelions. Each of the four globular domains are linked to the others by four 33 nm struts. Each of these links contains three triskelion arms. It was concluded that in conditions that do not favor the formation of the standard clathrin cages, low affinity interactions lead to closed assemblies of four triskelions.

Proteins associated with clathrin are essential for the polymerization reaction. One of these, a 114 kDa molecule, was previously characterized. We now have purified and characterized a second assembly protein, designated AP_{180}, with a molecular weight close to that of clathrin based on SDS gel analysis. Sedimentation equilibrium analysis, however, showed AP_{180} to have a molecular weight of 115,000 and a sedimentation coefficient, $S_{20,w}$ of 3.5. A content of 25% helical structure and 41% β-structure was found by circular dichroism. The rate of polymerization of clathrin is greatly enhanced in the presence of AP_{180}, forming baskets with 6 clathrin triskelions. This differs from the smaller size baskets formed in the presence of the 114 kDa associated protein.

Further studies with purified 8S clathrin and the 100 kDa - 110 kDa group of associated proteins have shown that ratio of these components determines the size of the baskets. Unlike the heterogeneous population of baskets formed under low pH conditions (0.1 Mes, pH 6.0-6.2), polymerization at pH 8 (0.5 M Tris) gives a homogeneous population. By varying the ratio of clathrin to associated proteins, three distinct sizes of baskets are produced with sedimentation coefficients of 150S, 220S and 300S. The larger baskets form at the lower ratios of clathrin to associated proteins. It has also been concluded that the 150S baskets are intermediates in polymerization of clathrin to larger size baskets. (Prasad, Lippoldt)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 45000-20 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Thyroxine-Protein Interactions

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

PI:	J. Robbins	Chief CEB, NIDDK
Others:	S. Benvenga	Guest Researcher CEB, NIDDK
	H.J. Cahnmann	Scientist Emeritus CEB, NIDDK

COOPERATING UNITS (if any)

University of Messina, Italy (S. Benvenga).
Dr. R.E. Gregg, Division of Intramural Research, NHLBI

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Hormone Metabolism and Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.9	.7	.2

CHECK APPROPRIATE BOX(ES)

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

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

Further studies have confirmed our recent report that the thyroid hormones, and reverse $T_3(rT_3)$, bind to the 3 major lipoprotein classes. They now show a greater overall binding of T_3 compared to T_4, which is greater than rT_3; i.e., the order of their biological potency. Displacement studies with iodothyronines and drugs indicate that at least part of this binding is the result of interaction between thyroid hormones and the apolipoprotein moieties.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45004-16 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of Polypeptide and Protein Hormones

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

PI: K. Prasad — Visiting Associate, CEB, NIDDK

Others: R. E. Lippoldt — Health Services Ofcr., CEB, NIDDK

COOPERATING UNITS *(if any)*
Washington University School of Medicine, St. Louis, MO (Dr. J.Heuser); Temple University School of Medicine, Philadelphia, PA (Dr. J.H.Keen)

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Protein Structure Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

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

We have previously reported the formation of an intermediate polymer of clathrin (8S) with a sedimentation coefficient of 27S. Deep etch electron microscopy of this polymer revealed it as a closed tetrahedron consisting of four globular domains, each linked to the others by three 33 nm struts.

A clathrin assembly protein AP_{180} that has near identical electrophoretic mobility to clathrin has been purified by a simpler procedure than has been reported. It has been characterized by equilibrium centrifugation as having a molecular weight of 115,000 instead of 180,000 as seen on SDS gels. It is found to react in stoichiometric amounts with clathrin to polymerize clathrin into the smallest size baskets with a sedimentation coefficient of 130S.

We have examined the role of the yet another group of clathrin associated proteins (100kDa-110kDa) previously identified, to account for the variability in the size and sedimentation coefficient distribution of the baskets formed when they are added to pure clathrin. Lower ratio of clathrin to associated proteins gives rise to smaller size baskets (150S) whereas higher ratio gives rise to predominantly large size baskets (300S). It has also been concluded that the 150S type baskets are intermediates in the formation of larger size baskets.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45009-20 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	J. Robbins	Chief, Clinical Endocrinology Branch CEB, NIDDK
Others:	M. Lakshmanan	Medical Staff Fellow CEB, NIDDK
	M. Phyillaier	Biologist CEB, NIDDK
	S. Benvenga	Guest Researcher, CEB, NIDDK
	K. Ain	Medical Staff Fellow, CEB, NIDDK

COOPERATING UNITS *(if any)*
University of Messina, Italy (Benvenga); Dr. J.Norton, Surgery Branch, NCI; Dr. J.Reynolds, Nuclear Medicine, CC

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Hormone Metabolism and Action Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	.3

CHECK APPROPRIATE BOX(ES)

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

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

An unusual case of pelvic radioiodine uptake in a 30 year old woman after thyroidectomy for papillary thyroid carcinoma was studied. Radioiodine and bone scanning localized the uptake to pelvic soft tissue and at surgery a teratoma was found in the wall of the rectum. The radioiodine was confined to this tumor and was localized to gastric mucosal epithelium.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45014-16 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	J. Wolff	Associate Chief CEB, NIDDK
Others:	D. L. Sackett	Staff Fellow CEB, NIDDK
	L. Knipling	Technician CEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Endocrine Biochemistry Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

This project has been kept in abeyance pending the outcome of other studies on lipid-tubulin and lipid-binding protein-tubulin interactions.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45016-17 CEB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Thyroid Hormone Secretion and the Function of Microtubules

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

PI:	J. Wolff	Associate Chief	CEB, NIADDK
Others:	D. L. Sackett	Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Endocrine Biochemistry Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.5	.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.)*

Recent evidence has suggested that the monomer-dimer equilibrium of tubulin under conditions of relative low concentration may have a substantial effect on the assembly of microtubules. To this effect we have discovered that the unchanged dye, nile red, binds to the surfaces of proteins with known hydrophobic domains with marked enhancement of fluorescence intensity and blue shifts that are presumed to indicate the polarity of the binding domain. The dye is sensitive to denaturation of the protein, conformational changes produced by other ligands and changes in polymerization. Tubulin fluorescence shows hypsochromic and quantum shift changes that can identify the components of this monomer/dimer equilibrium. The equilibrium seen by fluorescence agrees with that seen by the more cumbersome hydrodynamic analysis. Agents that shift the equilibrium also shift the fluorescence behavior. Our current efforts are directed toward confirming these findings by other physical parameters.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45018-12 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Adenylate Cyclase and Other Extracellular Products of B. Pertussis

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

PI:	J. Wolff	Associate Chief CEB, NIDDK
Others:	L. Knipling	Technician CEB, NIDDK
	F. Gentile	Visiting Fellow, CEB, NIDDK
	A. Raptis	Visiting Fellow, CEB, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Biochemistry, NCI (D. Newton and Dr. C. Klee)

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Endocrine Biochemistry Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 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.)*

Conditions have been devised that permit study of the entry of Bordetella pertussis extracellular adenylate cyclase into a variety of host cells in culture. It is important to guard against errors deriving from the extracellular production of cAMP formed from secreted ATP. Extracellularly generated cAMP appears not to enter the cells although transport in the opposite direction can occur in some cell lines. Purified enzyme does not enter cells but crude preparations show rapid, dose-dependent huge accumulations of intracellular cAMP not originating from the outside but ascribable to cyclase that has crossed the plasma membrane. This entry appears not to occur by classical endocytosis as judged by inhibitor studies with chloroquin, dansyl cadaverine, monensin ammonium chloride or methyl amine chloride. These agents are effective protectors against other toxins such as diptheria toxin. Adenylate cyclase may require the participation of microtubules (rather than microfilaments) and appears to be quite different from the entry of other bacterial toxins. The data suggest the existence of a helper factor that is currently being identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45020-11 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Synthesis of Thyroxine Transport Proteins

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

PI: J. Robbins Chief CEB, NIDDK

Others: M. Phyillaier Biologist CEB, NIDDK

COOPERATING UNITS *(if any)*

University of Pisa, Italy (L. Bartalena)

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Hormone Metabolism and Action Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.2	.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The role of glycosylation in the secretion of thyroxine-binding globulin (TBG) was investigated by the use of swainsonine, an inhibitor of α-mannosidase in the Golgi. The drug caused incomplete glycosylation of TBG in cultured human hepatoma cells (Hep G2), accompanied by accelerated TBG secretion. This demonstrates that complete oligosaccharide processing is not required for TBG secretion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45028-09 CEB

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Thyroid Hormone-Cell Interactions

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

PI:	J. Robbins	Chief CEB, NIDDK
Others:	M. C. Lakshmanan	Medical Staff Fellow CEB, NIDDK
	A. Pontecorvi	Visiting Fellow CEB, NIDDK
	M. Centanni	Guest Researcher CEB, NIDDK
	M. Phyillaier	Biologist CEB, NIDDK
	E. Goncalves	Visiting Fellow CEB, NIDDK
	D. Foti	Visiting Fellow CEB, NIDDK

COOPERATING UNITS *(if any)*: University of Rome, Rome, Italy (Pontecorvi, Centanni); University of Porto Allegra, Porto Allegra, Brazil (Goncalves); University of Catania (Foti)

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Hormone Metabolism and Action Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.2	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The mechanism and significance of intracellular entry of thyroid hormones has been studied in cultured cell lines. These have included rat myoblasts, mouse and human neuroblastoma cells, human medulloblastoma cells, and human glioma cells. All cells tested possessed a saturable, energy dependent transport system for T_3 located at the plasma membrane that is important for uptake of the hormone by the cell nucleus. In addition, rat myoblasts and mouse neuroblasts showed stereospecific uptake and the latter showed saturable uptake of T_4 as well as T_3. Differentiation of neuroblastoma cells, induced by butyrate, was shown to be accompanied by a transient increase and then a decrease in T_3 transport. The myoblasts were also used for a study of mechanism of induction of creatine kinase during cell differentiation. This appeared to depend on one or more short lived proteins, encoded by short lived mRNA(s) that selectively regulate the stability of the inducible mRNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45033-04 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	V. M. Nikodem	Visiting Scientist	CEB, NIDDK
Others:	H. Morioka	Guest Researcher	CEB, NIDDK
	G. Tennyson	Staff Fellow	CEB, NIDDK
	S. Usala	Medical Staff Fellow	CEB, NIDDK
	K. Petty	Medical Staff Fellow	CEB, NIDDK

COOPERATING UNITS *(if any)*
Dr. Scott Young, NIMH

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Hormone Metabolism and Action Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.3	.2

CHECK APPROPRIATE BOX(ES)

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

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

We have completed the structural map of the malic enzyme gene. This gene is more than 115 kb long and split into 14 exons.

We have also determined the sequence of the 914 base pairs of DNA 5' of the coding region of the malic enzyme gene. This region possesses neither TATA nor CCAAT sequences but it is rich in G/C residues. Promoter activity of chimeric genes that contain various parts of the 5' flanking region of the malic enzyme gene placed upstream of the coding sequence of the chloramphenicol acetyl was tested in transient transfection assays using several cell lines. Deletion analyses have revealed that sequences +1 to -41 are sufficient to initiate expression, although inclusion of sequences up to -177 is necessary for maximal promoter activity.

The chromatin structure of the malic enzyme gene has been analyzed in different thyroidal states. There are four hypersensitive sites in the 5' flanking region of the gene at positions -50 bp, -170 bp, -310 bp, and at approximately -4.1 Kb. There are five hypersensitive sites spaced approximately equidistantly Triiodothyronine increased the proportion of chromatin displaying malic enzyme gene hypersensitivity. We have shown that this might be due to "recruitment" of a population of hepatocytes after triiodothyronine treatment, by localizing malic enzyme mRNA in hepatocytes using in situ hybridization histochemistry.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 45034-04 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Specific Rat Liver mRNAs by Thyroid Hormone

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

PI:	V. M. Nikodem, Ph.D.	CEB, NIDDK
Others:	D. Greico, M.D.	CEB, NIDDK
	M. H. Song	CEB, NIDDK
	H. Morioka	CEB, NIDDK
	T. Mitsuhashi	CEB, NIDDK

COOPERATING UNITS *(if any)*
Dr. S.M. Aloj and Dr. L.Kohn, LBM, NIDDK

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Hormone Metabolism and Action Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.8	3.6	.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 mRNA of the lipogenic malic enzyme in FRTL-5 cell line is post-trancriptionally regulated by the thyroid stimulating hormone. The malic enzyme mRNA level increases about 5 fold within 6 hours after thyroid stimulating hormone addition and stays at this level for 18 hours there after. This increase is followed by a gradual decline, reaching the basal level at 72 hours after addition of the hormone. The re-addition of this hormone did not prevent the decrease. The treatment of the cells with the hormone and cyclohexamide did not alter the level of the malic enzyme mRNA, contrary to the treatment with actinomycin D. Actinomycin D added 23 hours after the hormone addition abolished the decrease in the malic enzyme mRNA level for 48 hours. Thus, it appears that ongoing protein synthesis is required to alter the stability and degradation rate of this message. Experiments are underway to establish if these changes are a cell cycle related.

Selection of the malic enzyme-specific intronic probes was performed in order to investigate nuclear stabilization of the rat liver malic enzyme transcript by thyroid hormone. Among 3 intronic probes tested so far, one probe hybridizes with the malic enzyme RNA only, and shows 11-fold stimulation by hormone. The increase is in agreement with that of cytoplasmic mRNA. Two more such intronic probes are under search. Northern blot analyses of nuclear transcripts and kinetic studies will be performed with these specific probes.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45035-04 CEB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Photoaffinity Labeling of Thyroid Hormone-Specific Binding Proteins

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

PI : V. M. Nikodem Visiting Scientist CEB, NIDDK

Others: H. J. Cahnmann Scientist Emeritus CEB, NIDDK

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Hormone Metabolism and Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.8	.6	.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.)

Findings in last year's report have been consistently supported by many additional analyses of rat liver extracts and isolated rat liver nuclei under a variety of experimental conditions. In addition, the analysis of photoaffinity-labeled whole cells (rat hepatocytes) showed only one predominant electrophoretic band (≈ 60 kDa) and one minor one (≈ 57 kDa) supporting the concept that any binding proteins with higher electrophoretic mobility previously observed in high-salt nuclear extracts represent breakdown products formed in the course of working up rat liver homogenates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 45037-02 CEB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

PI:	A. Pontecorvi	Visiting Fellow CEB, NIDDK
Others:	M. Phyillaier	Biologist CEB, NIDDK
	J. Robbins	Chief CEB, NIDDK
	J. Tata	Fogarty Scholar, CEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH: Clinical Endocrinology Branch

SECTION: Hormone Metabolism and Action Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.7	.5	.2

CHECK APPROPRIATE BOX(ES)

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

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

The rapid induction of muscle-specific gene products accompanying the fusion of cultured myoblasts to myotubes that is seen after removal of the mitogenic stimulus of serum and the addition of insulin has been intensively studied. We investigated the effect of removal and re-addition of insulin on the stability of induced mRNAs during and after differentiation of rat L_6A_1 myoblast cells in culture. Addition of insulin caused an 80 fold increase in creatine phosphokinase (CK) activity, a similar increase in CK mRNA, and a parallel increase in myosin heavy chain (MHC) mRNA. Removal of insulin caused CK mRNA, but not MHC mRNA, to be rapidly degraded, the effect being reversed when the hormone was added back. Cycloheximide and actinomycin D mimic the action of the inducer also in a reversible manner, indicating that a short-lived protein(s) encoded by a short-lived mRNA(s) selectively regulates the stability of inducible mRNA. Such factor(s) may be an important requirement for normal developmental processes.

GPO 914-918

Recognition of Previous Achievements

The Diabetes Branch continues to pursue a broad based program which encompasses clinical research, studies on the mechanism of insulin action, with special emphasis on the nature and function of the insulin receptor, studies on the evolution of hormones and their function as messenger molecules, gene sequencing of insulin and insulin-like growth factors I and II, studies on morphological interaction of hormones with cells, and detailed studies of the biosynthesis of the insulin receptor.

Grants were made to members of the Diabetes Branch from the Juvenile Diabetes Foundation. An additional fellowship was received from the Pharmacology Research Associate Program of the National Institute of General Medical Sciences. There is also a U.S.- Spain Cooperating Project which in part is used to support a Spanish Fellow. Dr. Joshua Shemer, Visiting Fellow, received the 1987 Caroline tum Suden Professional Opportunity Award given by the Committee on Women in Physiology of the American Physiological Society.

INSULIN RECEPTORS AND RELATED HORMONES

Phosphorylation of the Insulin and Insulin-like Growth Factor I Receptors

The alpha-subunit of the insulin receptor is the major binding subunit of the receptor and that the beta-subunit is a protein kinase, capable of autophosphorylating itself and a number of exogenous substrates. Many details of this phosphorylation reaction in both blood cells and other cells have been under continued study. It is clear that each tissue receptor has both a binding and protein kinase region and in that sense, all insulin receptors are similar; they are somewhat different, however, in their molecular weight based on migration on polyacrylamide gel electrophoresis. In studies carried out on guinea pigs, chickens, rats, lizards, frogs, and alligators, it is clear that these differences in structure are maintained both ontogenetically and phylogenetically. Further, similar studies have been carried out in neuroblastoma cell lines from adult rat tissue as well as primary cultured rat neuronal and glial cells. In addition to insulin receptors, the IGF-I receptors, which resembles the insulin receptors, are also found on neuronal tissues.

Both insulin and IGF-I receptors on neural tissues undergo autophosphorylation of the beta subunit following binding of the respective ligand to the predominantly extracellular alpha subunit. In addition, this results in stimulation of tyrosine kinase activity. Thus neuronal receptors, despite their differences in structure, are functional, and their presence suggests a role for insulin and IGF-I in the nervous system.

In previous studies we demonstrated that the tyrosine kinase domain of the insulin receptor possesses structural homology to tyrosine kinases encoded by viral oncogenes such as v-src and v-erb B. A growing body of evidence

strongly supports the hypothesis that the tyrosine kinase activity of the insulin receptor plays a key role in mediating the biological action of insulin.

We have identified a Mr-120,000 kDa glycoprotein (pp120) in rat liver membranes which can be phosphorylated on tyrosine-residues by solubilized insulin receptors in vitro. pp120 fulfills the necessary conditions to be considered as a physiologically relevant substrate for the insulin receptor-associated tyrosine kinase; first, it can be phosphorylated directly by the insulin receptor in a cell-free system; and second, insulin stimulates tyrosine-specific phosphorylation of pp120 in intact target cells for insulin action. pp120 can also be phosphorylated by solubilized EGF receptors. The protein has been partially purified, and a rabbit antiserum to pp120 has been obtained. Using the anti-pp120 antiserum, we have demonstrated that insulin stimulates phosphorylation of tyrosine residues in pp120 in intact hepatoma cells cultured in vitro.

Our investigations of Type II diabetes have led to the interesting discovery of a generalized defect in the relationship of the alpha and beta subunit functions in untreated patients. Specifically, the amount of kinase activity stimulated by insulin per unit of insulin binding is decreased in these patients compared to normals or obese non-diabetics. These data may indicate a defect in tyrosine kinase data or in the interaction of the two subunits of the insulin receptors of these patients. We are pursuing these interpretations by means of studies of the subunit interactions, i.e., effects of reductants which break the covalent attachments between the subunits.

Biosynthetic Labeling of Insulin & Insulin-like Growth Factor Receptors

The biosynthesis of the insulin receptor has been studied in cultured cells using an experimental approach based on biosynthetic labeling of the receptor with radioactive sugars or amino acids, immunoprecipitation with anti-receptor antibodies, and analysis by $NaDSO_4$/polyacrylamide gel electrophoresis.

The insulin receptor is an integral membrane protein composed of two major subunits, α and β, of apparent molecular weights of 135,000 and 95,000, respectively. The α and β subunits of the receptor contain covalently attached carbohydrate chains of the asparagine N-linked type. The α subunit appears to be more heavily glycosylated than the β subunit. Both α and β subunits contain oligosaccharide side chains of the complex and high mannose types. Pulse-chase labeling studies with radioactive sugars and amino acids followed by immunoprecipitation with anti-receptor antibodies and analysis on SDS/polyacrylamine gel electrophoresis have demonstrated the existence of a single polypeptide chain precursor of the insulin receptor, i.e., a proreceptor with an apparent molecular weight of 190,000. Our model for the biogenesis of the insulin receptor proposes that the single chain polypeptide-precursor is translated and the high mannose core is added co-translationally to the nascent polypeptide in the endoplasmic reticulum of the cell.

We have also explored the role for carbohydrate chain processing of the insulin receptor by inhibiting the removal of glucose residues from core oligosaccharides. Culture human lymphocytes were treated with either castanospermine, a plant alkaloid that inhibits glucosidase I, or 1-deoxynojirimycin, an antibiotic that inhibits glucosidase I and II.

Our results are consistent with the conclusion that prevention of glucose removal from core oligosaccharides retards processing of the insulin receptor and produces a marked decrease in cell surface receptors. However, proteolytic cleavage of the proreceptor is not blocked, although it takes place at a slower rate, and further processing of some of the carbohydrate chains is not completely inhibited. Furthermore, the processed receptors are inserted into the plasma membrane and their binding affinity is normal despite the presence of an undetermined number of glucosylated chains. In addition to carbohydrates, several membrane receptors contain covalently linked fatty acids. We investigated whether the insulin receptor also contains covalently linked fatty acids. Both [^{3}H]myristic acid and [^{3}]palmitic acid are found attached to the receptor subunits. The incorporation of fatty acids into the insulin receptor is dependent on protein synthesis and is also detectable in the M_r = 190,000 proreceptor form. Fatty acylation is thus a newly identified post-translational modification of the insulin receptor.

Insulin and IGF-I receptors are integral membrane glycoproteins of similar size consisting of two dissimilar subunits. The insulin receptor is processed from a high molecular weight precursor and contains both complex and high mannose oligosaccharide side chains. By using glycosylation mutant cell lines the carbohydrate portion of the insulin receptor was shown to be important for receptor affinity. The IGF-I receptor, biochemically similar and phylogenetically related to the insulin receptor, does not seem to be similarly sensitive to changes in the oligosaccharide portion of the receptor and/or other glycoproteins. Both insulin and IGF-I receptors are present in Chinese hamster ovary (CHO) cells. Two mutant CHO cell lines with specific defects in protein glycosylation exhibit anomalous insulin but not IGF-I binding.

Syndromes of Extreme Insulin Resistance

We have identified several classes of patients, each of which appears to have a different mechanism of insulin resistance:

(1) Quantitative receptor defects. In some patients with genetic forms of extreme insulin resistance, the cause of insulin resistance is a marked (≥90%) reduction in the number of cell surface receptors.

As described above, insulin receptors are composed of two major glycoprotein subunits (apparent molecular weight [M_r] of 135 and 95 kDa), which are both derived from a common precursor molecule with M_r of 190 kDa. In one patient there was a marked reduction in the biosynthesis of both the 190-kDa precursor and the mature receptor, i.e., the defect appears to occur early in the biosynthetic pathway. In contrast, in two sisters with type A extreme insulin resistance, biosynthesis of the 190-kDa precursor proceeds at a normal rate. However, there appears to be a defect subsequent to the biosynthesis of the 190-kDa precursor, but before the insertion of the mature receptor in the plasma membrane. These data suggest the existence of at least two distinct types of biosynthetic defects which may give rise to a marked reduction in the number of insulin receptors on the cell surface.

Fibroblasts cultured from severely insulin resistant patients showed that in a group of eight patients with partial or total lipoatrophic diabetes, three had levels of labeled ^{125}I-IGF-I binding significantly higher than control levels. Fibroblasts from an infant with leprechaunism, previously shown to bind insulin with low affinity, has completely normal IGF-I binding.

The patient with the Rabson-Mendenhall Syndrome (RM-1) had a similar defect similar to type-A patients since with either lactoperoxidase-catalyzed radioiodination of cell surface receptors or biosynthetic labeling of receptors with glucosamine, we demonstrated an 80-90% decrease in the number of insulin receptors.

We have used the cloned human insulin receptor cDNA to investigage the nature of the mutations causing the reduction in the number of insulin receptors in EBV-lymphocytes. Within the normal population, there is a close correlation between the number of insulin receptors on the surface of EBV-lymphocytes and the cellular content of insulin receptor mRNA. In one patient with leprechaunism there is a marked reduction in the level of receptor mRNA, which probably accounts for the extremely slow rate of receptor biosynthesis measured in this patient's cells. Two patients with type A extreme insulin resistance (sisters are the products of a consanguineous marriage), have normal levels of insulin receptor mRNA. In addition, we have analyzed restriction fragment length polymorphisms (RFLPs) of the insulin receptor gene in the family of these two patients including the parents who are first cousins as well as four unaffected siblings. Because of consanguinity, it seems likely that insulin resistance is caused by inheritance of an autosomal recessive mutation. Genotypes for two RFLP's have been identified with the restriction endonucleases Sac I and Eco RI. Both the mother and the father possess an allele identified by these polymorphisms as Sac I (-) and Eco RI (-); this allele appears to be identical by descent from a common grandparent. Both patients are homozygous for this allele. The probability of this occurring at random is ≤ 0.005. These data are consistent with the hypothesis that the mutation causing diabetes is genetically linked to the insulin receptor gene.

(2) Qualitative receptor defects. We have identified a patient who has a normal number of receptors, although the receptors are themselves qualitatively abnormal. The abnormality can be recognized in several ways: (a) decreased sensitivity to changes in temperature; (b) decreased sensitivity to changes in pH; (c) abnormally high binding affinity for insulin; and (d) absence of positive cooperative site-site interactions. We have also studied this patient's insulin receptor (Epstein-Barr virus transformed lymphocytes) for tyrosine kinase activity and found it to be normal. This suggests that the defect causing this patient's insulin resistance is independent of the receptor-associated tyrosine kinase.

Autoantibodies to the Insulin Receptor

Antibodies directed against the insulin receptor have played a central role in investigations of the insulin receptor structure and function. Initially, these antibodies were identified in the serum of patients with an autoimmune form of extreme insulin resistance. All of the anti-receptor autoantibodies in the original studies shared the ability to inhibit insulin binding. More recently, however, we have identified a patient whose serum contained anti-receptor antibodies which immunoprecipitated the insulin

receptor without inhibiting insulin binding.

We have synthesized peptides corresponding to specific structural domains in the receptor. Rabbits have been immunized with these peptides in order to develop anti-receptor antibodies directed against specific sites in the receptor. The antibodies have been employed to define the functions of these structural domains. In addition, anti-receptor antibodies have been used to identify structural abnormalities in patients with insulin resistant diabetes mellitus.

HYPOGLYCEMIA ASSOCIATED WITH NON-ISLET CELL TUMORS

We have previously reported elevated levels of plasma IGF-II-like material in 32% of patients with hypoglycemia associated with non-islet cell tumors. Recently a patient was cured by removal of a hemangiopericytoma. This tumor is rich in mRNA for IGF-II. In two lines of Hep G2 cells (human hepatoblastoma) and in human placenta, two tissues previously reported to be rich in IGF-II mRNA, significant levels of IGF-II mRNA were present in bands 7Kb, 3.3Kb and 2.4Kb in length; "these same" band lengths are also found in this patient's tumor tissue. The tumor in comparison to these tissues had 2.8-4.8 fold higher levels of IGF-II mRNA as determined by densitometric scanning of Northern blots. Hybridization of total RNA from the tumor to ^{32}P-labeled cDNA's of rat insulin and rat IGF-I failed to demonstrate any hybridizing bands. Thus, a hemangiopericytoma in a patient with fasting hypoglycemia produced markedly increased levels of IGF-II mRNA relative to other normal adult tissues as well as to Hep G2 cells. Determination of tumor and plasma IGF-II peptide levels will help to confirm the previous report of over-production of IGF-II-like material in 6 of 7 hemangiopericytomas evaluated.

HUMAN GROWTH HORMONE AND ITS RECEPTOR

Using standard crosslinking techniques with bi-or functional reagents, we have recently been able to cross-link growth hormone to its specific cellular receptor on cultured human lymphocytes. These studies are analogous to the studies using the insulin receptor in this cell. The cross-linked growth hormone receptor is electrophoresed under reducing conditions yielding an approximate 140kDa protein as the predominant band. Under non-reducing conditions, however, a molecular weight component ~ 270 kDa is seen. Further attempts were made to see if the growth hormone receptor was a protein kinase or a substrate for kinase activity; this does not appear to be the case. Thus, the growth hormone receptor is not analogous to the insulin receptor in this respect.

In addition, the glycoprotein nature of the hGH receptor has been examined. hGH receptor complex binds to wheat germ but reacts poorly with a panel of several other lectins. Treatment with neuraminidase diminishes the apparent molecular weight suggesting that sialic acid is involved in the oligosaccharide linkage. Endonuclease H digestion decreases mobility suggesting that most of the carbohydrate is in a complex form. However, endonuclease F digestion decreases mobility suggesting that most of the carbohydrate is N-linked. Thus, the growth hormone receptor seems to have more complex carbohydrates in the mature receptor than does the insulin receptor.

Acromegaly

Acromegalic patients have continued to be followed with respect to pituitary irradiation. Further, we are evaluating the effects of transsphenoidal hypophysectomy in these patients and comparing them to the pituitary-irradiated patients. Therapeutic studies in acromegaly have been continued in three ways: a) surgical therapy, b) conventional supervoltage irradiation and c) pharmacological treatment.

We have studied the use of the long-acting somatostain analogue, SMS 201-995, in acromegaly, patients with TSH secreting pituitary tumors and glucagonomas. These studies have defined 1) an appropriate dose and schedule for control of TSH secretion from TSH secreting pituitary adenoma and its resultant hyperthyroidism, 2) an appropriate subgroup of acromegaly patients in whom this analogue, given thrice daily, controls GH hypersection, 3) the effects of the drug in glucagonoma syndrome in terms of control of glucagon hypersecretion and correction of hypoaminoacidemia. Our current studies have focused on the long term use of this agent in acromegaly and patients with TSH secreting tumors and the correlation of hormonal effects with symptomatic benefit. In addition, our studies indicate that all patients while receiving treatment develop thickened bile accumulation in the gallbladder which may progress to gallstones.

Morphologic Studies of Ligand Binding to Cells

This work represents over 10 years of collaboration between the Diabetes Branch and the Institute of Histology and Embryology at the University of Geneva. The initial observations demonstrated that polypeptide hormones are taken up by the cell through a process of receptor-mediated endocytosis similar to other biologically important ligands that bind to the cells. In the present study we find there is an anatomical correlation between the dissociation of ^{125}I-insulin and its localization on the cell surface. These studies have now been extended to include an insulin resistant cell line which has dissociation characteristics which could be explained by this abnormal surface leading to a higher association of ligand to the non-villous portion of the cell surface. We have demonstrated that receptor-mediated endocytosis also appears to be regulated in hypoinsulinemic states, i.e., in both rat and in human type I diabetes there is an inhibition of ^{125}I-insulin internalization in the hyperglycemic state; the normal state is restored by insulin treatment. We have studied the role intracellular calcium on the endocytotic process as well as the relationship of stimulators of protein kinase C to internalization of both insulin and unrelated ligands such as transferrin. In addition, we are studying the function of the small non-coated invaginations in receptor-mediated endocytosis.

INSULIN AND INSULIN-LIKE GROWTH FACTORS (I AND II) RECEPTORS IN BRAIN/CNS AND EMBRYOS

Specific insulin receptors are widely distributed throughout the rat brain. The brain receptors are very similar to insulin receptors previously characterized in other tissues. Using fresh frozen sections of brain from adult (Sprague-Dawley) rats, we visualized insulin receptors by autoradiographic techniques. While insulin binding was widely distributed throughout the brain, heavy concentration of receptors were noted in the (i) choroid plexus, (ii) the olfactory bulb and closely related olfactory areas,

(iii) limbic areas such as the hippocampus and amygdala, and (iv) the cerebellum as well as other secondary motor areas. Competition studies with unlabeled analogues showed that the binding visualized on the tissue slices was to a typical insulin receptor.

Applying autoradiographic techniques, we have studied the binding of ^{125}IGF-I and ^{125}-rIGF-II to brain receptors and compared the IGFs' binding patterns to insulin's binding pattern. Like insulin, the IGF receptors were widely distributed throughout the brain. Especially dense were regions similar to those of insulin i.e. choroid plexus, olfactory bulb, limbic regions, and cerebellum. In each area, the binding of each of the three peptides conforms to well defined cytoarchitectonic boundaries. However, each of the three peptides binds to a distinctive region within each area. Thus, except for the choroid plexus, it appears that the receptors for the three peptides are binding to nearby but distinctly different groups of cells. In addition, competition studies were performed in those regions of rat brain which exhibited high density of binding for the IGFs.

Brain insulin and IGF-I receptors have unique structures when compared to their peripheral, non-neural counterparts. We have previously demonstrated that the unique brain insulin receptor is phylogenetically and developmentally conserved being present in human, rat, guinea pig, chicken and lizards. In extending these findings we have also studied alligators and frogs and similar results were obtained.

To determine whether the brain insulin receptor is unique to central nervous system tissues, or whether all neural tissues express this type of receptor, we examined retinal tissues and peripheral nerves including the trigeminal nerve and the superior cervical ganglia. In peripheral nervous tissues the insulin receptor is similar to insulin receptors on liver, adipocytes, and placenta namely, its apparent Mr on SDS-PAGE is larger than that of the brain insulin receptor. Retinal insulin receptors had both "brain-type" and non-brain type insulin receptors. Since insulin and IGF-I receptors are similar in structure, it was important to distinguish these receptors on the nervous tissues being studied in order to dissect out the function of each receptor. Having previously characterized the insulin receptor on neuronal and glial cells, we investigated the IGF-I receptors on these primary cultured cells from 1 day old neonatal rats. The IGF-I receptors on neuronal cells demonstrated an apparent Mr on SDS-PAGE similar to brain IGF-I receptors namely 10 kDa less than that of IGF-I receptors from placenta. Glial cells, on the other hand, express IGF-I receptors with apparent Mr similar to that of peripheral, non-neural tissues. These differences are reminiscent of the differences in Mr of the insulin receptor from neuronal and glial cells. In these cultured cells IGF-I stimulated thymidine incorporation in a dose dependent manner, suggesting that IGF-I may play a role in growth of both neuronal and glial cells. Insulin on the other hand stimulated glucose uptake in glial cells and inhibited catecholamine uptake in neuronal cells suggesting that it had different functions from that for IGF-I.

Previously, we described the appearance of insulin receptors in chick embryos heads as well as bodies by day 4 of egg incubation. In present studies using labeled insulin-like growth factor I and II and crude membrane preparations of developing chick embryos, there appears to be specific binding to a growth factor receptor (IGF-I). The IGF binding is present in "heads" and

brains of embryos day 2-18. Further, the binding pattern is different than observed for insulin. The structure of IGF and insulin binding sites charac terized and compared in developing tissues procedures using crosslinking and SDS-PAGE under reducing conditions were carried out. Insulin and IGF receptors are present in multiple chick embryo tissues at early stage of development, but each tissue has a distinct time-related pattern of binding, possibly reflecting different roles. Despite developmental changes in binding, both IGF-I and IGF-II appear to bind to a single type I α subunit, which shares with the subunit of the insulin receptor similar tissue-dependent mobilities therefore reinforcing the relationship between these sites.

The physiologic function of insulin in early embryonic life is unknown. To define insulin's role in the chick embryo, we exposed 2-day-old chick embryos to anti-insulin antibodies and followed their development up to day 5. Antibody-treated embryos had a higher rate of growth retardation and death by days 3-5 of embryogenesis compared with controls. Among the survivors, biochemical maturation was delayed at days 4 and 5; weight, protein, total creatine kinase activity, and creatine kinase-MB were decreased in antibody-treated embryos.

In contrast, insulin (50 ng/embryo) administered to 2-day-old embryos yielded nearly symmetrical stimulatory results. These findings suggest that endogenous insulin plays a probable physiologic role regulating growth and differentiation in early embryos. In addition, the findings provide some clues to a possible function for insulin produced outside the organism's own beta cells.

CELLULAR HORMONE-LIKE PEPTIDES AND GENE ISOLATION

The existence in invertebrates, unicellular eukaryotes, and prokaryotes of materials that resemble several vertebrate peptide hormones led to the suggestion that these peptide messengers may have arisen earlier in evolution than had previously been thought. Consistent with this hypothesis, we have found material in two plants, spinach and Lemna gibba G3, that is very similar to mammalian insulin, yet distinctive. In each of the early purification steps, the immunoactive material from plants resembled the common vertebrate insulins. The protein nature of the material was suggested by its destruction by pronase but not by the inactivated enzyme. In addition, on TSK chromatography it eluted earlier, and thus was more hydrophilic than most of the common mammalian insulins, including pork insulin. The interaction of the plant materials with anti-insulin antibodies in a radioimmunoassay was confirmed by using an affinity column of anti-insulin antibodies. The plant insulin-like material was distinguished immunologically from chicken insulin by homologous and heterologous radioimmunoassays. The plant insulin-like material bound to insulin receptors on IM-9 lymphocytes and stimulated glucose oxidation and lipogenesis in isolated adipocytes from young rats. The bioactivity was neutralized in the presence of anti-insulin antibodies, but not in the presence of normal guinea pig IgG. The role of this insulin-like material in plants is unknown but its existence is consistent with an early evolutionary origin of the insulin messenger peptide family. Alternatively, we cannot exclude a later convergent development of this family or introduction of vertebrate DNA into plants.

Based upon the discovery of vertebrate hormone-like materials in lower organisms, we are currently attempting to isolate and analyze the pertinent

gene sequences from Saccharomyces cerevisiae and Drosophila melanogaster. To date, we have been concentrating on insulin, insulin-like growth factors, and somatostatin, and the characterization of their counterparts in these species. Our experimental approach involves the screening of cDNA expression libraries in λgtll with antibodies to intact mammalian insulin, insulin B chain and somatostatin. Positive clones have been isolated from two libraries one constructed with yeast DNA and the other with Drosophila head cDNA, using non-purified anti-insulin B chain and antisomatostatin antibodies. Further characterization of these clones by DNA sequence analysis is currently being pursued.

In view of the possibility that the insulin-like molecules found in these more primitive organisms may be more similar to molecules such as insulin-like growth factor-I (IGF-I), than insulin itself, we have cloned the rat IGF-I cDNA's for use as a hybridization probe to screen the various banks described above. This was accomplished by probing a rat liver cDNA library with a previously characterized human IGF-I cDNA clone at high stringency. A number of independent rat IGF-I cDNA clones were isolated and their DNA sequences determined. These data revealed the high degree of conservation of IGF-I nucleic acid and amino acid sequences among mammalian species, and the characterized clones provide an array of probes with which to screen banks from unrelated organisms for the presence of insulin-like sequences, which may not be detectable with probes for insulin itself. As part of the characterization of the rat IGF-I probes, we also demonstrated differential splicing of rat IGF-I mRNA and transcriptional regulation of IGF-I gene expression by rat growth hormone in both liver and in extra-hepatic tissues. In addition to these findings we've also detected insulin immunoactivity and bioactivity in whole chick embryos before pancreatic β cell differentiation. Since insulin is apparently a requirement for normal development, we are investigating the pattern of expression of the insulin gene in the developing chicken pancreas and in the whole embryo at pre-pancreatic stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47001-06 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Phosphorylation of the Insulin Receptor

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

P.I.	D. LeRoith	Section Chief	DB/NIDDK
Others:	J. Shemer	Guest Researcher	DB/NIDDK
	M. Adamo	Guest Researcher	DB/NIDDK
	G.L. Wilson	Biologist	DB/NIDDK
	A. Ota	Visiting Fellow	DB/NIDDK
	R. Waldbillig	Guest Researcher	DB/NIDDK

COOPERATING UNITS *(if any)*

University of Florida, Gainesville, FL (M. Raizada)

LAB/BRANCH

Diabetes Branch

SECTION

Molecular and Cellular Physiology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	3.5	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Brain insulin receptors and IGF-I receptors are similar to their peripheral, non-neural counterparts, being comprised of two alpha subunits and two beta subunits in a heterotetrameric formation. On sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) they have smaller apparent Mrs compared to the peripheral receptors. These unique receptors are phylogenetically and ontogenetically conserved.

These unique insulin and IGF-I receptors have been studied in membranes prepared from whole retina, brain, peripheral nerves, as well as from neural-derived cultured cells. Primary cultures of neuronal cells contain unique insulin and IGF-I receptors resembling those of whole brain. Peripheral nerves and glial cells on the other hand contain insulin and IGF-I receptors similar to those found in non-neural tissues.

Both insulin and IGF-I receptors on neural tissues undergo autophosphorylation of the beta subunit following binding of the respective ligand to the predominantly extracellular alpha subunit. In addition, this results in stimulation of tyrosine kinase activity. Thus neuronal receptors, despite their differences in structure, are functional, and their presence suggests a role for insulin and IGF-I in the nervous system.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 DK 47005-15 DB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Insulin Receptors in Circulating Cells in Man

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

P.I.:	R. J. Comi	Senior Staff Fellow	DB, NIDDK
Others:	S. I. Taylor	Medical Officer	DB, NIDDK
	J. L. Young	Biol. Lab. Tech.	DB, NIDDK
	P. Gorden	Section Chief	NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section; Biochemistry and Molecular Pathology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

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

The present work continues prior investigations of the tyrosine kinase activity of the insulin receptor found on circulating cells in patients with insulin resistance. A defect in tyrosine kinase activity has been found in receptors of patients with Type II diabetes, who have mild resistance to insulin as well as in lipoatrophic diabetics, who exhibit more extreme insulin resistance. The effects of diet, fasting and treatment on these observed receptor abnormalities are under investigation.

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

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47007-12 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Antibodies to Receptors: Detection in Disease States and Use as Probes

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

P.I.: S.I. Taylor Chief, Bio. & Mol. Path. Section DB/NIDDK

Others:	B. Marcus-Samuels	Chemist	DB/NIDDK
	A. Cama	Visiting Fellow	DB/NIDDK
	A. Ota	Visiting Fellow	DB/NIDDK
	D. LeRoith	Visiting Scientist	DB/NIDDK
	D. Polomis	Medical Student	DB/NIDDK
	J. Roth	Chief	DB/NIDDK

COOPERATING UNITS *(if any)*

University of Naples (N. Perrotti) - Foreign

LAB/BRANCH

Diabetes Branch

SECTION

Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Antibodies directed against the insulin receptor have played a central role in investigations of the insulin receptor structure and function. Initially, these antibodies were identified in the serum of patients with an autoimmune form of extreme insulin resistance. All of the anti-receptor autoantibodies in the original studies shared the ability to inhibit insulin binding. More recently, however, we have identified a patient whose serum contained anti-receptor antibodies which immunoprecipitated the insulin receptor without inhibiting insulin binding.

In addition, based on the recently elucidated primary sequence of amino acids in the human insulin receptor, we have synthesized peptides corresponding to specific structural domains in the receptor. Rabbits have been immunized with these peptides in order to develop anti-receptor antibodies directed against specific sites in the receptor. The antibodies have been employed to define the functions of these structural domains. In addition, anti-receptor antibodies have been used to identify structural abnormalities in patients with insulin resistant diabetes mellitus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47014-18 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Acromegaly and Growth Hormone

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

P.I.:	P. Gorden	Section Chief	DB, NIDDK
Others:	C. M. Hendricks	Biol. Lab. Tech.	DB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	.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.)*

Acromegalic patients have continued to be followed with respect to pituitary irradiation. Further, we are evaluating the effects of transsphenoidal hypophysectomy in these patients and comparing them to the pituitary-irradiated patients.

Recently, human growth hormone has been cross-linked to its specific cellular receptor on IM-9 cultured lymphocytes. Under non-reducing conditions, however, a molecular weight component of ~ 270K is observed. Further attempts were made to see if the growth hormone receptor was a protein kinase or a substrate for this kinase activity; it is not. Thus, the growth hormone receptor is not analogous to the insulin receptor.

The heterogeneity of circulating growth hormone in plasma has been studied. Pituitary growth hormone was injected in normal volunteers and the individual growth hormone components isolated by gel filtration. It was found that the half-time of the "little" (22,000 Dalton) growth hormone component was faster than the "big" and the "pre-big" growth hormone components, respectively. This is compatible with a receptor-mediated type of removal of these components; previous studies have shown that the high molecular weight forms have lower radioreceptor activity than the 22,000 Dalton growth hormone preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 47018 10 DB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

P.I.	J. Roth	Chief, DB	NIDDK
Others:	D. LeRoith	Section Chief	DB/NIDDK
	M.A. Lesniak	Chemist	DB/NIDDK
	E. Collier	Senior Staff Fellow	DB/NIDDK
	C.T. Roberts	Expert	DB/NIDDK
	G.L. Wilson	Biologist	DB/NIDDK
	G. Delahunty	IPA	DB/NIDDK
	W.L. Lowe	Med. Staff Fellow	DB/NIDDK

COOPERATING UNITS *(if any)*
Laboratory of Cellular and Development Biology, NIDDK (J. Shiloach)
Lab. Neurophysiology, NINCDS (A.E. Schaffner)
Smithsonian Institute, Rockville, MD (C. Cleland)
W. Virginia State College, Institute, W.V. (S. Lasky)

LAB/BRANCH
Diabetes Branch

SECTION
Molecular and Cellular Physiology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

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

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

Substances similar to insulin, ACTH and somatostatin are present in unicellular organisms and higher plants. The studies were extended to further characterize the insulin-related molecule in spinach, Lemna and rye. Using gel chromatography and high performance liquid chromatography the insulin-related materials were partially purified and the activity demonstrated by specific radioimmunoassay and bioassays.

To isolate the genes encoding these peptide hormones in multicellular nonvertebrates and unicellular organisms recombinant DNA technology is being used. Using lambda gtII expression vectors, Drosophilia and yeast DNA was probed for the expression of insulin and insulin-related peptides using anti-insulin antibodies. In addition rat IGF-I cDNA was cloned and sequenced to be used as an additional tool in the search for insulin-related genes in primitive eukaryotes and prokaryotes.

GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47019-10 DB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Morphologic Studies of Ligand Binding to Cells

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

P.I.: P. Gorden Section Chief DB, NIDDK

COOPERATING UNITS (if any)
Institute of Histology and Embryology, University of Geneva School of Medicine, Geneva, Switzerland. (J. L. Carpentier, A.Robert, L. Orci) - Foreign

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

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

This work represents over 10 years of collaboration between the Diabetes Branch and the Institute of Histology and Embryology at the University of Geneva. The initial observations demonstrated that polypeptide hormones are taken up by the cell through a process of receptor-mediated endocytosis similar to other biologically important ligands that bind to cells. In the present study we find a) there is an anatomical correlation between the dissociation of ^{125}I-insulin and its localization on the cell surface. This work has now been extended to include an insulin resistant cell line which has dissociation characteristics which could be explained by this abnormal surface leading to a higher association of ligand to the non-villous portion of the cell surface. In b) we have demonstrated that receptor-mediated endocytosis also appears to be regulated in hypoinsulinemic states, i.e, in both rat and in human type I diabetes there is an inhibition of ^{125}I-insulin internalization in the hyperglycemic state, the normal state is restored by insulin treatment. In c) we have studied the role of intracellular calcium on the endocytotic process as well as the relationship of stimulators of protein kinase C to internalization of both insulin and unrelated ligands such as transferrin. d) In addition, we are studying the function of the small non-coated invaginations in receptor-mediated endocytosis.

FORMERLY Z01 AM 47019-08 DB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47021-09 DB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cultured Cell Model for Hormone Receptors

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

P.I.:	J. M. Podskalny	Biologist	DB, NIDDK
Other:	D. G. Rouiller	Visiting Associate	DB, NIDDK
	P. Gorden	Section Chief	DB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

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

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

The insulin-like growth factor-I receptor of Chinese hamster ovary (CHO) cells has been characterized in wild type and two mutant CHO lines. The oligosaccharide portion of the IGF-I receptor and/or other non-receptor glycoproteins does not appear to affect IGF-I receptor affinity. This is in sharp contrast to the situation with insulin receptors which appear to be significantly affected by glycosylation changes. Glucose starvation, which forces the wild type cells to become phenotypically identical to one of the mutant cells lines, has no effect on IGF-I binding while it significantly increased insulin binding due to an increase in affinity. A monoclonal antibody to the human IGF-I receptor, alpha-IR3, reacts with the hamster receptor equally well in all CHO lines. By using CHO cells, both wild type and mutants, and various lectins, we have determined indirectly that the carbohydrates may play a role at cell surface while others may express more intrinsic properties. In studies with fibroblasts cultured from patients with severe insulin resistance, significantly elevated levels of IGF-I binding was found in 3 of 8 patients with lipoatrophic diabetes. Fibroblasts from an infant with leprechaunism and her phenotypically normal mother both had normal levels of IGF-I binding despite their abnormal insulin binding. Monoclonal antibody alpha-IR3 was able to partially inhibit 125I-insulin binding to both cell lines and the presence of alpha-IR3 in insulin competition curves shifted the dose response curve to the right.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47022-08 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Insulin Receptors in Syndromes of Extreme Insulin Resistance

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

P.I.: S.I. Taylor Chief, Bio. & Mol. Path. Section DB/NIDDK

Others:	C. McKeon	Sen. Staff Fell.	DB/NIDDK	P. Gorden	Sec. Chief DB/NIDDK
	K. Ojamaa	Visit. Fellow	DB/NIDDK	T. Kadowaki	Visit. Fell. DB/NIDDK
	B. Samuels	Chemist	DB/NIDDK	C. Frapier	Visit. Fell. DB/NIDDK
	V. Moncada	Guest Worker	DB/NIDDK	C. Bevins	Prat Fellow DB/NIDDK
	A. Cama	Visiting Fellow	DB/NIDDK	C. Hendricks	Biotech DB/NIDDK
	D. Accili	Visiting Fellow	DB/NIDDK		
	R. Comi	Sen. Staff Fellow	DB/NIDDK		

COOPERATING UNITS *(if any)*

Genentech, South San Francisco, CA (Axel Ullrich)
University Utah School of Medicine (Steven Elbein)
Washington Univ. School of Medicine (M. Alan Permutt)

LAB/BRANCH

Diabetes Branch

SECTION

Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9.4	7.4	2.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Insulin resistance contributes to the pathogenesis of several human diseases such as obesity and non-insulin-dependent diabetes mellitus. We have investigated patients with genetic forms of extreme insulin resistance to gain insight into biochemical defects which give rise to disease.

1. Decreased receptor biosynthesis. In some patients, cells contain a decreased level of insulin receptor mRNA. This, in turn, leads to decreases in the rate of receptor biosynthesis and the number of insulin receptors on the cell surface. It seems likely that the primary defect is a mutation in the rate at which the receptor gene is transcribed. We are characterizing the regulatory regions of the insulin receptor gene.

2. Impaired transport to the plasma membrane. In some insulin resistant patients whose cultured cells possess normal levels of receptor mRNA and appear to biosynthesize receptors at a normal rate. There appears to be an impediment to the insertion of the receptors in the plasma membrane. Analysis of the inheritance of restriction fragment length polymorphisms has suggested that the mutation causing insulin resistance is linked to the insulin receptor gene.

3. Defect in transmembrane signalling. With some insulin resistant patients, the cultured cells possess a normal number of insulin receptors, but the receptors are qualitatively abnormal. These defects have been identified either because of abnormalities in binding affinity or defects in the receptor-associated tyrosine kinase activity.

We are presently attempting to obtain cDNA clones encoding patients' insulin receptors to identify mutations in the structural gene for the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47024-08 DB

PERIOD COVERED: October 1, 1986 to September 31, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Biosynthetic Labeling of the insulin receptor

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

P.I.	J. A. Hedo	Visiting Associate	DB, NIDDK
Others:	D. G. Rouiller	Visiting Associate	DB, NIDDK
	R. F. Arakaki	Medical Staff Fellow	DB, NIDDK
	E. Collier	Senior Staff Fellow	DB, NIDDK
	P. Gorden	Section Chief	DB, NIDDK

COOPERATING UNITS (if any): None

LAB/BRANCH: Diabetes Branch

SECTION: Clinical Cellular Biology Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

We have studied the biosynthesis of the insulin receptor in human IM-9 lymphocytes. The alpha (135,000) and beta (95,000) subunits of the receptor are synthesized in the endoplasmic reticulum as a single M_r = 190,000 glycoprotein with only high mannose oligosaccharide chains. This proreceptor is then transported to the Golgi complex where it undergoes proteolytic cleavage and carbohydrate processing. Direct analysis by high performance liquid chromatography of the carbohydrate chains of the insulin proreceptor demonstrate that the largest oligosaccharide found in control cells is $Glc_1MangGlcNac_2$ which represents only a small fraction (3%) of the total. The predominant proreceptor oligosaccharides are $MAN_9GLCNAc_2$ (25%) and $MAN_8GLCNAc_2$ (48%). Since a $GLc_3MAN_9GLUNAc_2$ species is transferred cotranslationally, cabohydrate processing of the proreceptor is very rapid and limited to removal of the three glucoses and one mannose. Furthermore, in the presence of glucosidase inhibitors, castanospermine and 1-deoxynojirimycin, an abnormal precursor of M_r = 205,000 is synthesized. The processing of this precursor to mature subunits is delayed and there is a reduction in cell surface insulin receptors. Thus, glucose removal is an important signal for processing of the insulin receptor.

Additionally, we have found that the insulin receptor contains covalently linked fatty acids. Both the alpha and the beta subunits incorporate [^{3}H]myristic and [^{3}H]palmitic acids. The incorporation of fatty acid is dependent on protein synthesis and is found in the M_r = 190,000 precursor. Thus, fatty acylation is a newly identified post translational modification of the insulin receptor.

GPO 914-618

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47025-04 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Tissue Receptors for Insulin and Insulin-like Growth Factors

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

P.I.:	M. A. Lesniak	Chemist	DB/NIDDK
	F. de Pablo	Visiting Scientist	DB/NIDDK
	J. Roth	Chief	DB/NIDDK
Others:	C. L. Bevins	Pratt Fellow	DB/NIDDK
	J. Serrano	Visiting Fellow	DB/NIDDK
	M. Rojeski	Visiting Researcher	DB,NIDDK

COOPERATING UNITS (if any) University of Barcelona, Sao Paolo, Barcelona, Spain (Ll. Bassas, M. Girbau) U.S. - Spain Cooperative Project NIMH, Clinical Neuroscience Branch (J.M. Hill, C.B. Pert); NIMH, Laboratory of Cell Biology (W.S. Young).

LAB/BRANCH

Diabetes Branch

SECTION

Receptor and Hormone Action Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Insulin immunoactivity and bioactivity are detected in whole chick embryos before pancreatic β cell differentiation. Since insulin is apparently a requirement for normal development, we are investigating the pattern of expression of the insulin gene in the developing chicken pancreas and in the whole embryo at pre-pancreatic stages.

Insulin-like growth factor binding also is being studied in chick embryo tissue. Using labeled insulin-like growth factor I and II (multiplication stimulating activity) and unlabeled homologous and heterologous peptides in competion binding assays, it appears that there are a specific insulin-like growth factor I receptor but not insulin-like growth factor II receptor in the developing chick embryo. The growth factor binding pattern is different from insulin binding in several chick embryo tissues.

To define insulin's role in early development anti insulin antibodies were injected into fertilized eggs and the effect of antibodies was studied.

Insulin receptor and insulin-like growth factors (I and II) receptors structure studies have been extended in rat brain. The binding of labeled peptides to thin sections of frozen fresh rat brain was visualized with autoradiography. By several criteria including structure-activity relationship analysis, these brain peptide receptors were qualitatively indistinguishable from peptide receptors previously characterized on brain and other more typical target tissues and distinct from each other. Each peptide exhibits its own distinctive binding pattern - i.e., each peptide binds to cytoarchitectonic structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 47026-03 DB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Tyrosine-Specific Protein Kinase Activity Associated with the Insulin Receptor

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

P.I.: S.I. Taylor Chief, Bio. & Mol. Path. Section DB/NIDDK

Others: S. Brown-Phillips Guest Worker DB/NIDDK
D. Accilli Visiting Fellow DB/NIDDK

COOPERATING UNITS *(if any)* East Carolina University (Jose F. Caro)
Columbia University College of Phys. & Surgns. (Dr. Robert Rees-Jones)
Howard University, Washington, D.C. (D. Semina, R. Margolis)
University of Naples (N. Perrotti) - Foreign

LAB/BRANCH

Diabetes Branch

SECTION

Biochemistry and Molecular Pathophysiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.6	2.6	1.0

CHECK APPROPRIATE BOX(ES)

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

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

In first step of insulin action, insulin binds to its receptor on the surface of the target cell. The insulin receptor is a transmembrane protein which possesses a tyrosine-specific protein kinase. When insulin binds to the extracellular domain of the receptor, this activates the receptor's tyrosine kinase. A growing body of evidence suggests that the activation of the tyrosine kinase is a necessary step in initiating the biological actions of insulin. Accordingly, we have embarked upon a search for intracellular proteins which are substrates for phosphorylation by the receptor-associated tyrosine kinase. We have identified one such substrate in rat liver plasma membranes: a glycoprotein with an apparent molecular weight of 120,000 daltons (pp120). pp120 is present in liver from several species, but has not been identified in other tissues.

Using cultured H-35 hepatoma cells, we have demonstrated that insulin induces tyrosine-specific phosphorylation of pp120 in intact cells. In addition, we have demonstrated that pp120 is a substrate for phosphorylation by the solubilized epidermal growth factor receptor.

Presently, studies are underway to elucidate the structure and function of pp120, as well as the physiologic significance of its phosphorylation. In addition the ontogenesis of pp120 is being studied in the rat liver.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 47027-02 DB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of SMS 201-995 in Hormone Secreting Tumors

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

P.I.:	P. Gorden	Section Chief	DB, NIDDK
Others:	R. J. Comi	Senior Staff Fellow	DB, NIDDK
	R. F. Arakaki	Medical Staff Fellow	DB, NIDDK
	B. Weintraub	Chief	MCNEB, NIDDK
	N. Gesundheit	Senior Staff Fellow	MCNEB NIDDK

COOPERATING UNITS *(if any)*
none

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

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

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

We have studied the use of the long-acting somatostatin analogue, SMS 201-995, in acromegaly, patients with TSH secreting pituitary tumors and glucagonomas. These studies have defined 1) an appropriate dose and schedule for control of TSH secretion from TSH secreting pituitary adenoma and its resultant hyperthyroidism, 2) an appropriate subgroup of acromegaly patients in whom this analogue, given thrice daily, controls GH hypersection, 3) the effects of the drug in glucagonoma syndrome in terms of control of glucagon hypersecretion and correction of hypoaminoacidemia. Our current studies have focused on the long term use of this agent in acromegaly and patients with TSH secreting tumors and the correlation of hormonal effects with symptomatic benefit. In addition, our studies indicate that all patients develop thickened bile accumulation in the gallbladder while receiving treatment, which may progress to gallstones.

GPO 914-918

ANNUAL REPORT OF THE CLINICAL HEMATOLOGY BRANCH

National Institute of Diabetes and Digestive and Kidney Diseases

I. Study of Immunology of Blood Cell Deficiencies

A. Identification and characterization of receptors for Platelet-associated IgG, IgM and IgA

The purpose of this study was to define the attributes of normal serum immunoglobulin reactions that are visualized by labeled antiglobulin reagents on Western blots (WB) of platelets and to characterize platelet receptors mediating these reactions. We have found that the IgG components of the majority of normal sera reacted with platelet proteins in the 90-95kD range and less frequently at 200kD, 170kD, 120kD and 60-65kD ranges. In addition, IgM and IgA components in 40-50% of normal sera reacted in the 90-95kD range. The 90kD receptor is membrane-bound but not detected by immunofluorescent staining of intact platelets. Antibodies directed against it cannot be detected by adsorption and elution from whole platelets, further suggesting that it is an internal protein. It is distinct from GPIIIa by several criteria It is present in Bernard Soulier platelets and not thrombin sensitive indicating that it is distinct from GPV. There are many recent report concerning platelet-specific autoantibodies identified exclusively by WB data in a variety of thrombocytopenic disorders. Some of these disorders, e.g., thrombocytopenia associated with sepsis, had not previously been considered to represent immune-mediated destructive processes, whereas others, e.g. PTP and drug purpura, had been attributed to clearly identified antibodies against foreign antigens. In several instances the autoantibodies described reacted with platelet antigens in the 80-95kD and 120kD MW ranges. The present work raises the question as to whether certain antibodies identified by WB in thrombocytopenic disorders represent quantitative variations of normal patterns similar to "acute phase" reactivity rather than qualitatively abnormal antibodies. Moreover, information on binding of normal immunoglobulins by normal platelets has bearing on the, as yet obscure, significance of disease-induced variation in platelet-associated immunoglobulins and the mechanism of clearance of senescent platelets from the circulation by the reticuloendothelial system.

B. Studies on the Mechanism of Drug-induced Immune Thrombocytopenia

Studies done during the past year and extended during the present year have shown: 1) Drug antibodies associate specifically with drug in a weak haptenic reaction. 2) Cell interaction with drug is nonspecific but may be catalytic by generating a high local concentration of reactants at the liquid-solid interface. 3) Conformational changes in Fab induced by drug binding appear to cause complementarity between Fab and cell receptor and 4) Steric rearrangement of both Fab and cell receptor maximize complemen-

tarity and affinity.

We are currently evaluating the nature of platelet membrane components that are responsible for drug-antibody binding. Of special interest with respect to cell receptor specificity is the finding that platelets from patients with Bernard-Soulier syndrome (BSS) have decreased sensitivity to drug antibodies. BSS platelets are a rare variant in which proteins of the glycoprotein (GP) Ib "complex" are deficient. The specific proteins lacking in BSS are GP Ib, GP V, GP IX, and possibly GP 100. BSS platelets have been described as having no "receptor" for quinine- and quinidine-induced antibodies. When quinine- or quinidine-induced antibodies are measured by quantitative absorption, complement fixation, and immunofluorescence techniques under conditions of optimal drug concentration (10^{-3}M) rather than the suboptimal concentration used in the initial studies (10^{-5}M), we found reactions of BSS platelets with these antibodies were from one-third normal to nearly normal. This implies that components of the GP Ib "complex" are not the only membrane constituents that influence attachment of drug antibodies to cells. The fact that wheat germ agglutinin binds no more than 80% of drug antibody receptor activity but all of the GP Ib in Triton X-100 extracts of platelet membranes further supports this conclusion. GP Ib and GP IX, subjected to SDS-PAGE and then transferred to cellulose by Western blotting, did not bind drug-dependent antibodies. It appears that the entire structure of the GP Ib complex may be necessary to effect attachment of drug antibodies. This implies that there is a conformational requirement for binding, probably involving more than one component of the complex. We are currently using staphylococcal protein A to precipitate complexes formed by reaction of drug, drug antibody, and Triton X-100-solubilized radiolabeled platelet membranes from normal and BSS platelets.

C. Pathophysiology of Thrombocytopenia and Platelet-Associated Immunoglobulin Abnormalities in AIDS, Lupus and Primary Biliary Cirrhosis

The accepted concept that almost all nonthrombocytopenic SLE patients have increased platelet turnover with a "compensated thrombocytolytic state" is based on a study in which 5 of 5 patients had shortened platelet survival using a DF^{32}P label and 6 of 8 patients had megathrombocytosis presumed to indicate increased platelet production. In a group of 78 SLE patients with platelet counts >130,000/µl, we found 23 (29%) with elevated platelet-associated IgG (PAIgG). Elevated values of SLE patients ranged from 5-15 fg/platelet (normal 0.5-2.5 fg/platelet). PAIgG levels did not correlate with the severity or duration of SLE and 5 patients with high PAIgG when retested as soon as 3 weeks later, showed normal values without change in platelet count or clinical status. Survival studies using labeled autologous platelets, performed on 6 SLE patients, 3 with normal and 3 with elevated PAIgG all gave normal values and megathrombocytes were

not increased. Additionally, there was no electron micrographic evidence of platelet fragmentation in SLE patients in contrast to ITP patients whose high PAIgG levels appear to be attributable to IgG on circulating platelet debris. Fluorescence-activated cell sorting tests for possible correlation between elevated PAIgG in lupus and the presence of anti-cardiolipin antibodies are currently underway in collaboration with MD:NIDDK and AR:NIAMS.

Cooperative Study of the Diagnosis and Treatment of Neonatal Thrombocytopenia Caused by Alloimmune or Autoimmune Antibodies

During the past year we have participated as one of three U.S. clinics in an international cooperative study of the pathophysiology and treatment of neonatal immune thrombocytopenia (NIT). We have contributed 12 cases of allo- and autoimmune NIT which were documented with respect to titer and specificity of maternal antibodies by immunoprecipitation, ELISA, immunofluorescence, Western blotting and RIA. Outcome was correlated with use of I.V.IgG, and adrenocorticosteroids.

This study which so far involves 73 pooled cases, has defined degree of hemorrhage with respect to platelet count, frequency of occurrence of intracranial hemorrhage in utero, effect of bleeding on apgar scores, value of maternal versus random platelets, and the relative mildness of NIT resulting from maternal autoimmunity comparable to alloimmunity.

Pathophysiology, Treatment and Serology of Post-transfusion Purpura

We have studied 4 cases of PTP to determine whether we can detect circulating immune complexes.

Post-transfusion purpura (PTP) is a disease caused by a mismatched platelet transfusion in which antibodies appear against the foreign transfused platelet antigen and the sensitized patient develops thrombocytopenia. When we described the disease in 1959 we proposed that adsorption of antigen-antibody complexes on the patient's platelets were responsible for thrombocytopenia. Until recently immunologic techniques have not been sensitive enough to evaluate this theory. We have found that platelet antigens that cause PTP circulate free from platelets in donor plasma and can be adsorbed by platelets lacking the antigen. The amounts of free antigen or antigen-antibody complexes circulating in PTP patients when thrombocytopenia develops is too low to detect serologically directly in plasma. However, survival of antigen free from platelets in amounts below detection would nevertheless be compatible with providing sufficient Ag-Ab complexes per platelet (200 to 400) to cause platelet destruction. We have been unable to corroborate a report that sera from PTP patients contain an autoantibody that reacts with a 120,000 kD antigen on Western blot.

II. Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

A. A Class of Drugs that Suppress Platelet Release and Aggregation by Inhibiting Interaction of Thrombin with Platelets

Certain non-penetrating anionic aromatic compounds such as pyridoxal phosphate, probenecid, SITS, DIDS, and suramin are known to block anion transport and to inhibit exocytosis of secretory cells, including platelets. Inhibition of platelet release and aggregation by anion transport blockers (ATB's) has been attributed to suppression of anion flux thus interfering with osmotic lysis of secretory granules at the final stage of exocytosis. However, we have found that ATB's inhibit the thrombin-mediated platelet response at the initial step of stimulation by preventing binding of thrombin to platelets. In the presence of ATB's, platelet uptake of radiolabeled thrombin and thrombin-induced malonyl dialdehyde formation were decreased proprotional to decreases in the platelet release and aggregation reactions. However, these same platelet responses to A23187 and arachidonic acid were not decreased by ATB's nor was aggregation by ADP, epinephrine or collagen. Clotting of fibrinogen by thrombin was inhibited by ATB's to a similar degree over the same range of drug concentrations that inhibit platelet responses to thrombin. Hyperosmolality and decreased pH inhibited thrombin attachment to platelets and clotting of fibrinogen by thrombin to the same degree that the platelet release reaction was inhibited by these conditions. Moreover, one of the potent inhibitory compounds, DIDS, precipitated fibrinogen and agglutinated normal platelets but not platelets from patients with thrombasthenia, suggesting interaction of ATB's with platelet fibrinogen or GPIIb-IIIa. It is clear from these various findings that ATB's inhibit thrombin stimulation of platelets by interfering with the action of the agonist on platelets. Whether this is due primarily to inhibition of a reaction between fibrinogen and thrombin or to multiple effects of ATB's binding to cell membranes and soluble proteins remains to be determined.

B. Definition of the Epitope Responsible for von Willebrand Factor-dependent Ristocetin Aggregation

A platelet-reactive antibody in the serum of a polytransfused patient (proband) and a platelet-reactive antibody in the serum of a mother of an infant with neonatal thrombocytopenia served to establish the diallelic, platelet-specific alloantigen system, Pl^E by us in 1964. We now have evidence that the platelet-specific antibody in the serum of the proband, anti-Pl^{E1}, recognizes epitopes associated with the alpha subunit of glycoprotein (GP) Ib. By ^{51}Cr release, platelets from two of

three patients with the Bernard-Soulier syndrome (BSS) responded subnormally to anti-Pl^{E1}, and the apparently normal response of platelets from the last BSS patient was attributable to anti-HLA-A2 antibodies in the proband serum. These results suggested that the Pl^{E1} antigen is associated with the GPIb complex (glycoproteins Ib+IX) known to be absent from BSS platelets. This possibility was confirmed by ELISA using the purified GPIb complex or glycocalicin, the N-terminal fragment of GPIb alpha produced by proteolysis with endogenous platelet calpain, as solid-phase antigen. Anti-Pl^{E1} antibody bound specifically to both the GPIb complex and glycocalicin. ^{3}H-labeled platelet membrane glycoproteins with apparent molecular weights of 130k, 25k, and 21, (under reduced conditions) corresponding to GPIb alpha, GPIb beta, and BPIX were immunoprecipitated by anti-Pl^{E1} plasma. Finally, at a titer of 1:16, anti-Pl^{E1} completely inhibited ristocetin-induced platelet agglutination, a property of platelets mediated by GPIb.

C. Platelet Kinetic Studies of the Mechanism of Thrombocytopenia in Chronic Idiopathic Thrombocytopenic Purpura

The role of platelet production in the pathogenesis of ITP has been controversial. Some kinetic and morphological studies suggest that a number of patients have suppressed platelet production, whereas others report platelet turnover to be normal or markedly increased in ITP. These discrepancies have been attributed theoretically to a more marked effect of patients' antibodies on platelets or theoretically different effects of antibody on ^{51}Cr and ^{111}In labels. We have studied platelet survival in 12 patients with chronic ITP using ^{111}In-labeled autologous platelets. In each case we have found platelet survival proportional to the platelet level, indicating normal platelet production in ITP with thrombocytopenia accounted for by uncompensated platelet destruction. ^{51}Cr-labeled homologous platelets survived the same as ^{111}In-labeled platelets but only ^{111}In-labeled platelets permit evaluation of autologous platelet survival in patients with less than 30,000 platelets/µl. When autologous survivals are performed we found that the degree of red cell contamination of labeled platelets was roughly inversely proprotional to the patient's platelet level. Red cells are labeled along with platelets by ^{111}In and produce an apparant long survival or "foot" on the platelet decay curve if appropriate controls are not used. Spurious long survival measurements appear to account for falsely low turnover rates that would lead to the wrong conclusion that inhibition of platelet production rather than excessive destruction is responsible for some of the worst cases of ITP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 51,000-29 CHB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Immunology of Blood Cell Deficiencies

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

PI:	N. R. Shulman	Chief	CHB, NIDDK
Others:	D. M. Reid	Senior Staff Fellow	CHB, NIDDK
	M. Basta	Visiting Fellow	CHB, NIDDK
	C. Jones	Chemist	CHB, NIDDK
	S.E. Pillemer	Senior Staff Fellow	MDB, NIDDK
	M. Cronin	Medical Staff Fellow	MDB, NIDDK
	J. Hoofnagle	Medical Officer	DDB, NIDDK

COOPERATING UNITS *(if any)*
T. J. Kunicki, R. Aster (Blood Center of SE Wisconsin and Medical College of Wisconsin); A. W. Bracey (University of Texas Medical School, Houston, Texas); T. Bussell (New York Hospital)

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

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.)*

Idiopathic thrombocytopenic purpura (ITP), neonatal purpura (INT) post-transfusion purpura (PTP), drug-purpura and thrombocytopenia associated with infection or altered immune states are the major immunologic thrombocytopenias. Antibody reactions in these disorders are relevant to autoimmunity, histocompatibility, malignant surveillance, alloimmunity, pathogenicity of antigen-antibody complexes and cellular immune injury generally. We have developed more precise and sensitive assays than heretofore available for platelet-associated (PA)IgG, IgM, IgA and albumin and Western blot analysis(WB) of specific platelet receptors. We found that normal IgG, M and A reacted in WB with receptors of 90kD and 120kD These receptors were distinct from glycoproteins IIIa and V and were bound to internal membranes. The pathophysiology of post-transfusion purpura(PTP) was found to be compatible with adsorption of antigen-antibody complexes by quantitative adsorption of $P1^{A1}$-anti-$P1^{A1}$ on platelets and by PTP response to plasmapheresis. Neonatal thrombocytopenia cases, as well as PTP cases were a source of antibodies that led to clear identification of the $P1^{A2}$ antigen, the allele of $P1^{A1}$. Studies of Ag-Ab complexes, and PAIgG in AIDS, lupus erythematosus, and primary biliary cirrhosis, has helped clarify the mechanism of thrombocytopenia in these diseases. The platelet receptor for drug antibodies was found not to be solely dependent on GPIb, V, and IX as previously supposed.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 51,001-29 CHB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

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

PI:	N. R. Shulman	Chief	CHB:NIDDK
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	Charles E. Jones	Chemist	CHB:NIDDK

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

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.)*

The physiology of platelet secretion has many features in common with the secretory physiology of endocrine and neuronal cells; and a number of the biogenic amines synthesized, stored, and secreted by these different cell types are similar. Platelet membrane glycoproteins (GP) appear to be major factors determining cell-cell recognition, and secretion. We have identified a new epitope of GPIb that acts as a receptor for von Willebrand's factor which controls platelet endothelial interaction but does not interfere with platelet secretion or reaction with drug antibodies that also interact with GPIb. The role of platelet production in the pathogenesis of idiopathic thrombocytopenic purpura (ITP) has been controversial. We studied platelet turnover in ITP with ^{111}In-labeled autologous platelets and found approximately normal production rates in all 15 cases. We found that a platelet-specific antibody, anti-Pl^{E1}, that we identified in a patient with Bernard-Soulier Syndrome, reacted with the glycoprotein Ib complex and glycocalacin and inhibited ristocetin-induced platelet agglutination. We have found that the anion transport blockers, SITS, DIDS, and suramin inhibit exocytosis of platelets by interfering with the action of agonist on platelet receptors rather than by inhibiting anion flux.

GPO 914-918

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Biochemical Genetics Section

Dr. Proia and his colleagues have continued their studies of the lysomal enzymes β-hexosaminidase (a deficiency of which is responsible for Tay-Sachs disease). During the last year they have isolated human genomic clones covering the entire gene encoding for the beta chain of this enzyme. Comparison with the previously characterized a chain revealed an extensive conservation of intron position for 12 of the 13 introns in both genes. This demonstrates that the two genes were derived from a common ancestor.

In separate studies they have expressed the β-chain both in cultured cells and in a cell-free system. The ability to have the β-chain cDNA expressed in a completely cell-free system into enzymatically active dimer should enable them and others to study the relationship between structure and function of proteins in a relatively quick and straightforward manner.

Dr. Robbins and her colleagues have continued their analyses of endocytosis, glycoprotein biosynthesis and sorting using biochemical genetics. In the area of endocytosis, they have identified a protein by two-dimensional gel electrophoresis alterations which appear to be responsible for the End2 complementation group of CHO cell mutants. Extending their previous observations of the inter-dependence of Golgi and endocytic activities, they have shown that an Ltk$^-$ mutant isolated for cross-resistance to protein toxins and toxic lectins has a defect in its secretory pathway: viral envelope glycoproteins appear to move normally from ER to Golgi in this mutant, then slow as they proceed through the Golgi and from Golgi to the plasma membrane.

Finally, in order to examine the last essential step common to both protein glycosylation and enlongation of the lipid-linked oligosaccaharide, they are developing an in vitro assay system for translocation of the lipid-linked $GlcNAc_2Man_5$ intermediate from the cytoplasmic to the luminal face of the ER membrane.

Molecular Genetics Section

Dr. Ackerman and collaborators have continued their work on an oocyte specific gene product called OAX RNA for oocyte activated in Xenopus. OAX DNA is approximately 1% of the X. laevis genome and OAX appears to be a single-copy gene in X. borealis. Several OAX genes from X. laevis and the X. borealis clone have been sequenced.

They have continued their analysis of the Aspergillus toxin alpha-sarcin have compared its cleavage of the 28S ribisomal RNA to the mode of action of ricin and Shiga toxin.

Recently, they have initiated a project to investigate whether Xenopus oocytes are a good source of DNA repair activities.

Dr. Camerini-Otero and his colleagues have continued their studies of genetic recombination in eukaryotes. They have partially purified and characterized a strand exchange protein or recombinase from human cells. The product of this strand

exchange reaction is a joint molecule composed of a single-strand circular DNA joined to one end of a linear duplex DNA by a region of hetroduplex DNA. Formation of these hetroduplexes is accompanied by strand displacement.

Over the last year they have also partially purified and characterized similar proteins from mitotic S. cerevisiae and embryos of D. melanogaster. In all respects they have examined the Drosophila protein is similar to the human protein. The yeast protein, however, shares some properties with the human protein and some with E. coli protein, Rec A. Work is now in progress to purify these proteins to homogeneity and to clone the genes encoding them.

Finally, they have investigated the possible role of these recombinases in immunoglobulin heavy-chain class switching. They have obtained joint molecules from DNA bearing the μ switch site and either the ε or γ switch sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 52008-08 GBB

PERIOD COVERED
October 1, 1986 to September 30, 1987

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

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

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Others:	C. S. Camerini-Otero	Med. Staff Fellow	GBB, NIDDK
	A. Eisen	Med. Staff Fellow	GBB, NIDDK
	P. Hsieh	Senior Staff Fellow	GBB, NIDDK
	M. S. Meyn	Med. Staff Fellow	GBB, NIDDK
	F. Mills	Guest Researcher	GBB, NIDDK
	P. Pittman	Guest Researcher	GBB, NIDDK
	P. Gotwals	Bio. Lab. Tech.	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Genetics and Biochemistry Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	6.5	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.)*

Genetic recombination is a multistep process involving many gene products. In order to dissect the biochemical steps involved we have chosen to focus on a key early step: strand exchange between homologous parental DNAs. To date, the ability to carry out a strand exchange between a linear duplex DNA and a homologous circular single-strand DNA is unique to recombination proteins. The product of this strand exchange reaction is a joint molecule composed of a single-strand circle joined to one end of a linear duplex. Three proteins responsible for this step have been purified: uvsx from phage T4; Rec A from E. coli; and rec 1 from U. maydis.

We reported about 18 months ago the partial purification and characterization of a similar recombinase or strand-exchange protein from nuclear extracts of the human B cell lymphoblastoid line RPMI 1788. The protein had two noteworthy characteristics: (1) it did not require ATP (unlike Rec A and rec 1); and (2) its direction of strand-displacement (3′ to 5′) was similar to that of rec 1 but opposite to that of Rec A.

Over the last 18 months we have partially purified and characterized two similar proteins from mitotic S. cerevisiae and embryos of D. melanogaster. In all respects we have examined the D. melanogaster protein is similar to the human protein. The S. cerevisiae protein, however, appears to be different from the human and fruit-fly protein in that its direction of strand displacement is similar to that of Rec A.

Finally, we have investigated the possible role of these recombinases in immunoglobulin heavy-chain class switching. We have obtained joint molecules from DNAs bearing the μ switch site and either the ε or γ switch sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52009-08 GBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Endocytosis, Secretion and Compartmentalization in Mutant CHO Cells

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

PI:	A. R. Robbins	Research Geneticist	GBB, NIDDK
Others:	C. W. Hall	Research Chemist	GBB, NIDDK
	C. F. Roff	Senior Staff Fellow	GBB, NIDDK
	S. M. Laurie	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS *(if any)*

Department of Biochemistry, School of Public Health, Johns Hopkins University (S. S. Krag)

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.3	4.0	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Our approach to dissecting the processes of endocytosis, glycoprotein biosynthesis and sorting is through isolation and analysis of mutants.

By hybridization we have shown that the majority of CHO cell endocytosis mutants isolated by ourselves and others fall into two genetic complementation groups, End1 and End2. Phenotypes of these two classes of mutants are essentially identical, both defects resulting in decreased acidification of early endosomes. Comparing mutant and parental cells by two-dimensional electrophoresis we have identified a membrane-associated protein that may represent End2.

In the End1 and 2 CHO cell mutants a single genetic defect results in alterations of both endosomal and Golgi activities, specifically of the late Golgi-*trans* Golgi reticulum region. An LTk- cell mutant, altered in endocytic activity but apparently normal with respect to endosomal acidification, appears to transport viral envelope glycoproteins at a decreased rate once those proteins have entered the early regions of the Golgi.

The lipid-linked oligosaccharide that is transferred *en bloc* to protein in N-linked glycosylation is thought to be assembled in stages, with the $Man_5GlcNac_2$ built on the cytoplasmic face of the ER using GDP-mannose, then elongated on the luminal face using ManP-dolichol. We have isolated a mutant whose phenotype is consistent with a defect in the translocation of the lipid-linked Man_5 intermediate. Development of an *in vitro* assay system for translocation is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52011-03 GBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Oocyte Specific Genes in Amphibian Embryogenesis

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

PI:	Eric Ackerman	Senior Staff Fellow	GBB, NIDDK
Others:	Shailendra K. Saxena	Visting Fellow	GBB, NIDDK
	John Hays	Sabbatical Professor	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

I. No gene transcription occurs in fertilized eggs of the amphibian Xenopus until the mid-blastula transition, (approximately 4000 cells). Therefore all of the appropriate gene products necessary for early development must be stored in the oocyte or egg prior to fertilization. In order to understand the molecular embryology of early amphibian development, we must obtain genes whose products are expressed only in oocytes. We have been working on an oocyte specific gene product called OAX RNA for oocyte activated in Xenopus. This RNA is 181 nucleotides long, present approximately in 10,000 copies/oocyte and is in a cytoplasmic complex of approximately 50S size. This RNA is not found in adult somatic tissue.

II. The Aspergillus toxin alpha-sarcin produces a precise cut near the 3'-end of 28S ribosomal RNA in vitro only if the ribosomes are pre-treated with puromycin and EDTA. Alpha-sarcin can also behave as a general nuclease in vitro under appropriate conditions. In order to investigate alpha-sarcin's in vivo activity, we injected it into living Xenopus oocytes and analyzed the resulting RNA. We have also investigated whether ricin and Shiga toxin produce similar effects in oocytes.

III. During early development Xenopus replicates its DNA nearly as fast as E. coli in log phase; perhaps indicating that oocytes may be an excellent source of DNA repair activity. We have investigated pyrimidine dimer repair by microinjecting uv-irradiated DNA into oocytes and assaying for repair using 2 methods: (1) Transformation of repair deficient E. coli mutants with the microinjected DNA; (2) Absence of pyrimidine dimers using UV-Endonuclease and denaturing agarose gels.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 52012-03 GBB

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Structure-Function Relationships of Lysosomal Enzymes

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

PI:	Richard L. Proia	Senior Staff Fellow	GBB, NIDDK
Others:	Sybille Sonderfeld-Fresko	Guest Researcher	GBB, NIDDK
	Emilia Soravia	Visiting Fellow	GBB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.3	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.)*

The lysosomal enzyme β-hexosaminidase has been studied (A.) at the level of it's genetic organization and (B.) by in vivo and in vitro expression of it's cDNAs.

A. Genetic Organization. The gene encoding the β-chain of β-hexosaminidase has been isolated and it's genomic organization precisely characterized by restriction endonuclease mapping and sequencing of the intron-exon junctions. Comparison with the previously characterized α-chain gene revealed extensive similarities with some interesting differences. Both genes are about 40 kilobases in length, contain 14 exons and 13 introns, and have compact 3' ends and expanded 5' ends. The respective coding sequences are interrupted at homologous positions by 12 of the 13 introns. The most 5' intron of the two genes, which is the only intron to occur at a non-homologous position, interrupts both sequences at possible proteolytic processing sites. The extensive conservation of intron positions between the two genes demonstrates that they were derived from a common ancestor.

B. In Vivo and In Vitro Expression. The β-chain cDNA has been expressed in transfected COS cells under the control of the SV-40 late promotor. The expressed protein is enzymatically active and undergoes proteolytic processing consistent with transport to lysosomes. An in vitro expression system has been developed in which β-chain mRNA, generated by transcription with SP6 polymerase, is translated in a rabbit reticulocyte lysate supplemented with microsomes. Within the microsomes the translated product assumes a native structure as indicated by reactivity with a conformation-sensitive antibody, assembly of the subunits into dimers and acquisition of catalytic activity. In addition, by expressing genetically engineered forms of the β-cDNA, we have shown that the β-chain contains two functional initiator AUG codons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 52013-03 GBB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

The Genetic Lesions of Tay-Sachs Disease

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

PI: R. Myerowitz Senior Staff Fellow LBM, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical and Genetics Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

This project transferred to the Laboratory of Biochemistry and Metabolism. The new project number is Z01 DK 17024-01 LBM.

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

SUMMARY OF BRANCH ACTIVITIES

The Digestive Diseases Branch has two sections (Section on Gastroenterology and the Liver Diseases Section). The Liver Diseases Section has 2 senior physicians and 3 medical staff fellows; the Section on Gastroenterology has 3 senior physicians and 5 medical staff fellows. The Digestive Diseases Branch also has 5-10 guest investigators from other laboratories.

Detailed summaries of the activities of each section precede the individual project reports. Both sections are engaged in investigations of basic biologic processes (e.g., hormone action, membrane transport, cellular and humoral immunology) and are attempting to apply this information to understand the pathophysiology of various disorders involving the liver and gastrointestinal tract. Both sections are also involved in attempts to improve therapy of clinical disorders such as neoplasms associated with overproduction of gastrointestinal hormones, hepatitis and fulminent hepatic failure.

Section on Gastroenterology

The Section on Gastroenterology is currently following approximately 120 patients with Zollinger-Ellison syndrome (ZES, gastrin-producing neoplasm, hypergastrinemia and increased secretion of gastric acid). All patients are currently being treated with oral medication that inhibits gastric acid secretion.

Although histamine H_2-receptor antagonists are effective inhibitors of gastric acid secretion in patients with Zollinger-Ellison syndrome, these agents must be taken in large doses and at frequent intervals. Omeprazole a new antisecretory agent that inhibits gastric H^+,K^+-ATPase was tested for therapeutic efficacy in patients with Zollinger-Ellison syndrome. A single dose of omeprazole inhibited gastric acid secretion for more than 48 hours in patients with Zollinger-Ellison syndrome. In 90% of patients with Zollinger-Ellison syndrome, gastric acid secretion could be adequately inhibited by a single daily dose of omeprazole. Omeprazole was free of detectable toxicity during three years of therapy. Because of its long duration of action, omeprazole offers an advance in convenient medical therapy for Zollinger-Ellison syndrome compared with histamine H_2-receptor antagonists.

In patients with Zollinger-Ellison syndrome and metastatic gastrinoma to the liver, significant benefits can be obtained by aggressive resection of all detectable tumor plus chemotherapy with streptozotocin, adriamycin and 5-fluorouracil. Although most patients have been followed for only a short period of time, initial results are extremely promising.

Approximately fifty percent of patients with Zollinger-Ellison syndrome in whom a pancreatic tumor can be demonstrated by visualization studies can be cured by surgery. Selective celiac angiography and computerized axial tomography of the abdomen are comparably sensitive in detecting pancreatic gastrinoma; however, angiography is better than tomography at detecting metastatic gastrinoma in the liver. Selective transhepatic portal venous sampling for gastrin is no better than celiac angiography or abdominal tomography in detecting pancreatic gastrinoma.

Although most patients with Zollinger-Ellison syndrome can be treated effectively with histamine H_2-receptor antagonists, many patients require large doses of drug to adequately inhibit gastric acid secretion. Thirty percent of patients with Zollinger-Ellison syndrome have been found to have parietal cell resistance to cimetidine and another fifty percent have delayed cimetidine absorption. These abnormalitiies of cimetidine pharmacokinetics account for the high doses of cimetidine required to inhibit gastric acid secretion in patients with Zollinger-Ellison syndrome.

It is known that gastrinomas may produce other peptides besides gastrin. During this past year we have established that in approximately 5 percent of patients with sporadic gastrinoma the tumor also secretes significant amounts of ACTH giving rise to a florid form of Cushing's syndrome. In contrast to patients with sporadic gastrinoma, 20% of patients with gastrinoma and MEN-I have Cushing's syndrome resulting from pituitary overproduction of ACTH.

During this past year we have systematically evaluated the role of the sulfate ester in cholecystokinin (CCK), gastrin and structurally related peptides in influencing biologic activity on pancreatic acinar cells. Removing the sulfate ester from CCK causes a 300-fold decrease in potency with no change in efficacy. Removing the sulfate ester from gastrin causes a 10-fold decrease in potency with no change in efficacy. Removing the sulfate ester from a C-terminal fragment of CCK that has partial agonist activity abolishes the efficacy and causes a 7-fold decrease in potency of the peptide. Removing the sulfate ester from a C-terminal fragment of CCK that is a CCK receptor antagonist does not alter efficacy but causes a 10-fold decrease in potency.

In dispersed acini prepared from mouse pancreas, cholecystokinin and structurally related peptides cause significant stimulation of enzyme secretion. During this past year we made the surprising observation that when acini are first incubated with cholecystokinin and then washed and reincubated, enzyme secretion during the second incubation is 30 percent greater than when cholecystokinin is added directly to the incubation. The basis for this phenomenon is not clear; however, its elucidation should provide insight into the regulation of receptor-effector coupling in mouse pancreatic acinar cells.

Previously we showed that analogues of substance P function as substance P receptor antagonists as well as bombesin receptor antagonists. During this past year we showed that D-Phe^{12} analogues of bombesin function as specific bombesin receptor antagonists with no substance P receptor antagonist activity. These new bombesin receptor antagonists should be particularly valuable in elucidating the physiologic significance of bombesin and structurally related peptides.

Studies measuring the crosslinking of radiolabeled vasoactive intestinal peptide (VIP) to its membrane receptors on pancreatic acinar cells indicate that VIP interacts with two membrane substituents. One component (a major band on gel electrophoresis) has an apparent molecular weight of 45,000 and a second component (a minor band on gel electrophoresis) has an apparent molecular weight of 30,000. Future studies will be required to establish the significance of these two VIP-binding components to VIP receptor action.

Studies using ^{125}I-secretin and ^{125}I-vasoactive intestinal peptide (^{125}I-VIP) indicate that pancreatic acinar cells possess three classes of receptors that interact with VIP and secretin. One class (A) has a high affinity for VIP and a low affinity for secretin; one class (B) has an intermediate affinity for VIP and a low affinity for secretin, and one class (C) has a high affinity for secretin and a low affinity for VIP. Occupation of class A increases cyclic AMP and stimulates pancreatic enzyme secretion. Occupation of class B has no known biologic effect. Occupation of class C increases cellular cyclic AMP but does not alter any known cellular function.

The Liver Diseases Section is currently conducting eight principal studies.

I. Studies Relating to the Pathogenesis of Hepatic Encephalopathy

The abnormal pattern of visual evoked responses (VERs) in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembles that associated with coma induced by a barbiturate, a benzodiazepine (BZ) or a gamma-amino-butyric acid (GABA) agonist but differ fundamentally from those associated with ether-induced coma or encephalopathies induced by infusing a variety of potential neurotoxins in liver failure (e.g. ammonia, a mercaptan precursor, a short chain fatty acid). As barbiturates, BZs and GABA agonists induce neural inhibition by potentiating GABAergic inhibitory neurotransmission as a consequence of their interaction with specific binding sites on the GABA/BZ receptor complex on postsynaptic neural membranes, these findings suggest that the pattern of neuronal activity in HE may resemble that associated with activation of the GABA inhibitory neurotransmitter system. To take account of the rapid metabolism of GABA, a modified Oldendorf technique, which employed the use of a vascular marker, has been used to demonstrate that the brain uptake index of plasma GABA is increased in the rabbit model of HE. Ameliorations of HE (both clinical and electrophysiologic [VER waveform]) were induced in rabbits with FHF by a GABA receptor antagonist, a BZ receptor antagonist and a chloride channel blocker and in rats with FHF by a BZ receptor antagonist and a partial inverse agonist of the BZ receptor. Rabbits with HE exhibited increased resistance to the convulsion effects of the GABA-receptor antagonist. The spontaneous activity of Purkinje neurons of rabbits in HE exhibited increased sensitivity to depression by a GABA agonist and a BZ agonist, but was excited by BZ receptor antagonists. These findings suggest that in HE due to FHF: (i) There is increased GABAergic tone which is neither species nor model dependent; (ii) Blockading GABA or BZ receptors ameliorates HE, (iii) BZ receptor antagonists may be of clinical value in treating HE; and (iv) An endogenous BZ receptor agonist may contribute to HE.

In rats the construction of a large end-to-side portacaval anastomosis (PCA) is followed by marked liver atrophy but no overt encephalopathy. Rats with a PCA who are gavaged with blood develop overt encephalopathy. Rats with a PCA who are gavaged with blood and have (CCl_4-induced) cirrhosis or a 50% hepatectomy develop more severe encephalopathy. The encephalopathy in these animals appears to be a model of portal systemic encephalopathy (PSE). [E.A. Jones, J. Vergalla, K.D. Mullen, D.B. Jones, M. Roessle, S.H. Gammal, P. Martin, A. Basile, P. Skolnick].

II. Studies of Cellular Immune Function in Primary Biliary Cirrhosis

The role of abnormal mechanisms in the pathogenesis and perpetuation of the hepatobiliary lesion of primary biliary cirrhosis (PBC) is being studied. Cytotoxicity mediated by circulating natural killer (NK) cells is reduced in PBC. This defect can be partially corrected by incubating PBC NK cells with interferon and appears to be due to a functional defect of cytolytic effector cells. Complement receptor function in PBC has been assessed by quantitating the ability of peripheral blood monocytes to form rosettes with complement-coated sheep erythrocytes. PBC monocytes have a normal capacity to form rosettes but PBC serum in the presence or absence of normal serum inhibits rosette formation. This inhibition is probably mediated by an abnormally immunoreactive IgM present in PBC serum and does not depend on complement. An IgM that binds to receptors for C3b affords a potential explanation for the C3b-specific clearance defect by fixed macrophages in PBC. Defects of humoral immunity attributable to activation of small subpopulations of B cells occur in PBC. For example, in PBC there is evidence compatible with an expanded clone of B cells that synthesizes mitochondrial antibodies with different antigenic specificities from those synthesized (under appropriate conditions) by normal B cells. Recently with the use of monoclonal antibodies it has become apparent that CD4 (T4) T cells can be subdivided into subpopulations having unique functions. The subpopulation expressing the antigen recognized by anti-Leu-8 is of particular interest. CD4 positive, Leu-8 positive T cells have been demonstrated to have direct suppressor function, as well as the capacity for inducing CD8 (T8) suppressor cells. In addition, it has been shown that the CD4 positive, Leu-8 positive T cell population is the predominant autoreactive T cell subpopulation in peripheral blood. Thus the activation of autoreactive cells and suppressor T cell function may involve common mechanisms mediated by a single T cell subset. Since a defect in suppressor function and a defect in the autologous MLR have been shown to be present in patients with PBC, it seems likely that the function of the CD4 positive, Leu-8 positive T cell subset may be abnormal in patients with PBC. To address this issue, further studies are being undertaken to assess the function of these cells in patients with PBC and appropriate controls. The results of these studies will probably provide further insights into the cellular immune basis of autoimmune phenomena in patients with PBC. [E.A. Jones, T. Suou, M. Civeira, S.P., James, M.I. Avigan, J.H. Hoofnagle, W. Strober].

III. Studies of Protease Inhibitor (Pi) Phenotypes

Pi phenotypes and serum α_1-antitrypsin (α_1AT) concentrations have been determined in 80 unselected southern African Black patients with hepatocellular carcinoma and 103 age, sex and tribally matched control subjects. Non-MM phenotypes were present in 8.7% of patients with hepatocellular carcinoma and 12.6% of controls. The heterozygous PiZ carrier state was present in 5.0% of patients with hepatocellular carcinoma and 1.9% of controls; no subjects had the homozygous PiZZ phenotype. No patients with hepatocellular carcinoma had a subnormal serum α_1AT concentration as assessed by rocket immunoelectrophoresis. The four patients with the heterozygous PiZ phenotype did not have fibrolamellar carcinomas. It is inferred that α_1AT deficiency does not play an etiologic role in hepatocellular carcinoma in southern African Blacks. [E.A. Jones, J. Vergalla, M.C. Kew (not NIH].

IV. Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by slowly progressive intrahepatic cholestatis due to non-suppurative, presumably autoimmune, destruction of septal and the larger interlobular bile ducts. A controlled trial of chlorambucil therapy for patients with symptomatic PBC has been conducted. Twenty-four patients (23 women, 1 man; ages 34-63) were admitted to this trial: 13 were randomized to receive chlorambucil therapy (0.5-4.0 mg/day) and 11 to the control (no treatment) group. The dose of chlorambucil was adjusted to reduce the peripheral blood lymphocyte count by 50% and maintain the polymorphonuclear leukocyte count above 1000 per c.mm. All patients have been followed for 2-6 years (mean = 4.1 years). During follow-up, two patients have died: both in the control group. The mean serum bilirubin levels remained almost constant in the treated group but increased by an average of about 50% each year in the control group. Mean serum albumin values increased slightly in treated patients but decreased in control patients. Mean serum transaminase levels were significantly less in treated patients than in controls. Mean serum immunoglobulin (IgM and IgG) levels decreased from elevated values to values within the normal range in all chlorambucil-treated patients, but did not change appreciably in control patients. Liver biopsy histopathology after one, and/or two years revealed significantly less inflammation, slightly less fibrosis and less progression of the stage of disease in the treated than in the control patients. Potential side effects of chlorambucil therapy included onset of menopause, localized herpes simplex or zoster and persistent leukopenia or thrombocytopenia. These findings strongly suggest that chlorambucil therapy retards the progression of PBC. [E.A. Jones, J.H. Hoofnagle, D.B. Jones, V.R. Rustgi, K.D. Mullen, R.N.M. MacSween (not NIH].

V. Studies of the Natural History and Treatment of Chronic Type B Hepatitis

A cohort of patients with chronic type B hepatitis is being evaluated and followed to determine the long-term natural history of this common form of chronic liver disease. Selected patients have been entered into therapeutic trials in which antiviral or immunomodulatory agents have been administered. Eight patients have been entered into a study of the treatment of chronic type B hepatitis with recombinant human alpha and gamma interferon. Alpha interferon has a more pronounced inhibitory effect than gamma interferon on serologic markers of HBV replication. Because of its immunomodulatory and anti-viral effects, gamma interferon may have a role as an adjunct to other therapies in the treatment of chronic type B hepatitis. [J.H. Hoofnagle, A.M. Di Bisceglie, C. Kassianides, M. Lisker-Melman, E.A. Jones].

VI. Studies of the Natural History and Treatment of Chronic Non-A, Non-B Hepatitis

Patients with well-documented chronic non-A, non-B hepatitis are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients is available to evaluate experimental therapies for this disease. To date 12 patients with chronic non-A, non-B hepatitis have been treated with recombinant human alpha interferon for periods ranging from 2 months to one year. In 10 of the 12 patients there has been a dramatic decrease in serum aminotransferase levels during therapy, the levels falling from values 3 to 10 times the upper limit of the normal range to normal (7 patients) or near normal (3 patients). Follow up liver biopsies, obtained from 8 patients, demonstrated marked improvement in the hepatitis disease activity (a decrease in both inflammation and hepatocellular necrosis). A prospective randomized, placebo-controlled trial of alpha interferon therapy for chronic non A, non B hepatitis is underway. So far fifteen patients of a planned total of 35 have been entered. [J.H. Hoofnagle, A.M. Di Bisceglie, C. Kassianides, M. Lisker-Melman, E.A. Jones].

VII. Immunologic Studies of Chronic Viral Hepatitis

Immunological factors seem to be important in determining the course and outcome of both acute and chronic viral hepatitis. Furthermore promising therapies for chronic viral hepatitis have profound effects on immune function and sustained responses to therapy may depend largely on restoration of normal immune responsiveness. The role of immunologic mechanisms in determining the course of and ultimate outcome of viral hepatitis is being studied and the effects of antiviral and immunomodulatory therapies on the immune system are being evaluated. Serial studies of cellular immune function have been performed on patients with chronic type B hepatitis treated with interferon. [J.H. Hoofnagle, E.A. Jones, A.M. Di Bisceglie, C. Kassianides, M. Lisker-Melman].

VIII. Studies of the Natural History and Treatment of Duck Hepatitis B Virus Infection

Duck hepatitis B virus (DHBV) infection is a potentially useful experimental model of human hepatitis B infection. New antiviral and immunomodulatory agents are being assessed for their ability to suppress DHBV replications in ducks. It is anticipated that the ability of a drug to suppress DHBV replication will be shown to be a satisfactory screening test for new effective therapies for chronic type B hepatitis in man. Care of DHBV-infected ducks as well as methods for obtaining serum and liver tissue from ducks have been standardized. Reproducible assays for quantitating DHBV DNA and DNA polymerase in serum have been established. Data from the first animals that have been treated with adenine arabinoside monophosphate and 2,3 dideoxycytidine are being analysed. [E.A. Jones, C. Kassianides, J.H. Hoofnagle].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53001-17 DDB

PERIOD COVERED

October 1, 1986 to Sept. 30, 1987

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

Studies of Membrane Function

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

PI:	Jerry D. Gardner	Chief	DDB, NIDDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. A. Wank, R. Vinayek, H. Frucht	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, M. Younes, D-H. Yu	Visiting Fellows	DDB, NIDDK
	D. Kasbekar, D. Menozzi	Guest Workers	DDB, NIDDK
	P. Heinz-Erian, T. von Schrenck	Guest Workers	DDB, NIDDK
	S. W. Jones, V. E. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Section on Gastroenterology

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.6	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The broad categories which are included in the project are: 1) Characterizing functionally the mechanism by which various substrates cross the plasma membrane of different mammalian cells; 2) identifying the metabolic and humoral factors which influence the transport of various substrates across the plasma membrane; 3) developing techniques which will distinguish between binding of a substrate to the membrane and translocation of the substrate across the membrane; 4) characterizing the mechanism by which the membrane transport of various substrates is altered in certain diseases; and 5) relating these alterations of membrane transport to the pathogenesis and clinical manifestations of the disease.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53002-15 DDB

PERIOD COVERED
October 1, 1986 to Sept. 30, 1987

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

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

PI:	J. D. Gardner	Chief	DDB, NIDDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. Wank, R. Vinayek, H. Frucht	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, D-H. Yu	Visiting Fellows	DDB, NIDDK
	D. Kasbekar. M. Younes, D. Menozzi	Guest Workers	DDB, NIDDK
	P. Heinz-Erian, T. von Schrenck	Guest Workers	DDB, NIDDK
	S. Jones, V. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*
Dept. of Chemistry, Case-Western Reserve Univ., Cleveland, Ohio
Div. of Cellular Biology, Kennedy Institute for Rheumatology, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	4.0	1.6

CHECK APPROPRIATE BOX(ES)

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

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

In vitro systems are being used to study the mechanism of action of gastrin, secretin, cholecystokinin, bombesin, substance P and vasoactive intestinal peptide with their specific membrane receptors.

Clinical investigators are directed toward developing alternative forms of therapy for and elucidating the pathogenesis of disorders characterized by ectopic production of gastrointestinal hormones (e.g., Zollinger-Ellison syndrome and pancreatic cholera).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 53004-15 DDB

PERIOD COVERED
October 1, 1986 to Sept. 30, 1987

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

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

PI:	Jerry D. Gardner	Chief	DDB, NIDDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIDDK
	P. N. Maton	Visiting Scientist	DDB, NIDDK
	S. Wank, R. Vinayek	Medical Staff Fellows	DDB, NIDDK
	Z-C. Zhou, D-H. Yu	Visiting Fellows	DDB, NIDDK
	D. Kasbekar, M. Younes. D. Menozzi	Guest Workers	DDB, NIDDK
	T. von Schrenck, P. Heinz-Erian	Guest Workers	DDB, NIDDK
	S. Jones, V. Sutliff	Chemists	DDB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Gastroenterology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.6	5.0	1.6

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

In vitro systems are being used to characterize the mechanism by which cyclic nucleotides alter cell function and to explore the mechanism of action of agents whose effect on cell function is mediated by cellular accumulation of cyclic nucleotides.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53501-14 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies Relating to the Pathogenesis of Hepatic Encephalopathy

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	J. Vergalla	Chemist	LDS, NIDDK
	K.D. Mullen	Medical Staff Fellow	LDS, NIDDK
	D.B. Jones	Medical Staff Fellow	LDS, NIDDK
	M. Rossle	Guest Researcher	LDS, NIDDK
	S.H. Gammal	Guest Researcher	LDS, NIDDK
	P. Martin	Visiting Associate	LDS, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Theoretical and Physical Biology, NICHHD (P.J. Munson)
Laboratory of Neuroscience, NIDDK (P. Skolnick and A. Basile)
Division of Cancer Biology and Diagnosis, NCI (D. Covell)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The abnormal pattern of visual evoked responses (VERs) in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembles that associated with coma induced by a barbiturate, a benzodiazepine (BZ) or a gamma-amino-butyric acid (GABA) agonist. As these drugs induce neural inhibition by potentiating GABAergic inhibitory neurotransmission as a consequence of their interaction with specific binding sites on the GABA/BZ receptor complex on postsynaptic neural membranes, these findings suggest that the pattern of neuronal activity in HE may resemble that associated with activation of the GABA inhibitory neurotransmitter system. To take account of the rapid metabolism of GABA, a modified Oldendorf technique, which employed the use of a vascular marker, has been used to demonstrate that the brain uptake index of plasma GABA is increased in the rabbit model of HE. Ameliorations of HE (both clinical and electrophysiologic [VER waveform]) were induced in rabbits with FHF by a GABA receptor antagonist, a BZ receptor antagonist and a chloride channel blocker and in rats with FHF by a BZ receptor antagonist and a partial inverse agonist of the BZ receptor. The spontaneous activity of Purkinje neurons of rabbits in HE exhibited increased sensitivity to depression by a GABA agonist and a BZ agonist, but was excited by BZ receptor antagonists. These findings suggest that in HE due to FHF: (i) There is increased GABAergic tone which is neither species nor model dependent; (ii) Blockading GABA or BZ receptors ameliorates HE; (iii) BZ receptor antagonists may be of clinical value in treating HE; and (iv) An endogenous BZ receptor agonist may contribute to HE.

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 53503-13 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Immunologic Studies of Primary Biliary Cirrhosis

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	J.H. Hoofnagle	Medical Officer	LDS, NIDDK
	J. Vergalla	Chemist	LDS, NIDDK
	T. Suou	Guest Researchers	LDS, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Tumor Cell Biology, NCI (M. Civeira)
Laboratory of Clinical Investigation, NIAID (S.P. James and W. Strober)

LAB/BRANCH: Digestive Diseases Branch

SECTION: Liver Diseases Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

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

Abnormal immune mechanisms are being studied in patients with primary biliary cirrhosis (PBC). T cell-mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis by B cells have been studied using radioimmunoassays to measure IgG and IgM synthesized by cultures containing appropriate mixtures of different lymphocyte subpopulations in vitro. The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated B cells (mixed lymphocyte reactions) has been assessed. Results of these studies include the demonstration in PBC of (i) a diminished capacity of T cells to inhibit immunoglobulin synthesis in vitro and (ii) a deficiency of the autologous but not the allogeneic mixed lymphocyte reaction. These findings suggest that in PBC there is a fundamental defect in the interaction between autoreactive T cells and surface antigens on autologous non-T cells which leads to diminished activation of suppressor T cells and hence predisposes to a state of immune hyperresponsiveness. The coexistence of IgA deficiency and PBC has been documented. It is possible that IgA deficiency may contribute to the development of PBC, but the pathogenesis of PBC does not require IgA-dependent mechanisms. Sera from patients with PBC have been shown to contain a factor, probably an abnormally immunoreactive IgM, which blocks the binding of C3b-opsonized erythrocytes by monocytes. This finding affords a potential explanation for the C3b-receptor specific clearance defect by fixed macrophages in PBC. Patients with PBC have been shown to have diminished natural killer cell activity due to a functional defect of cytolytic effector cells. Defects of humoral immunity due to activation of subpopulations of B cells occur in this disease. For example, in PBC there is evidence compatible with the existence of an expanded clone of B cells that synthesize mitochondrial antibodies with different antigenic specificities from those synthesized by normal B cells. A disease-specific immunologic defect has yet to be defined in PBC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53505-12 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies of Alpha-1-Antitrypsin Phenotypes and Metabolism

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	J. Vergalla	Chemist	LDS, NIDDK

COOPERATING UNITS *(if any)*

University of the Witwatersrand, Johannesburg, South Africa
(Dr. M. C. Kew)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0	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.)*

Protease inhibitor (Pi) phenotypes have been determined using isoelectric focusing on polyacrylamide gel in populations of normal subjects and patients with rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and hepatocellular carcinoma. Of 80 unselected southern African Black patients with hepatocellular carcinoma, the incidence of aberrant (non-MM) phenotypes was 8.7%. In 103 age, sex and tribally-matched control subjects the corresponding incidence was 12.6%. None of the patients or controls had the PiZZ phenotype. 5% of patients and 1.9% of controls were heterozygous carriers of the Z gene. No patient with hepatocellular carcinoma had a sub-normal serum concentration of alpha-1-antitrypsin, as assessed by rocket immuno-electrophoresis. The four patients with the heterozygous Z phenotype did not have fibrolamellar carcinomas. These findings suggest that alpha-1-antitrypsin deficiency does not play an etiologic role in hepatocellular carcinoma in southern African Blacks.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53508-10 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies of Hepatic Receptors for Glycoproteins

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	J. Vergalla	Chemist	LDS, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Biochemistry and Metabolism, NIDDK (G. Ashwell)

LAB/BRANCH Digestive Diseases Branch

SECTION Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

The cellular location and carbohydrate specificities of a glycoprotein recognition system on rat hepatic sinusoidal cells have been determined. Purified preparations of endothelial, Kupffer and parenchymal cells have been prepared by in situ collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein, was selectively and specifically taken up in vitro by endothelial cells. Glucose and a glucose-albumin conjugate competitively inhibited this uptake process over a wide range of concentrations. Uptake by cells from fasted rats was enhanced, but uptake by cells from fasted or fed diabetic rats was normal. The in vivo hepatic uptake and catabolism of ^{125}I-AGOR were slower in diabetic than normal rats. It is inferred that 1) the hepatic receptors which recognize N-acetylglucosamine/mannose terminated glycoproteins are located predominantly on endothelial cells, 2) these receptors are glucose sensitive, 3) fasting increases the number of these receptors and 4) diabetes mellitus abolishes this effect of fasting and impairs the function of this receptor in vivo. These findings suggest a mechanism for abnormal glycoprotein metabolism in diabetes mellitus. This carbohydrate recognition system may play an important role in the removal of potentially autodestructive glycoprotein lysosomal hydrolases and other glycoprotein enzymes from the circulation under normal physiological conditions and in disease states.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53509-09 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies of the Natural History and Treatment of Chronic Type B Hepatitis

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

PI:	J.H. Hoofnagle	Medical Officer	LDS, NIDDK
Others:	E.A. Jones	Chief	LDS, NIDDK
	C. Kassianides	Visiting Associate	LDS, NIDDK
	M. Lisker-Melman	Visiting Associate	LDS, NIDDK
	A. Di Bisceglie	Visiting Associate	LDS, NIDDK

COOPERATING UNITS *(if any)*

Georgetown University, Washington, D.C. (J. Gerin)
Laboratory of Infectious Diseases, NIAID (S. Feinstone)
Walter Reed Army Institute of Research, Washington, D.C. (M. Sjogren)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

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

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

A cohort of patients with chronic type B hepatitis is being evaluated and followed to determine the long-term natural history of this common form of chronic liver disease. Selected patients have been entered into therapeutic trials in which antiviral and/or immunomodulatory agents have been administered. Results of our randomized, controlled trial of alpha-interferon for chronic type B hepatitis indicate that 32% of patients had a favorable serum biochemical and serological response to therapy. Efforts are now being directed towards improving this response rate. A pilot study of alpha and gamma interferon in combination is underway. Patients are treated with 8 week courses of gamma interferon in gradually increasing doses followed by alpha interferon in increasing doses and finally by alpha and gamma interferon in combination. Changes in serum levels of hepatitis B DNA polymerase are monitored at frequent intervals to determine the efficacy of treatment. To date, 8 patients have been entered into this study and 4 have completed all 3 arms of therapy. DNA polymerase levels are consistently reduced in a dose-dependent fashion in all patients by both alpha and gamma interferons. Maximal inhibition with gamma interferon was 25% below pre-treatment values, whereas the corresponding inhibition for alpha interferon was 60%. So far, in only 1 patient subjected to this regimen has DNA polymerase in serum become undetectable. Thus these studies indicate that both alpha and gamma interferons are effective in inhibiting hepatitis B DNA polymerase. Further studies are required to determine if these agents, when given in combination, are more effective than when either is used individually.

GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53510-08 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies of the Natural History and Treatment of Chronic Non-A, Non-B Hepatitis

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

PI:	J.H. Hoofnagle	Medical Officer	LDS, NIDDK
Others:	E.A. Jones	Chief	LDS, NIDDK
	A. Di Bisceglie	Visiting Associate	LDS, NIDDK
	C. Kassianides	Visiting Associate	LDS, NIDDK
	M. Lisker-Melman	Visiting Associate	LDS, NIDDK

COOPERATING UNITS *(if any)*

NIH Blood Bank (H.J. Alter)
Division of Digestive Diseases and Nutrition, NIDDK (J. Everhart)
Armed Forces Institute of Pathology, Washington, D.C. (K. Ishak)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

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

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

☐ (a1) Minors

☐ (a2) Interviews

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

Patients with well-documented chronic non-A, non-B hepatitis are being evaluated to determine the long-term natural history of this common form of chronic liver disease. A cohort of such patients are available to evaluate experimental therapies for this disease. To date 12 patients with chronic non-A, non-B hepatitis have been treated with recombinant human alpha interferon for periods ranging from 4 to 15 months. Ten of the 12 patients have shown a dramatic decrease in serum aminotransferase levels during therapy, their levels falling from values 3 to 10 times the upper limit of the normal range to normal (7 patients) or near-normal (3 patients). Follow up liver biopsies have been obtained from nine patients, which demonstrate marked improvement in the hepatitis disease activity (a decrease in both inflammation and hepatocellular necrosis). The optimum dose appears to be 2 million units (mu) given three times per week. A prospective, randomized, placebo-controlled, double-blind trial of a six month course of human alpha interferon in patients with chronic non-A, non-B hepatitis is underway. Patient with chronic non A, non B hepatitis are evaluated in the Outpatient Clinic and then on the ward, where a percutaneous liver biopsy is obtained. Approximately 1 week later, therapy is commenced with either interferon 1 mu sc or placebo sc three times a week. After 6 months the treatment will be discontinued. Patients who received placebo will then be offered interferon therapy.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53511-08 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Controlled Trial of Chlorambucil in Primary Biliary Cirrhosis

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	J.H. Hoofnagle	Medical Officer	LDS, NIDDK
	K.D. Mullen	Medical Staff Fellow	LDS, NIDDK
	V.R. Rustgi	Medical Staff Fellow	LDS, NIDDK
	D.B. Jones	Visiting Associate	LDS, NIDDK

COOPERATING UNITS *(if any)*

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LAB/BRANCH Digestive Diseases Branch

SECTION Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by slowly progressive intrahepatic cholestasis due to non-suppurative, presumably autoimmune, destruction of septal and the larger interlobular bile ducts. Because certain other autoimmune diseases appear to respond favorably to alkylating agents, a controlled trial of chlorambucil therapy for patients with symptomatic PBC has been conducted. Twenty-four patients were admitted to this trial: 13 were randomized to receive chlorambucil therapy (0.5-4.0 mg/day) and 11 to the control (no treatment) group. The dose of chlroambucil was adjusted to reduce the peripheral blood lymphocyte count by 50% and maintain the poly-morphonuclear leukocyte count above 1000 per c.mm. All patients have been followed for 2-6 years (mean = 4.1 years). During follow-up, two patients died: both were controls. Mean serum bilirubin levels decreased slightly in the treated group but increased significantly in the controls. At 2 years the mean serum albumin was significantly improved in treated patients but was decreased in controls. Mean serum transaminase levels were significantly less in treated patients than in controls. Mean serum immunoglobulin (IgM and IgG) levels decreased from elevated values to values within the normal range in all chlorambucil-treated patients, but did not change appreciably in controls. Liver biopsy histopathology after one and/or two years revealed significantly less inflammation, slightly less fibrosis and less progression of the stage of disease in the treated than in the control patients. Potential side effects of chlorambucil therapy included the onset of menopause in two patients, localized herpes simplex or zoster in 3 and, in 4 patients, persistent leukopenia or thrombocytopenia requiring discontinuation of the drug. These findings strongly suggest that chlorambucil therapy retards the progression of PBC, and they provide an impetus to search for safer (e.g. noncarcinogenic) and more effective immunosuppressive regimes for the treatment of this disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53514-04 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Immunological Studies in Chronic Viral Hepatitis

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

PI:	Jay H. Hoofnagle	Medical Officer	LDS, NIDDK
Others:	E.A. Jones	Chief	
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	V. Rustgi	Medical Staff Fellow	LDS, NIDDK
	A. Di Bisceglie	Visiting Associate	LDS, NIDDK
	T. Suou	Guest Researcher	LDS, NIDDK

COOPERATING UNITS *(if any)*

Laboratory of Immunoregulation, NIAID (Dr. Julian Ambrus)
Washington University School of Medicine (Dr. Marion Peters)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Immunological factors seem to be important in determining the course and outcome of both acute and chronic viral hepatitis. Furthermore, promising therapies for chronic viral hepatitis have profound effects on immune function and sustained responses to therapy may depend largely on restoration of normal immune responsiveness. The role of immunologic mechanisms in determining the course and ultimate outcome of viral hepatitis is being studied and the effects of therapies on the immune system are being evaluated. Serial studies of cellular immune function were performed on patients with chronic type B hepatitis. In addition, the immunological status of patients with chronic type B hepatitis has been assessed and the effect of immunosuppressive as well as antiviral therapy on immunological function in these patients has been studied prospectively.

GPO 814-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 53515-01 DDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

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

Studies of the Natural History and Treatment of Duck Hepatitis B Virus Infection

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

PI:	E.A. Jones	Chief	LDS, NIDDK
Others:	C.K. Kassianides	Visiting Associate	LDS, NIDDK
	J.H. Hoofnagle	Medical Officer	LDS, NIDDK

COOPERATING UNITS *(if any)*

Comparative Animal Unit (D. Matthews)
Hepatitis Virus Section, NIAID (R. Miller)
Clinical Oncology Program, NCI (H. Mitsuya)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1	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.)*

There are many similarities in structure and properties between the human hepatitis B virus and duck hepatitis B virus (DHBV). This makes DHBV infection in ducks a very useful experimental model of human HBV infection, particularly as HBV cannot be grown readily in cell culture. Ducks infected with DHBV at birth become chronic carriers of the virus, although they may not develop overt hepatitis. Some DHBV-infected ducks have been reported to develop hepatocellular carcinoma, a tumor strongly linked etiologically in humans with chronic hepatitis B infection. New antiviral immunomodulatory agents are being assessed for their ability to suppress DHBV replication in ducks as a screening test for new effective therapies for chronic type B hepatitis in man.

GPO 914-918

ANNUAL REPORT OF THE

MOLECULAR, CELLULAR, AND NUTRITIONAL ENDOCRINOLOGY BRANCH

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

The MCNEB continues basic and clinical investigations in the areas of molecular regulation and neuroendocrinology (Molecular Regulation and Neuroendocrinology Section, Bruce D. Weintraub, Chief); experimental diabetes, metabolism and nutrition (Experimental Diabetes, Metabolism and Nutrition Section, Samuel W. Cushman, Chief); and growth and development (Growth and Development Section, Matthew M. Rechler, Chief). The Branch has had many visiting fellows and associates, as well as international collaborations with the University of Milan, Italy; University of Marseilles, France; Karolinska Institute, Sweden; Institute of Organic Chemistry, Padova, Italy; Nankai University, Tianjin, Peoples Republic of China; Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, CSSR; Postgraduate School of Obstetrics and Gynaecology, University of Auckland, New Zealand; University of Naples, Italy; Department of Medicine, University of Gothenburg, Sweden; Endocrine Institute, Rambam Medical Center, Haifa, Israel; Department of Biochemistry, The University of Newcastle upon Tyne, England.

I. GLYCOPROTEIN HORMONES: MOLECULAR BIOLOGY, SYNTHESIS, PROCESSING REGULATION, ACTION AND PATHOPHYSIOLOGY

A. Analysis and Heterogeneity of TSH Carbohydrate Structure

We have previously used serial lectin affinity chromatography to fractionate TSH glycopeptides into distinct classes based on their carbohydrate structure. In order to examine TSH oligosaccharides in more detail without using harsh chemical methods, we have developed techniques to deglycosylate TSH enzymatically. We have demonstrated that endoglycosidase F, an enzyme that removes complex oligosaccharides with a biantennary structure, can completely deglycosylate purified bovine and human TSH under both denaturing and non-denaturing conditions. Further, we have shown that peptide:N-glycosidase F, an enzyme with broader deglycosylating specificity, can be used to remove selectively one oligosaccharide chain from TSH-α. These enzymes have permitted the characterization of TSH oligosaccharides by high performance liquid chromatography (HPLC) and the study of the role of TSH carbohydrate in receptor binding and in vitro bioactivities as measured by adenylate cyclase activity in human thyroid membranes and cyclic AMP production and iodide uptake in FRTL-5 cells.

Using these two deglycosylating enzymes we have examined the oligosaccharides released from secreted mouse TSH. By anion-exchange HPLC we have shown that secreted TSH-α oligosaccharides contained structures with 0, 1, or 2 negative charges while TSH-β contained these groups as well as > 20% with 3 or more negative charges. More than 9 different charged species were apparent on TSH-β alone. The TSH-α oligosaccharide chain released by peptide:N-glycosidase F appeared to contain more highly charged groups than the site resistant to this enzyme, which was enriched in neutral groups. The oligosaccharides in TSH-β that

contained 3 or more negative charges were particularly enriched in sialic acid residues. Oligosaccharides from the free α subunit demonstrated a profile on anion-exchange HPLC intermediate between TSH-α and -β. These oligosaccharides were also characterized by ion-suppression, amine-adsorption HPLC which permits size separation of molecules differing in charge. These studies showed that oligosaccharide chains on TSH-β were of greater average size than those on TSH-α, while those from the free α subunit were of intermediate size. These studies demonstrate greater heterogeneity of TSH oligosaccharide structure and charge than had been previously appreciated including the presence of novel triply charged structures on TSH-β.

. . . . N. Gesundheit, P. W. Gyves, B. D. Weintraub

B. Developmental Regulation on TSH Carbohydrate Structure

Research performed during the past year has elucidated alterations in the carbohydrate structure of secreted rat thyrotropin during ontogenesis using a combination of concanavalin A (con A) affinity chromatography as well as anion-exchange and ion suppression HPLC. The proportion of secreted glycopeptides that did not bind to con A increased during ontogenesis from 29% in prenatal animals to 58% in mature animals. There was a corresponding decrease during development in the percentage of TSH glycopeptides that bound to con A and were eluted with α-methylglucoside from 67% in prenatal animals to 38% in mature animals. There was no appreciable alteration in the percentage of TSH glycopeptides that bound to con A and were eluted with α-methylmannoside during ontogenesis.

By anion-exchange and ion suppression HPLC the majority of oligosaccharides of secreted TSH from prenatal animals consisted of two or less negative charges, and contained sulfate as the predominant anionic species. In contrast, mature animals contained a predominance of oligosaccharide structures with two or more negative charges, as well as a relative increase in the proportion of species containing sialic acid.

HPLC analysis was also performed on separated TSH α and β subunits. In both prenatal and mature animals, the α subunit was found to contain the majority of oligosaccharide species with one negative charge. In contrast, the β subunit contained a greater proportion of oligosaccharide structures with two or more negative charges. In addition, the progression towards more charged groups in older animals, when compared to younger animals, was more pronounced in the β than α subunit.

These data suggest that alterations in secreted TSH carbohydrate structure, particularly sialylation, occur during ontogenesis in the rat. Correlation of these structural changes with bioactivity is an important adjunct to these studies and is currently under investigation.

. . . . P. W. Gyves, N. Gesundheit, T. Taylor, B. D. Weintraub

C. Endocrine Regulation of TSH Carbohydrate Structure

TSH carbohydrate chains consist of mixed structures and selected synthesis may be regulated by neuropeptides. We have previously shown that rats with hypothalamic hypothyroidism created by paraventricular nuclear (PVN) lesions, had significantly altered carbohydrate chain characteristics in secreted TSH as compared to sham lesioned animals. In addition, the TSH carbohydrate chains from rats with hypothalamic hypothyroidism were markedly different from those in rats with primary hypothyroidism suggesting that these alterations may be due to TRH or other hypothalamic peptides. To further study the role of certain neuropeptides in altering TSH carbohydrate structure, adult rats received either sham or PVN lesions. At 10 days, SC osmotic pumps infusing saline, 1 mg/kg/d TRH, or TRH and 0.25 mg/kg/d somatostatin (SRIF) were placed. At 14 days, pituitaries were incubated with labeled glucosamine for 24 hours. Plasma free T4 was lower in the lesion + saline than sham + saline group (1.6 ± 0.4 vs. 5.2 ± 0.1 ng/dl, p 0.001). TRH without and with SRIF in lesioned groups normalized free T4 but had no effect in the sham groups. Secreted TSH glycopeptides in the lesion + saline as compared to sham + saline group had fewer unbound forms (43 ± 4 vs. 57 ± 1%, p. 0.05), and more weakly bound forms (50 ± 4 vs. 35 ± 2%, p. 0.05). TRH +/- SRIF normalized the binding pattern in the lesion but had no effect in the sham. In both the lesion and sham + saline, TSH α subunit demonstrated both unbound and bound forms but TSH-β subunit had a predominance of unbound forms. In summary, hypothalamic hypothyroidism alters TSH carbohydrate structures and *in vivo* TRH normalizes the structures in parallel with the normalization of serum T4. Current studies are applying HPLC analysis to characterize further the structural alterations and ultimately correlate them with TSH function.

. . . . T. Taylor, N. Gesundheit, P. W. Gyves, B. D. Weintraub

D. Receptor-Binding and Bioactivity of Heterogeneous Forms of Human, Bovine and Rodent Thyrotropins.

We continue to study the biological action of heterogeneous TSH forms derived from various physiological and pathophysiological states in man and in animal models. Currently, we have developed a number of *in vitro* assays to study such activity, including adenylate cyclase stimulation of bovine thyroid membranes, cyclic AMP production and iodide trapping in rat FRTL-5 cells, as well as release of ^{125}I-labeled T4 and T3 from mice *in vivo*. We find that different forms of thyrotropin have different actions in various bioassays. In the cAMP production bioassay, immunoaffinity purified serum TSH showed increased bioactivity in patients with primary hypothyroidism and with a TSH-secreting pituitary tumor compared to normal subjects, while in the iodide uptake bioassay, minimal differences were detected among the different groups. Other types of rodent TSH show different responses in each assay, which is being correlated with carbohydrate structure.

. . . . M. Nissim, B. D. Weintraub

E. Receptor-Binding and Bioactivity of Deglycosylated Human and Bovine Thyrotropins.

To investigate further structure-function relationships of TSH we studied the effects of deglycosylated purified pituitary bovine and human TSH in various bioassays. Using two new enzymes, peptide-N-glycosidase (PNGase F) and endo-β-N-acetylglucosaminidase F (Endo F) we removed one carbohydrate chain from TSH-α and all three chains from TSH, respectively. In the rat iodide uptake and human adenylate cyclase bioassay both enzymes induced a 50-80% decrease in TSH biopotency, while in the rat cAMP production bioassay this decrease was only present with PNGase F treated TSH. TSH receptor binding to human thyroid membranes was not affected by enzyme treatment, indicating that deglycosylation affected post-receptor steps in hormone action.

. . . . K. O. Lee, M. Nissim, N. Gesundheit, B. D. Weintraub

F. Study of Growth Failure in Children with Thyroid Hormone Resistance.

We currently follow approximately 20 children with pituitary and peripheral thyroid hormone resistance from eight kindreds. Some kindreds are characterized by inappropriate TSH secretion, short stature and markedly delayed bone maturation. In addition, a retrospective analysis of our adult patients from the same affected kindreds suggests that ultimate adult height may be compromised. Therefore, we have begun to study in more detail the mechanism(s) of altered growth in these affected children including the peripheral actions of thyroid hormone and secretion dynamics of growth hormone. Patients with thyroid hormone resistance and the characteristic short stature have been enrolled in a clinical trial to assess whether additional thyroid hormone, to levels that produce nearly complete pituitary TSH suppression, will improve growth and accelerate bone maturation. These patients are readmitted to the clinical service every 3 months at which time careful growth measurements are performed and thyroid and growth hormone status are evaluated. This study is estimated to required 2-5 years from completion of the initial phase.

. . . . N. Gesundheit, P. W. Gyves, B. D. Weintraub

G. Cloning of the Human TSH-β Subunit Gene.

The complete gene for the human TSH-β subunit has not previously been cloned. A 17 kilobase human genomic DNA fragment containing the human TSH-β gene was isolated from a human leukocyte EMBL3 library using a mouse TSH-β cDNA probe. Detailed restriction mapping and sequence analysis revealed that his gene contains two protein coding exons and one 5' untranslated exon. The 5' untranslated exon is separated from the two protein coding exons by a 3.9 kilobase intron and is thus similar in structure to the rat TSH-β gene.

To characterize further the 5' untranslated exon and promoter region of this gene, we constructed a human pituitary cDNA library from postmortem human pituitary RNA using a specific oligonucleotide primer complementary to most 5' region of the second exon. We are currently

characterizing this library in order to obtain definitive boundaries for the 5' untranslated exon. In addition, primer extension and S1 nuclease experiments indicate that this exon is approximately 40-50 nucleotides in length and may contain two transcriptional start sites as has been noted in both the rat and mouse TSH-β gene.

Finally, transfection of constructs containing the entire TSH-β gene into both rat pituitary (GH3) and mouse thyrotropic tumor cells resulted in expression of human TSH-β RNA. Moreover, we were able to inhibit expression of human TSH-β RNA 4-fold after addition of 5 nM T3 to cell media. Transfection experiments utilizing chimeric plasmids containing the promoter of human TSH-β and the chloramphenicol acetyl transferase gene are also in progress to complement these studies. Deletion and site-directed mutagenesis of this promoter and 5' flanking region will allow a detailed study of the molecular mechanisms by which mediators such as thyroid hormone, glucocorticoids, and TRH regulated TSH-β expression.

. . . . F. E. Wondisford, S. Usala, M. Castren, V. Nikodem, B. D. Weintraub

H. Synthetic Human TSH from Cell Culture after Gene Transfection.

No species of TSH has been synthesized by either stable or transient gene transfection. Human TSH was synthesized in cell culture after transfection with viral expression vectors containing the human CGα cDNA and the two protein coding exons of human TSH-β. While a variety of viral expression vectors were utilized, we found that an adeno-associated viral vector transfected into embryonal human kidney cells (293) gave the highest level of TSH expression. In addition, a plasmid containing the VA1 adenovirus gene increased protein expression several fold. This gene has previously been shown to increase the rate of translation by inhibiting the phosphorylation and inactivation of eucaryotic initiation factor 1.

The TSH synthesized in cell culture was both larger in apparent molecular weight and had a slightly different binding pattern on lectin chromatography than standard human pituitary TSH. This suggests a different glycosylation pattern such as more sialylation. However, the synthetic TSH was equivalent to the same standard in an *in vitro* TSH bioassay. This novel form of TSH may display differences in *in vivo* bioactivity and metabolic clearance not noted in the *in vitro* TSH bioassay.

. . . . F. E. Wondisford, S. Usala, M. Castren, J. P. Trempe, G. S. DeCherney, V. Nikodem, B. J. Carter, B. D. Weintraub.

II. NEUROENDOCRINE PEPTIDES: BIOSYNTHESIS, FOLDING AND FUNCTION

A. Molecular mechanisms in neuroendocrine peptide and protein pathways

Molecular mechanisms underlying the neuroendocrine occurrence, biosynthesis, molecular characteristics and function of neuroendocrine peptides and proteins are being studied, with emphasis on the neuropeptide hormones oxytocin and vasopressin, associated neurophysins (NP's), and their biosynthetic precursors. An hypothesis has been examined that biosynthetic precursors of the neurohypophysial hormones adopt a defined conformational organization upon completion of translation and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. Chemical methods have been devised to produce biosynthetic precursors and both sequence-designed and site-specific mutants. These methods have been used to prepare semisynthetic oxytocin/neurophysin I and Arg 8 vasopressin/neurophysin II precursor analogs. Evaluation of structural characteristics of the semisynthetic precursors shows that the precursors are well-ordered, folded molecules which can form self-associated species. The latter are concluded to be the prevailing forms in neurosecretory granules in which enzymatic processing occurs. Evaluation of the impact of these characteristics on enzymatic processing has been made by comparing rates and products of processing of intact precursors to these properties for synthetic fragments containing processing sites. Separately, evaluation has been extended of the relationship between occurrence of the hormone/NP neuroendocrine system in the ovary versus that in hypothalamo-neurophysial system. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. In addition to neurophysin and oxytocin, a newly identified neurophysin-binding species has been found in both sites and is currently being characterized. The data obtained in this study are being used to help define the relationship between molecular mechanisms which occur in different neuroendocrine sites.

. . . . S. Ando, G. Fassina, I. M. Chaiken

B. Mechanisms of Peptide and Protein Recognition, Assembly, Function

Principles which govern surface recognition, intra- and intermolecular assembly and function of peptides and proteins are being studied. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in determining molecular order and function. A major project is in progress to understand the newly described phenomenon that peptides encoded in anti-sense DNA have unexpected and potentially provocative interaction properties, including an ability both to bind to corresponding sense peptides (those encoded in sense DNA) and to elicit antibodies which bind to cellular receptors of sense peptides. An experimental paradigm has been established to characterize sense-antisense peptide binding and used to reveal quantitative properties of this binding process. Separately, underlying principles which determine surface recognition and consequent molecular

order are being evaluated by studying the effect of synthetic sequence mutation on the peptide-protein assembly of semisynthetic ribonuclease-S, using high resolution structure of a modeled semisynthetic ribonuclease-S as a starting point. The data are being used to examine rules of protein self-assembly and to establish general guidelines for protein engineering. And a study continues of the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules, and the hormone-neurophysin precursors which also appear to self-associate into forms likely to exist in granules before processing. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes have been studied, using natural hormones and hormone mutants obtained by chemical synthesis. In addition, sequence-variant mutants of precursor have been prepared by semisynthesis and their interaction properties are being studied.

. . . . G. Fassina, S. Ando, Y. Shai, I. M. Chaiken

C. Biorecognition Technology

Bioaffinity methods are being developed which can be used for characterizing functional interaction properties, including multi-molecular assembly, of biological macromolecules and to design polypeptides de novo which recognize protein surfaces. A major study has been designed to evaluate the potential to adapt bioaffinity chromatography to extant high performance liquid chromatography technology. Silica-based matrices are being used to measure protein-protein, peptide-protein, and peptide-peptide, antibody-protein interactions, for neuroendocrine peptides and proteins and their precursors as well as well-understood "model" peptides and proteins. Analytical high performance affinity chromatography also is being used as an evaluative tool to design and chemically synthesize peptides de novo which can recognize protein surfaces specifically and ultimately be used for protein isolation and for diagnostic characterization of macromolecular recognition properties. Overall analytical high performance affinity chromatographic methods which result from this study provide potentially important analytical biochemistry tools both for characterizing basic properties of macromolecules and for microscale molecular profiling and diagnosis.

. . . . G. Fassina, Y. Shai, P. Caliceti, I. M. Chaiken

IV. STUDIES OF THE MECHANISM OF THE INSULIN ACTION AND ITS PERTURBATION IN ALTERED METABOLIC STATES

A. Insulin-Cell Interaction

The phosphorylation state of insulin receptors and their tyrosine kinase activity in membrane fractions from insulin-treated isolated rat adipose cells have been studied. The results suggest that insulin receptors retain their kinase activity on internalization. However, if the

internalized receptor kinase mediates insulin's effect on glucose transport, only a portion of its maximum activity appears to be necessary for full glucose transport stimulation.

. . . . T. M. Weber, H. G. Joost, I. A. Simpson, S. W. Cushman

B. Insulin's Regulation of Glucose Transport

3T3-L1 fibroblasts differentiate in culture to resemble adipose cells both morphologically and biochemically. The number of glucose transporters has been measured in subcellular membrane fractions from these cells during differentiation. The data suggest that the glucose transporter undergoes differential processing and that functional, insulin-responsive glucose transporters may be different from the insulin-insensitive (basal) glucose transporter. In a preliminary series of experiments, insulin appears to stimulate glucose transport in isolated human adipose cells by a translocation mechanism similar to that observed in rat adipose cells and diaphragm. Conditions have been established which allow the isolation of rat adipose cell plasma membranes retaining a large part of the stimulatory effect of insulin in intact cells. In these membranes, the magnitude of glucose transport stimulation in response to insulin was compared with the concentration of glucose transporters as measured with the cytochalasin B binding assay or by immunoblotting with an antiserum against the human erythrocyte glucose transporter. The results suggest that in addition to stimulating the translocation of glucose transporters to the plasma membrane, insulin appears to induce a structural or conformational change in the glucose transporter manifested in an altered activation energy for plasma membrane glucose transport and possibly in an altered immunoreactivity as assessed by Western blotting.

. . . . S. W.Cushman, I. A. Simpson, B. D. Kahn, H. G. Joost, T. M. Weber, M. J. Zarnowski, D. R. Yver, A. D. Habberfield, T. L. Jones, J. Saltis

C. Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

The effects of insulin therapy on the glucose transport response to insulin in adipocytes from stretozotocin diabetic rats have been examined. The results suggest that insulin therapy produces markedly hyperresponsive insulin-stimulated adipocyte glucose transport but only in part by increasing intracellular glucose transporters and insulin-stimulated glucose transporter translocation to the plasma membrane. The remaining hyperresponsiveness appears to be due to concurrently augmented glucose transporter intrinsic activity.

. . . . B. B. Kahn, S. W. Cushman, T. L. Jones

D. Alterations in Insulin's Action with Chronic Hyperinsulinemia

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells have been studied. The results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of

cells. In these membranes, the magnitude of glucose transport stimulation in response to insulin was compared with the concentration of glucose transporters as measured with the cytochalasin B binding assay or by immunoblotting with an antiserum against the human erythrocyte glucose transporter. The results suggest that in addition to stimulating the translocation of glucose transporters to the plasma membrane, insulin appears to induce a structural or conformational change in the glucose transporter manifested in an altered activation energy for plasma membrane glucose transport and possibly in an altered immunoreactivity as assessed by Western blotting.

. . . . S. W.Cushman, I. A. Simpson, B. D. Kahn, H. G. Joost, T. M. Weber, M. J. Zarnowski, D. R. Yver, A. D. Habberfield, T. L. Jones, J. Saltis

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. . . . B. B. Kahn, S. W. Cushman, T. L. Jones

D. Alterations in Insulin's Action with Chronic Hyperinsulinemia

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells have been studied. The results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose without changing the cells' sensitivity to insulin. The mechanism of increased insulin-stimulated glucose transport in adipocytes from chronically hyperinsulinemic rats has also been examined. These results suggest that chronic hyperinsulinemia in the rat enhances insulin's stimulatory action on glucose transport in adipocytes by increasing the intracellular pool of glucose transporters through a generalized effect on the net synthesis of intracellular protein.

. . . . B. B. Kahn, S. W. Cushman

E. Insulin's Regulation of Hormone Binding

A comparison of insulin's effects on glucose transport and cell surface IGF-II receptors has been undertaken in rat adipose cells using 3-O-methylglucose transport as a measure of glucose transport activity and Scatchard analysis of IGF-II binding in the presence of KCN to determine cell surface IGF-II receptor number. These results demonstrate that while the characteristics of the stimulatory action of insulin on glucose transport activity and cell surface IGF-II receptor number are qualitatively similar, quantitative differences are clearly demonstrable

which suggest that the subcellular cycling of these two integral membrane proteins occurs by distinct processes. The effects of adenosine, isoproterenol, and glucose have now been examined on both steady state insulin responsiveness and sensitivity in this cell type prepared in the presence of saturating adenosine (200 nM). The results show that the stimulatory effect of insulin on IGF-II binding to rat adipose cells is modulated not only by counterregulatory hormones, but also by glucose, a major substrate of insulin action.

. . . . S. W. Cushman, I. A. Simpson

F. Counterregulation of Insulin's Action by Catecholamines

The modulation of insulin-stimulated glucose transport activity in rat adipose cells by ligands for receptors (R) that mediate stimulation (R_s; lipolytic) or inhibition (R_i; antilipolytic) of adenylate cyclase has been examined. The results suggest that 1) R_i- and R_s-mediated effects on glucose transport are independent of changes in cAMP, 2) these cAMP-independent effects are mediated by GTP-binding proteins, N_i and N_s, and 3) R_i and R_s ligands modulate the intrinsic activity of the glucose transporter in the plasma membrane. The mechanism of modulation of insulin-stimulated glucose transport activity in isolated rat adipose cells by lipolytic and antilipolytic agents has been further examined by measuring glucose transport activity in plasma membranes. The data indicate that modifications of glucose transport activity produced by lipolytic and antilipolytic agents in intact adipose cells can be fully retained in plasma membranes isolated under appropriate conditions, further supporting the concept that the effects of these agents occur through a modification of glucose transporter intrinsic activity. The effects of β-adrenergic stimulation and different analogues of cAMP on insulin-stimulated IGF-II binding have also been studied. The results indicate that β-adrenergic stimulation and high levels of cAMP markedly impair both sensitivity and responsiveness to insulin suggesting an antagonistic effect on insulin's signalling mechanism. Furthermore, adenosine appears to exert a potent modulating effect through N_i, while activation of phosphodiesterase by insulin appears to play a crucial role for the expression of insulin action under conditions of elevated cAMP levels. The counterregulatory action of catecholamines on insulin-stimulated glucose transport and its relation to glucose transporter phosphorylation have been studied in isolated rat adipose cells. The results suggest that the phosphorylation state of the glucose transporter does not appear to be involved in either signalling glucose transporter translocation or triggering changes in glucose transporter intrinsic activity.

. . . . H. G. Joost, I. A. Simpson, T. M. Weber, S. W. Cushman, M. J. Zarnowski

G. Alterations in Insulin's Action with Fasting/Refeeding

Rapid alterations in glucose transport and metabolism have been shown in rat adipose cells after fasting and refeeding. The mechanism for this was examined in rats fasted for 48 h and sacrificed ± 6 d of refeeding. The results suggest that insulin resistance at the glucose transport level induced by fasting is due to a depletion of intracellular glucose

transporters. In contrast, the hyperresponsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters and may also involve modulation of glucose transporter intrinsic activity.

. . . . B. B. Kahn, S. W. Cushman, I. A. Simpson

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55000-15 MCNE

PERIOD COVERED

October 1, 1986 to September 30, 1987

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

Biosynthesis and Glycosylation of Thyrotropin

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

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	N. Gesundheit	Senior Medical Staff Fellow	MCNEB, NIDDK
	P. W. Gyves	Guest Researcher	MCNEB, NIDDK
	T. Taylor	Guest Researcher	MCNEB, NIDDK
	B. S. Stannard	Biologist	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	4.1	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.)*

Glycoproteins constitute the most common class of viral and protozoal envelope proteins, mammalian serum proteins, plasma membrane receptors, transporters and ion channels. Although it has been suggested that such proteins may have heterogeneity of carbohydrate structures, the specific chemical nature and significance of such heterogeneity is not known. Using newly developed methods of lectin analysis of glycopeptides as well as high performance liquid chromatography (HPLC) analysis of enzyme-released sugars, we have elucidated at least 9 different carbohydrate variants of secreted TSH. Moreover, for the first time in any glycoprotein system we have elucidated major developmental and endocrine regulation of such glycosylation, and are currently studying the functional correlates of such regulated heterogeneity. These studies may reveal a novel post-translational control mechanism that may explain various aspects of thyroid physiology and pathophysiology.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55001-11 MCNE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation and Action of Thyrotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	K. O. Lee	Visiting Fellow	MCNEB, NIDDK
	M. Nissim	Visiting Fellow	MCNEB, NIDDK
	N. Gesundheit	Senior Medical Staff Fellow	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	2.7	.2

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thyrotropin (TSH) is he primary regulator of thyroid function and it is important to determine factors that control its regulation and action. Heterogeneous forms of TSH differing in carbohydrate structure display different actions in different assay systems. Enzymatic deglycosylation causes inhibition of TSH activity in most bioassays, primarily at a post-receptor step. Thus, carbohydrate modulates the action of TSH in a qualitative manner, in addition to the primary transcriptional control of TSH synthesis which regulates the quality of the hormone. Certain human diseases such as thyroid hormone resistance cause abnormal regulation of TSH as well as decreased growth in children. We are currently treating such patients with large doses of thyroid hormone in an attempt to correct both the pituitary and growth defect.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55002-07 MCNE

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Chief	MCNEB, NIDDK
Others:	F. E. Wondisford	Medical Staff Fellow	MCNEB, NIDDK
	G. S. DeCherney	Guest Researcher	MCNEB, NIDDK
	M. Castren	Visiting Fellow	MCNEB, NIDDK
	S. Usala	Medical Staff Fellow	CEB, NIDDK
	V. Nikodem	Senior Investigator	CEB, NIDDK
	J. P. Trempe	Staff Fellow	LMCB, NIDDK
	B. J. Carter	Chief	LMCB, NIDDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.3	2.1	1.2

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Currently natural human pituitary hormones such as thyrotropin (TSH) are available in only limited supply and these preparations may be contaminated with pathologic viruses. Thus it has not been possible to prepare detailed biochemical, physicochemical, biologic or therapeutic studies with such preparations. Moreover, the factors controlling pituitary hormone gene expression are poorly understood. We have recently cloned and sequenced the complete human TSH-beta subunit gene and have partially defined its thyroid hormone regulatory region. Using cotransfection experiments with a human alpha subunit cDNA and human TSH-beta genomic DNA, we have also produced synthetic human pituitary TSH. Although apparently containing more sialic acid than natural TSH, this synthetic TSH is equally active in vitro and will be used to define additional biochemical aspects and therapeutic actions of the hormone. We also plan to define other regulatory regions of the gene to gain insight into the fundamental mechanisms of transcriptional control.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55003-14 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms in Neuroendocrine Peptide and Protein Pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Shoji Ando	Visiting Fellow	MCNEB, NIDDK
	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
Neurosciences Department, Johns Hopkins Medical School, Baltimore, MD; Laboratory of Biochemistry and Metabolism, NIDDK

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms underlying the neuroendocrine occurrence, biosynthesis, molecular characteristics and function of neuroendocrine peptides and proteins are being studied, with emphasis on the neuropeptide hormones oxytocin and vasopressin, associated neurophysins (NP's), and their biosynthetic precursors. An hypothesis has been examined that biosynthetic precursors of the neurohypophysial hormones adopt a defined conformational organization upon completion of translation and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. Chemical methods have been devised to produce biosynthetic precursors and both sequence-designed and site-specific mutants. These methods have been used to prepare semisynthetic oxytocin/neurophysin I and Arg 8 vasopressin/neurophysin II precursor analogs. Evaluation of structural characteristics of the semisynthetic precursors shows that the precursors are well-ordered, folded molecules which can form self-associated species. The latter are concluded to be the prevailing forms in neurosecretory granules in which enzymatic processing occurs. Evaluation of the impact of these characteristics on enzymatic processing has been made by comparing rates and products of processing of intact precursors to these properties for synthetic fragments containing processing sites. Separately, evaluation has been extended of the relationship between occurrence of the hormone/NP neuroendocrine system in the ovary versus that in hypothalamo-neurophysial system. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. In addition to neurophysin and oxytocin, a newly identified neurophysin-binding species has been found in both sites and is currently being characterized. The data obtained in this study are being used to help define the relationship between molecular mechanisms which occur in different neuroendocrine sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55004-17 MCNE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Peptide and Protein Recognition, Assembly, Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK
	Shoji Ando	Visiting Fellow	MCNEB, NIDDK
	Yechiel Shai	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)* Inst. of Organic Chem., Univ. of Padova, Italy; Biophysics Dept., Johns Hopkins Medical School, Baltimore, MD

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.7	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.)*

Principles which govern surface recognition, intra- and intermolecular assembly and function of peptides and proteins are being studied. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in determining molecular order and function. A major project is in progress to understand the newly described phenomenon that peptides encoded in anti-sense DNA have unexpected and potentially provocative interaction properties, including an ability both to bind to corresponding sense peptides (those encoded in sense DNA) and to elicit antibodies which bind to cellular receptors of sense peptides. An experimental paradigm has been established to characterize sense-antisense peptide binding and used to reveal quantitative properties of this binding process. Separately, underlying principles which determine surface recognition and consequent molecular order are being evaluated by studying the effect of synthetic sequence mutation on the peptide-protein assembly of semisynthetic ribonuclease-S, using high resolution structure of a modeled semisynthetic ribonuclease-S as a starting point. The data are being used to examine rules of protein self-assembly and to establish general guidelines for protein engineering. And a study continues of the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules, and the hormone-neurophysin precursors which also appear to self-associate into forms likely to exist in granules before processing. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes have been studied, using natural hormones and hormone mutants obtained by chemical synthesis. In addition, sequence-variant mutants of precursor have been prepared by semisynthesis and their interaction properties are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55005-17 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biorecognition Technology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin M. Chaiken	Research Chemist	MCNEB, NIDDK
Others:	Giorgio Fassina	Visiting Fellow	MCNEB, NIDDK
	Yechiel Shai	Guest Researcher	MCNEB, NIDDK
	Paolo Caliceti	Guest Researcher	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Molecular Regulation and Neuroendocrinology Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Bioaffinity methods are being developed which can be used for characterizing functional interaction properties, including multi-molecular assembly, of biological macromolecules and to design polypeptides de novo which recognize protein surfaces. A major study has been designed to evaluate the potential to adapt bioaffinity chromatography to extant high performance liquid chromatography technology. Silica-based matrices are being used to measure protein-protein, peptide-protein, and peptide-peptide, antibody-protein interactions, for neuroendocrine peptides and proteins and their precursors as well as well-understood "model" peptides and proteins. Analytical high performance affinity chromatography also is being used as an evaluative tool to design and chemically synthesize peptides de novo which can recognize protein surfaces specifically and ultimately be used for protein isolation and for diagnostic characterization of macromolecular recognition properties. Overall analytical high performance affinity chromatographic methods which result from this study provide potentially important analytical biochemistry tools both for characterizing basic properties of macromolecules and for microscale molecular profiling and diagnosis.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55006-14 MCNE

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Insulin-like Growth Factors (Somatomedins): Biosynthesis and Action

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M.M. Rechler	Chief, GD Section	MCNEB, NIDDK
Others:	A.L. Brown	Staff Fellow	MCNEB, NIDDK
	D.E. Graham	Expert	MCNEB, NIDDK
	C.C. Orlowski	Staff Fellow	MCNEB, NIDDK
	J.-F. Wang	Visiting Fellow	MCNEB, NIDDK
	Y.W-H. Yang	Staff Fellow	MCNEB, NIDDK
	J.A. Romanus	Biologist	MCNEB, NIDDK
	L.Tseng	Chemist	MCNEB, NIDDK

COOPERATING UNITS *(if any)*: DB, NIDDK (C. Roberts), MB NCI (S.P. Nissley, W. Kiess); Univ. Naples, Italy (C.B. Bruni, R. Frunzio, L. Chiariotti); Mt. Sinai Sch. Med., CUNY, NY, (G.T. Burke, P.G. Katsoyannis)

LAB/BRANCH: Molecular, Cellular and Nutritional Endocrinology Branch

SECTION: Growth and Development Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.75	4.75	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our study of the insulin-like growth factor, rat IGF-II. During the past year, we have demonstrated that: (1) multiple IGF-II RNAs (1.2 to 5 kb) arise from a single gene through the use of 2 promoters and alternate polyA addition sites; (2) the IGF-II gene is transcribed from both promoters in 11 fetal rat tissues with different efficiencies. Transcription from both promoters is high in the fetus and early neonate and negligible in adult tissues; (3) 1.2 kb IGF-II RNA is translated into Mr 22,000 pre-pro-rIGF-II, whereas 4 and 5 kb IGF-II RNAs are not translationally competent; (4) IGF-II RNA is translated in different tissues, and pre-pro-rIGF-II processed to Mr 7484 biologically active IGF-II; (5) the fetal/neonatal form of the IGF carrier protein is synthesized as a Mr 35,000 precursor in cell-free translation, and cotranslationally processed to the stable, secreted Mr 33,000 form; (6) translatable RNA encoding the Mr 35,000 carrier protein precursor is present in fetal and neonatal rat liver, but not in adult liver, suggesting that developmental regulation occurs at the level of transcription; (7) polyclonal antibodies to purified type II receptor do not stimulate or inhibit IGF actions in L6 rat myoblasts, suggesting that these effects are not mediated by the type II receptor; (8) nearly full-size type II IGF receptors circulate in fetal and neonatal rat serum, and that levels of circulating receptor decrease markedly in older rats; (9) IGF-II potently stimulates neurite outgrowth in sympathetic and sensory neurons cultured from chick embryos; (10) activation of human T lymphocytes results in increased expression of type I and type II IGF receptors, suggesting that the IGFs may participate in the activation cascade; (11) two-chain insulin-IGF hybrid molecules containing the B-domain of IGF-I have increased mitogenic activity and binding to type I IGF receptors but do not bind to IGF carrier proteins.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55007-09 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin-Cell Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. M. Weber	Staff Fellow	MCNEB, NIDDK
Others:	H. G. Joost	Guest Worker	MCNEB, NIDDK
	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

DB/NIDDK (S. DiPaolo).

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The phosphorylation state of insulin receptors and their tyrosine kinase activity in membrane fractions from insulin-treated isolated rat adipose cells have been studied. The results suggest that insulin receptors retain their kinase activity on internalization. However, if the internalized receptor kinase mediates insulin's effect on glucose transport, only a portion of its maximum activity appears to be necessary for full glucose transport stimulation.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55008-09 MCNE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin's Regulation of Glucose Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
	H. G. Joost	Guest Worker	MCNEB, NIDDK
	T. M. Weber	Staff Fellow	MCNEB, NIDDK
	M. J. Zarnowski	Biologist	MCNEB, NIDDK
	D. R. Yver	Chemist	MCNEB, NIDDK
	A. D. Habberfield	Visiting Fellow	MCNEB, NIDDK
	T. L. Jones	Medical Staff Fellow	MCNEB, NIDDK
	J. Saltis	Visting Fellow	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

Dept. Med., Univ. Gothenburg, Sweden (U. Smith); Endocrine Inst., Rambam Med. Center, Haifa, Israel (E. Karnieli); Dept. Surg., Bethesda Naval Hospital, Bethesda, MD (B. Chernow); Erie County Lab., Erie County Med. Center, Buffalo, NY (P.J. Hissin); PECRB/NIDDK (J. E. Foley); Dept. Biochem. and Mol. Biol., Univ. Florida Coll. Med., Gainesville, FL (S. C. Frost); Dept. Biol. Chem., The Johns Hopkins Univ. Sch. Med., Baltimore, MD (M. D. Lane).

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.1	5.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

3T3-L1 fibroblasts differentiate in culture to resemble adipose cells both morphologically and biochemically. The number of glucose transporters has been measured in subcellular membrane fractions from these cells during differentiation. The data suggest that the glucose transporter undergoes differential processing and that functional, insulin-responsive glucose transporters may be different from the insulin-insensitive (basal) glucose transporter. In a preliminary series of experiments, insulin appears to stimulate glucose transport in isolated human adipose cells by a translocation mechanism similar to that observed in rat adipose cells and diaphragm. Conditions have been established which allow the isolation of rat adipose cell plasma membranes retaining a large part of the stimulatory effect of insulin in intact cells. In these membranes, the magnitude of glucose transport stimulation in response to insulin was compared with the concentration of glucose transporters as measured with the cytochalasin B binding assay or by immunoblotting with an antiserum against the human erythrocyte glucose transporter. The results suggest that in addition to stimulating the translocation of glucose transporters to the plasma membrane, insulin appears to induce a structural or conformational change in the glucose transporter manifested in an altered activation energy for plasma membrane glucose transport and possibly in an altered immunoreactivity as assessed by Western blotting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 55010-06 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action in Insulin-Dependent Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
	T. L. Jones	Medical Staff Fellow	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of insulin therapy on the glucose transport response to insulin in adipocytes from stretozotocin diabetic rats have been examined. The results suggest that insulin therapy produces markedly hyperresponsive insulin-stimulated adipocyte glucose transport but only in part by increasing intracellular glucose transporters and insulin-stimulated glucose transporter translocation to the plasma membrane. The remaining hyperresponsiveness appears to be due to concurrently augmented glucose transporter intrinsic activity.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 55011-05 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action with Chronic Hyperinsulinemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK

COOPERATING UNITS *(if any)*
Metabolic Unit, Department of Medicine, University of Vermont College of Medicine, Burlington, VT (E. S. Horton).

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Experimental Diabetes, Metabolism and Nutrition

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells have been studied. The results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose without changing the cells' sensitivity to insulin. The mechanism of increased insulin-stimulated glucose transport in adipocytes from chronically hyperinsulinemic rats has also been examined. These results suggest that chronic hyperinsulinemia in the rat enhances insulin's stimulatory action on glucose transport in adipocytes by increasing the intracellular pool of glucose transporters through a generalized effect on the net synthesis of intracellular protein.

PHS 6040 (Rev. 1/84)

GPO 914-916

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 55012-05 MCNE

PERIOD COVERED: October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Insulin's Regulation of Hormone Binding

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK

COOPERATING UNITS (if any): Research Laboratories, A. H. Robins Company, Richmond, VA (K. C. Appell).

LAB/BRANCH: Molecular, Cellular and Nutritional Endocrinology Branch

SECTION: Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.01	0.01	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A comparison of insulin's effects on glucose transport and cell surface IGF-II receptors has been undertaken in rat adipose cells using 3-O-methylglucose transport as a measure of glucose transport activity and Scatchard analysis of IGF-II binding in the presence of KCN to determine cell surface IGF-II receptor number. These results demonstrate that while the characteristics of the stimulatory action of insulin on glucose transport activity and cell surface IGF-II receptor number are qualitatively similar, quantitative differences are clearly demonstrable which suggest that the subcellular cycling of these two integral membrane proteins occurs by distinct processes. The effects of adenosine, isoproterenol, and glucose have now been examined on both steady state insulin responsiveness and sensitivity in this cell type prepared in the presence of saturating adenosine (200 nM). The results show that the stimulatory effect of insulin on IGF-II binding to rat adipose cells is modulated not only by counter-regulatory hormones, but also by glucose, a major substrate of insulin action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55013-04 MCNE

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Counterregulation of Insulin's Action by Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. G. Joost	Guest Worker	MCNEB, NIDDK
Others:	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK
	T. M. Weber	Staff Fellow	MCNEB, NIDDK
	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
	M. J. Zarnowski	Biologist	MCNEB, NIDDK

COOPERATING UNITS *(if any)* Dept. Med., Univ. of Gothenburg, Sweden (P. Lönnroth, C. Wesslau, U. Smith); LCDB/NIDDK (C. Londos); Fermentation Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan (M. Kuroda); Research Laboratories, A. H. Robins Co., Richmond, VA (K. C. Appell); Dept. of Biochemistry, The Univ. of Newcastle upon Tyne, Newcastle upon Tyne, England (R. C. Honnor).

LAB/BRANCH

Molecular, Cellular and Nutritional Endocrinology Branch

SECTION

Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The modulation of insulin-stimulated glucose transport activity in rat adipose cells by ligands for receptors (R) that mediate stimulation (R_s; lipolytic) or inhibition (R_i; antilipolytic) of adenylate cyclase has been examined. The results suggest that 1) R_i- and R_s-mediated effects on glucose transport are independent of changes in cAMP, 2) these cAMP-independent effects are mediated by GTP-binding proteins, N_i and N_s, and 3) R_i and R_s ligands modulate the intrinsic activity of the glucose transporter in the plasma membrane. The mechanism of modulation of insulin-stimulated glucose transport activity in isolated rat adipose cells by lipolytic and antilipolytic agents has been further examined by measuring glucose transport activity in plasma membranes. The data indicate that modifications of glucose transport activity produced by lipolytic and antilipolytic agents in intact adipose cells can be fully retained in plasma membranes isolated under appropriate conditions, further supporting the concept that the effects of these agents occur through a modification of glucose transporter intrinsic activity. The effects of β-adrenergic stimulation and different analogues of cAMP on insulin-stimulated IGF-II binding have also been studied. The results indicate that β-adrenergic stimulation and high levels of cAMP markedly impair both sensitivity and responsiveness to insulin suggesting an antagonistic effect on insulin's signalling mechanism. Furthermore, adenosine appears to exert a potent modulating effect through N_i, while activation of phosphodiesterase by insulin appears to play a crucial role for the expression of insulin action under conditions of elevated cAMP levels. The counterregulatory action of catecholamines on insulin-stimulated glucose transport and its relation to glucose transporter phosphorylation have been studied in isolated rat adipose cells. The results suggest that the phosphorylation state of the glucose transporter does not appear to be involved in either signalling glucose transporter translocation or triggering changes in glucose transporter intrinsic activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 55014-04 MCNE

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action with Fasting/Refeeding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	MCNEB, NIDDK
Others:	S. W. Cushman	Chief, EDMNS	MCNEB, NIDDK
	I. A. Simpson	Visiting Scientist	MCNEB, NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Molecular, Cellular and Nutritional Endocrinology Branch

SECTION
Experimental Diabetes, Metabolism and Nutrition Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rapid alterations in glucose transport and metabolism have been shown in rat adipose cells after fasting and refeeding. The mechanism for this was examined in rats fasted for 48 h and sacrificed ± 6 d of refeeding. The results suggest that insulin resistance at the glucose transport level induced by fasting is due to a depletion of intracellular glucose transporters. In contrast, the hyper-responsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters and may also involve modulation of glucose transporter intrinsic activity.

PHS 6040 (Rev. 1/84)

GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF STRUCTURAL BIOLOGY
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

I. BIOLOGY OF COMPLEX CARBOHYDRATES

Cell surface carbohydrates change during development suggesting that they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant, and at various stages of development. To obtain cell-specific monoclonal antibodies, mice and rats have been immunized with various cell types in many laboratories. Some of the antibodies derived from hybridized spleen cells of the immunized animals that have an apparent specificity for certain cells and developmental stages are directed against carbohydrates. We have elucidated the structure of over 100 of these carbohydrate antigens. The antibodies are being used to study changes in cell surface carbohydrates during development in hopes of providing insights into the functions of glycoconjugates. Some are also being evaluated for their possible usefulness in serum tests for cystic fibrosis and various cancers. For example, antibody CC3C195 detects elevated levels of mucin in serum from many patients with cancer of the colon and pancreas, or with cystic fibrosis. The antibody binds to the sialylated human Le(a) blood group antigen like the previously reported antibody 19.9. However, unlike 19.9, CC3C195 also binds to the Le(a) antigen itself. This broader specificity may make CC3C195 more useful for diagnosis than 19.9.

.....Drs. V. Ginsburg, D. Roberts, S. Fukuta, S. Argyle

II. METABOLISM AND ROLE OF POLYSACCHARIDE SULFATES

The discovery of a novel sulfatase of unusual specificity and the synthesis of isomeric glucosamine sulfates of known structure have led to the discovery that heparin contains a unique 3-O sulfated glucosamine residue which is essential for its role as an anticoagulant. The enzyme has been partially purified from human urine.

Many polyanions, including heparin, induce allosteric changes in hemoglobin which markedly affect its solubility. In a study of allosteric effects of polyanions of controlled size, highly sulfated trehalose and stachyose have been prepared. These compounds bind with high affinity to hemoglobin-S and strongly decrease its affinity for oxygen. Studies of the effects of these and other highly sulfated sugars on the solubility of hemoglobin-S are being carried out.

.....Dr. I. G. Leder

III. EXPRESSION AND FUNCTION OF BACTERIAL CELL

The polysaccharide, or O-antigen (O-Ag), portion of lipopolysaccharide (LPS) of Salmonellae plays a crucial role in the killing of these bacteria by the host humoral system. Specifically, the O-Ag structure directly effects the rate and extent of deposition of the complement component C3b on the cell surface by affecting the initial amount of C3b deposited and by influencing the subsequent interaction of this C3b with factor b. O-Ag structure does not influence the inactivation of bound C3b.

Similarly, the O-Ag size and density on the surface of Salmonellae montevideo cells is responsible for the extent of killing of the cells by normal human serum. Survival in serum was associated with LPS that contained O-Ag side chains of at least 4-5 subunits in length and with about 20% of the LPS cores being substituted with O-Ag side chains of length more than 14 subunits. It is proposed that the O-Ag functions to provide serum resistance by sterically hindering access of the C5b-9 complex to the cell membrane.

E. coli cells grown in the presence of 5 mM sodium salicylate become phenotypically resistant to a variety of antibiotics. This resistance begins within 5 minutes after the addition of salicylate and does not involve marked alterations in the pattern of protein or LPS isolated from the outer membrane. We have developed an assay to show that the salicylate-induced drug resistance is due to a 75-80% decrease in the permeability of the outer membrane.

.....Drs. J. Foulds, V. Jiminez, S. Stickley

IV. STRUCTURE AND FUNCTION OF COMPLEX CARBOHYDRATES

During differentiation and oncogenic transformation the structures of complex carbohydrates in the cell change. Many monoclonal antibodies which detect differentiation or cancer-associated antigens are directed against these carbohydrates. Recently, more cancer-associated carbohydrate antigens were characterized. Antibody MOV2 binds the Le(a) oligosachride, whereas antibody ONC-M26 binds the SLe(x) heptasacharide. The latter antibody also strongly binds a novel disialylated Le(x) glycolipid. Another antibody, MOV15, detects difucosylated type 2 chain oligosacharides (Le(y)-active) on mucins elevated in the serum of cancer patients. Other antibodies are also useful for studying the function of specific carbohydrate sequences. For example, antibody LeoMel3 binds strongly to ganglioside GD2 and with lesser affinity to gangliosides GT3, GD3 and GQlb. This antibody binds melanoma cells and specifically blocks their killing by anomalous killer (AK) cells but not by classical cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. Thus, human anomalous killer cells may recognize and use these carbohydrate tumor markers as targets to kill melonoma cells. Other carbohydrate antigens may be used to study changes in development and function of specific glycoproteins. For example, the neural cell adhesion molecule, N-CAM, is a transmembrane glycoprotein that mediates adhesion among normal and tumor cells of neuroectodermal origin. Monoclonal antibodies produced against the polysialic acid of the capsular polysaccharides of Menningococcus B bacteria can distinguish the embryonic from the adult form of N-CAM. As the embryo develops into an adult, the length of polysialic acids on N-CAM decreases. Removal of sialic acid increases the adhesion among N-CAM molecules, suggesting that the developmental regulation of these oligosaccharides modulates the function of N-CAM.

.....Dr. J. Magnani

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 57000-22 LSB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biology of Complex Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Victor Ginsburg, Ph.D.	Chief, LSB	LSB	NIDDK
Others:	David D. Roberts, Ph.D.	Staff Fellow	LSB	NIDDK
	Shinji Fukuta, M.D.	Visiting Fellow	LSB	NIDDK
	Susan Argyle, M.D.	Visiting Fellow	LSB	NIDDK

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Structural Biology

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell surface carbohydrates change during development suggesting that they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant, and at various stages of development. To obtain cell-specific monoclonal antibodies, mice and rats have been immunized with various cell types in many laboratories. Some of the antibodies derived from hybridized spleen cells of the immunized animals that have an apparent specificity for certain cells and developmental stages are directed against carbohydrates. We have elucidated the structure of over 100 of these carbohydrate antigens. The antibodies are being used to study changes in cell surface carbohydrates during development in hopes of providing insights into the functions of glycoconjugates. Some are also being evaluated for their possible usefulness in serum tests for cystic fibrosis and various cancers. For example, antibody CC3C195 detects elevated levels of mucin in serum from many patients with cancer of the colon and pancreas, or with cystic fibrosis. The antibody binds to the sialylated human Le(a) blood group antigen like the previously reported antibody 19.9. However, unlike 19.9, CC3C195 also binds to the Le(a) antigen itself. This broader specificity may make CC3C195 more useful for diagnosis than 19.9.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 57001-10 LSB

PERIOD COVERED: October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Metabolism and Role of Polysaccharide Sulfates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Irwin G. Leder	Research Chemist	LSB	NIDDK

COOPERATING UNITS (if any)
Allen Minton, LBP, NIDDK
William Poillon, Center for Sickle Cell Disease, Howard University

LAB/BRANCH
Laboratory of Structural Biology

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The discovery of a novel sulfatase of unusual specificity and the synthesis of isomeric glucosamine sulfates of known structure have led to the discovery that heparin contains a unique 3-O sulfated glucosamine residue which is essential for its role as an anticoagulant. The enzyme has been partially purified from human urine.

Many polyanions, including heparin, induce allosteric changes in hemoglobin which markedly affect its solubility. In a study of allosteric effects of polyanions of controlled size, highly sulfated trehalose and stachyose have been prepared. These compounds bind with high affinity to hemoglobin-S and strongly decrease its affinity for oxygen. Studies of the effects of these and other highly sulfated sugars on the solubility of hemoglobin-S are being carried out.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DK 57002-13 LSB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression and Function of Bacterial Cell Surface Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	John Foulds, Ph.D.	Research Biochemist	LSB	NIDDK
Others:	Victor Jiminez, M.D., M.Sc.	Staff Fellow	LSB	NIDDK
	Susan Stickley, DDS	Guest Researcher	LSB	NIDDK

COOPERATING UNITS *(if any)*
Keith Joiner, LCI, NIAID
Judeh Rosner, LMB, NIDDK

LAB/BRANCH
Laboratory of Structural Biology

SECTION
Section on Membrane Biology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Md. 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.75	2.75	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polysaccharide, or O-antigen (O-Ag), portion of lipopolysaccharide (LPS) of Salmonellae plays a crucial role in the killing of these bacteria by the host humoral system. Specifically, the O-Ag structure directly effects the rate and extent of deposition of the complement component C3b on the cell surface by affecting the initial amount of C3b deposited and by influencing the subsequent interaction of this C3b with factor b. O-Ag structure does not influence the inactivation of bound C3b.

Similarly, the O-Ag size and density on the surface of Salmonellae montevideo cells is responsible for the extent of killing of the cells by normal human serum. Survival in serum was associated with LPS that contained O-Ag side chains of at least 4-5 subunits in length and with about 20% of the LPS cores being substituted with O-Ag side chains of length more than 14 subunits. It is proposed that the O-Ag functions to provide serum resistance by sterically hindering access of the C5b-9 complex to the cell membrane.

E. coli cells grown in the presence of 5 mM sodium salicylate become phenotypically resistant to a variety of antibiotics. This resistance begins within 5 minutes after the addition of salicylate and does not involve marked alterations in the pattern of protein or LPS isolated from the outer membrane. We have developed an assay to show that the salicylate-induced drug resistance is due to a 75-80% decrease in the permeability of the outer membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 57003-01 LSB

PERIOD COVERED: October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Structure and Function of Complex Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John L. Magnani, Ph.D. Research Chemist LSB, NIDDK

COOPERATING UNITS *(if any)*: None

LAB/BRANCH: Laboratory of Structural Biology

SECTION: Section on Biochemistry

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During differentiation and oncogenic transformation the structures of complex carbohydrates in the cell change. Many monoclonal antibodies which detect differentiation or cancer-associated antigens are directed against these carbohydrates. Recently, more cancer-associated carbohydrate antigens were characterized. Antibody MOV2 binds the Le(a) oligosachride, whereas antibody ONC-M26 binds the SLe(x) heptasacharide. The latter antibody also strongly binds a novel disialylated Le(x) glycolipid. Another antibody, MOV15, detects difucosylated type 2 chain oligosacharides (Le(y)-active) on mucins elevated in the serum of cancer patients. Other antibodies are also useful for studying the function of specific carbohydrate sequences. For example, antibody LeoMel3 binds strongly to ganglioside GD2 and with lesser affinity to gangliosides GT3, GD3 and GQ1b. This antibody binds melanoma cells and specifically blocks their killing by anomalous killer (AK) cells but not by classical cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. Thus, human anomalous killer cells may recognize and use these carbohydrate tumor markers as targets to kill melonoma cells. Other carbohydrate antigens may be used to study changes in development and function of specific glycoproteins. For example, the neural cell adhesion molecule, N-CAM, is a transmembrane glycoprotein that mediates adhesion among normal and tumor cells of neuroectodermal origin. Monoclonal antibodies produced against the polysialic acid of the capsular polysaccharides of Menningococcus B bacteria can distinguish the embryonic from the adult form of N-CAM. As the embryo develops into an adult, the length of polysialic acids on N-CAM decreases. Removal of sialic acid increases the adhesion among N-CAM molecules, suggesting that the developmental regulation of these oligosaccharides modulates the function of N-CAM.

PHS 6040 (Rev. 1/84) GPO 914-918

The LMCB now comprises several groups. One group, led by Barrie J. Carter, studies gene regulation in mammalian cell systems and is particularly interested in developing efficient vector systems for delivery of genes into cells. The second group, led by Takami Oka, is generally interested in the endocrine control of differentiation of the mouse mammary gland and has focused on physiological effects of EGF and the molecular biology of various genes which are important in this process. During the past year a third project led by Frank Tietze has been added to LMCB. This project is aimed at understanding the molecular basis of several human genetic defects which result in lysosomal storage diseases. This project is conducted in collaboration with workers in NICHD.

Function of DNA Virus Genomes in Animal Cells

The group led by B. Carter has continued to employ DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a small genome. AAV has also been developed as a eukaryotic expression vector. AAV normally grows in cells only in the presence of a helper virus (either adenovirus or herpesvirus). In the absence of any helper the AAV genome integrates into the cell chromosome. Thus, the AAV vector is useful as a transducing virus for high frequency integration of genes in mammalian cell chromosomes to yield stable expression. This vector also may be useful for therapy. Award of a patent for this vector system is imminent. We are now analyzing intensively the control of gene regulation in AAV vectors in order to maximize the expression of foreign genes introduced into mammalian cells using this vector. We have discovered a complex system of gene regulation mediated by products of the AAV rep gene which are required for replication of AAV DNA but also mediate transcriptional activation and also translational inhibition of some genes. Coding of all these functions in a single gene appears to be unique in eukaryotic systems. We are also studying adenovirus since this is the helper virus for AAV multiplication. This helper relationship is being analyzed. Also, both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus, this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits Ad12 oncogenesis in newborn animals. The mechanism of this inhibition of tumor induction by AAV is being studied at the molecular level in both cell culture and in animal experiments. We are also studying mutations in mouse 3T3 cells which render the cells resistant to malignant transformation by a single oncogene (ras) but allow malignant transformation by two oncogenes (ras, myc) acting in concert.

Hormonal Regulation of Cell Growth and Differentiation

Epidermal growth factor (EGF) is produced in large amounts by the mouse submandibular gland. It is also present in such biological fluids as plasma, milk, urine and saliva. EGF is a potent mitogen for a wide variety of cells in culture but its function in the body needs to be elucidated. Our previous

studies have demonstrated that EGF plays a key role in the development of the mammary gland during pregnancy and mammary tumorigenesis in female mice; in males it serves a role in spermatogenesis by stimulating the meiosis of spermatocytes. We have continued our studies to elucidate the physiological role of EGF by employing a variety of experimental approaches, including radioimmunoassay of EGF in tissues and biological fluids, EGF receptor assay and bioassay of EGF in cell culture. In addition, we have established the useful means of causing EGF deficiency in mice by removal of the submandibular gland and/or administration of anti-EGF antiserum. These procedures, combined with EGF replacement therapy have provided valuable information concerning the function of EGF in the body. Our studies have shown that the concentration of EGF in the submandibular gland and plasma of female mice increases significantly during pregnancy. Attentuation of the rise in EGF by sialoadenectomy and anti-EGF treatment resulted in increased rate of spontaneous abortion, suggesting that EGF is necessary for the normal course of pregnancy. In addition, EGF has been shown to have a physiological role in maintaining the normal structure of the epidermis. Our studies also have revealed that milk contains a high concentration of EGF which serves a physiological function by promoting neonatal eyelid opening.

Lysosomal Transport and Storage Disease

This work is being conducted by Dr. Frank Tietze. Degradation of cellular biopolymers such as proteins and polysaccharides takes place chiefly within the lysosome. The end-products of this degradation, viz., amino acids and monosaccharides, are presumed to exit the lysosome to the cytoplasm, where further metabolism or expulsion to the external medium occurs. To study the process of lysosomal transport, we have developed methods to load lysosomes of various cells with amino acids (e.g., cystine, tyrosine) or with a specific monosaccharide (viz., sialic acid) and to measure their rates of egress from the organelle. Our studies of cystine egress from lysosomes of human polymorphonuclear leukocytes and of tyrosine from cultured rat thyroid cell lysosomes have revealed these processes to be carrier-mediated and stereo-specific. The further demonstration that no egress of cystine could be detected from similarly loaded lysosomes from patients with the inherited disorder cystinosis indicated that this storage disease is due to a congenital defect of a specific lysosomal carrier. Similar studies on the egress of sialic acid from fibroblast lysosomes have suggested strongly that impaired lysosomal transport underlies another lysosomal storage disorder, free sialic acid storage disease. In addition to a carrier system specific for the lysosomal transport of tyrosine, preliminary evidence has indicated that lysosomes from cultured rat thyroid cells also possess a carrier for mono-iodotyrosine an end-product of the lysosomal catabolism of thyroglobulin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DK 57501-11 LMCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of DNA Virus Genomes in Animal Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Barrie J. Carter	Chief, Laboratory of Molecular and Cellular Biology	LMCB:NIDDK
Other:	Ella Mendelson	Visiting Associate	LMCB:NIDDK
	Christeine Lally	Visiting Fellow	LMCB:NIDDK
	James Trempe	I.R.T.A. Fellow	LMCB:NIDDK
	Nor Chejanovsky	Visiting Fellow	LMCB:NIDDK
	Irving Miller	Biologist	LMCB:NIDDK
	Brunhild Redemann	Guest Worker	LMCB:NIDDK

COOPERATING UNITS *(if any)*

V. Nikodem, S. Usala CEB, NIDDK; B. Weintraub, F. Wondisford, MCEB, NIDDK; M.G. Smith, Univ. Otago, New Zealand; J. Tratschin, Univ. Bern, Switzerland; E. Katz, Hebrew Univ., Jerusalem; N. Young, G. Kurtzman, NHLBI

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK:NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are employing DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of a human parvovirus, adeno-associated virus (AAV) since this virus has only a small genome. AAV has also been developed as a eukaryotic expression vector. AAV normally grows in cells only in the presence of a helper virus (either adenovirus or herpesvirus). In the absence of any helper, the AAV genome integrates into the cell chromosome. Thus, the AAV vector is useful as a transducing virus for high frequency integration of genes into mammalian cell chromosomes to yield stable expression. This vector also may be useful for gene therapy. We are now analyzing intensely the control of gene regulation in AAV vectors in order to maximize the expression of foreign genes introduced into mammalian cells using this vector. We have discovered a complex system of gene regulation mediated by products of the AAV rep gene which are required for replication of AAV DNA but also mediate transcriptional activation and also translational inhibition of some genes. Coding of all these functions in a single gene appears to be unique in eukaryotic systems. We are studying also adenovirus which is a more complex genome. Adenovirus is the helper virus for AAV multiplication. This helper relationship is being analyzed. Also, both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits Ad12 oncogenesis in newborn animals. Thus, AAV inhibits tumor induction. The mechanism of this inhibition of tumor induction is being studied at the molecular level in both cell culture and in animal experiments. We are also studying mutations in mouse 3T3 cells which render the cells resistant to malignant transformation by a single oncogene (ras) but allow malignant transformation by two oncogenes (ras, myc) acting in concert.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK57502-14 LMCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormonal Regulation of Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Oka, T.,	Senior Investigator,	LMCB, NIDDK
Others:	Borellini, F.	Visiting Fellow	LMCB, NIDDK
	Kasayama, S.	Visiting Fellow	LMCB, NIDDK (since May, 1987)
	Tsutsumi, A.	Guest Worker	LMCB, NIDDK (until June, 1987)
	Tsutsumi, O.	Visiting Fellow	LMCB, NIDDK (until June, 1987)
	Perry, J.W.	Biologist	LMCB, NIDDK
	Yoshimura, M.	Visiting Fellow	LMCB, NIDDK

COOPERATING UNITS *(if any)*

Dr. Charles Edwards, LCBG, NIDDK
Dr. G. Mezzetti, University of Modena, Italy
Dr. Y. Kubota, DB, NCI

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

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.)*

Epidermal growth factor (EGF) is produced in large amounts by the mouse submandibular gland. It is also present in such biological fluids as plasma, milk, urine and saliva. EGF is a potent mitogen for a wide variety of cells in culture but its function in the body needs to be elucidated. Our previous studies have demonstrated that EGF plays a key role in the development of the mammary gland during pregnancy and mammary tumorigenesis in female mice; in males it serves a role in spermatogenesis by stimulating the meiosis of spermatocytes. We have continued our studies to elucidate the physiological role of EGF by employing a variety of experimental approaches, including radioimmunoassay of EGF in tissues and biological fluids, EGF receptor assay and bioassay of EGF in cell culture. In addition, we have established the useful means of causing EGF deficiency in mice by removal of the submandibular gland and/or administration of anti-EGF antiserum. These procedures, combined with EGF replacement therapy have provided valuable information concerning the function of EGF in the body. Our studies have shown that the concentration of EGF in the submandibular gland and plasma of female mice increases significantly during pregnancy. Attentuation of the rise in EGF by sialoadenectomy and anti-EGF treatment resulted in increased rate of spontaneous abortion, suggesting that EGF is necessary for the normal course of pregnancy. In addition, EGF has been shown to have a physiological role in maintaining the normal structure of the epidermis. Our studies also have revealed that milk contains a high concentration of EGF which serves a physiological function by promoting neonatal eyelid opening.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK57503-14 LMCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

(formerly Z01 DK1800213 LBM)

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lysosomal Transport and Storage Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Tietze, F. Research Chemist, LMCB, NIDDK

COOPERATING UNITS *(if any)*

Gahl, William A. Research Chemist, Section on Human Biochemical and Developmental Genetics, NICHD

LAB/BRANCH

Laboratory of Molecular and Cellular Biology

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Degradation of cellular biopolymers such as proteins and polysaccharides takes place chiefly within the lysosome. The end-products of this degradation, viz., amino acids and monosaccharides, are presumed to exit the lysosome to the cytoplasm, where further metabolism or expulsion to the external medium occurs. To study the process of lysosomal transport, we have developed methods to load lysosomes of various cells with amino acids (e.g., cystine, tyrosine) or with a specific monosaccharide (viz., sialic acid) and to measure their rates of egress from the organelle. Our studies of cystine egress from lysosomes of human polymorphonuclear leukocytes and of tyrosine from cultured rat thyroid cell lysosomes have revealed these processes to be carrier-ediated and stereospecific. The further demonstration that no egress of cystine could be detected from similarly loaded lysosomes from patients with the inherited disorder cystinosis indicated that this storage disease is due to a congenital defect of a specific lysosomal carrier. Similar studies on the egress of sialic acid from fibroblast lysosomes have suggested strongly that impaired lysosomal transport underlies another lysosomal storage disorder, free sialic acid storage disease. In addition to a carrier system specific for the lysosomal transport of tyrosine, preliminary evidence has indicated that lysosomes from cultured rat thyroid cells also possess a carrier for mono-iodotyrosine an end-product of the lysosomal catabolism of thyroglobulin.

GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF ANALYTICAL CHEMISTRY

NATIONAL INSTITUTE OF DIABETES, DIGESTIVE AND KIDNEY DISEASES

SECTION ON INSTRUMENTATION

SERVICE FUNCTIONS AND INSTRUMENTATION

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH and to a limited extent to personnel of other government agencies. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, infrared, nuclear magnetic resonance, ultraviolet and flame photometry. Assistance in interpretation of spectra is rendered on request. Samples for microanalysis are handled by external contracts. (D.F. Johnson, H.J.C. Yeh, N. Whittaker, W. White).

APPLICATIONS OF NMR IN BIOCHEMICAL AND BIOLOGICAL SYSTEMS

The objective of this project is to develop and apply nuclear magnetic resonance for elucidating molecular structures and for studying the interactions within and between molecules in making contribution to the solution of various chemical problems.

Various nmr techniques have been employed: 1) to study the conformation and atropisomerism of colchicinoids; (2) to study the binding of the D and L isomers of 5-fluoro-tryptophan and the 2,3-dihydro derivatives to tryptophan synthase; (3) to assign configurations of both the tetrahydro epoxide and K-region arene oxide derivatives of polycyclic aromatic hydrocarbons; (4) to elucidate the structures of principal adducts formed from the deoxyguanosine residues of DNA upon reaction in vitro with four configurationally isomeric benzo[c]phenanthrene 3,4-diol-1,2-epoxides; (5) to assign structures of several fluorinated carbohydrates; and (6) to demonstrate the presence and heterogeneity of the phosphate residues on thyroglobulin preparations.

Colchicine, the major antimitotic alkaloid of the meadow saffron Colchicum autumnale, binds specifically and with high affinity to tublin dimer, the major protein subunit of microtubules. It has been suggested that binding of the drug to tublin is accompanied by a conformational change in the colchicine molecule. We have taken an NMR investigation on the conformational isomerism of colchicine and its derivatives. NMR spectral analysis of (-)-(7S)-colchicine, which shows high binding affinity, indicates that ring B of colchicine exists in a boat form with the acetamido group at C(7) chiral center oriented in a pseudoequatorial position. Using the Drieding model, such orientational preference of the C(7) acetamido group in ring B affords the "biaryl" system in a " " conformation, i.e. the dihedral angle between the planes defined by ring A and C is ca. +53° (assuming a plannar C ring). Spectral analysis of the unnatural (+)-(7R)-colchicine, which does not bind to tublin, reveals that the molecule undergoes conformational isomerization by flipping ring B and the "biaryl" system from the "S" to "R" (i.e. the dihedral angle -53°) conformation. Confirmation of the R-S conformational isomerism came from the study of 1-0-acetyl-1-0-demethylcolchicine which showed the rate of the R-S interconversion is in the order of 10^{-5} sec^{-1} at 22°C, corresponding

to a free energy of activation of ca. 22-24 Kcal/mol, which is in agreement with values observed for biaryl that undergo atropisomerization. The position of R-S equilibrium, which is decisive for the binding of drug to tublin, appears in colchicine to be controlled by ring B bearing the acetamido group which prefers a pseudoequatorial orientation. These results suggest that in addition to a structural requirement of the methoxy groups in rings A and C the spatial arrangement of the phenyl-tropolone unit in a "S" conformation is also crucial in the drug-tublin interaction.

P-31 NMR was used to investigate the nature of the phosphate residues on bovine thyroglobulin. The P-31 spectrum of the preparation of 19 S follicular bovine thyroglobulin showed three resonances centered at delta 1.5, 0.0, and -2.5 ppm, indicating a heterogeneity existing within the preparation. These resonances are assigned, respectively, to P-31 resonances (i) of a number of sugar 6-phosphates and/or serine O-phosphates (delta 1.5), (ii) of sugar 1-phosphates and/or inorganic phosphate (delta 0.0), and (iii) of phenyl phosphate, tyrosine-O-phosphate, or phosphodiesters such as present in pApA or the mannan core (delta -2.5). (H. Yeh, D.M. Jerina, A. Brossi, P. Kovac, C.P. Glaudemans, J.M. Sayer, S.K. Balani, D.E. Ryan, P.E. Thomas, A.H. Conney, W. Levin, D.R. Thakker, A.M. Acquaviva, E. Consiglio, S. Formisano, D. Liquoro, A. Gallo, E.W. Miles, L.A. Cohen, R.S. Phillips, H. Yagi, S.K. Agarwal, L.K. Pannell, P. Santisteban, M. De Luca, S. Shifrin, L.D. Kohn, and G.L. Jung).

SECTION ON STEROID HORMONES

NATURE OF STEROID-RECEPTOR INTERACTIONS

The objective of this project is to define the initial, intracellular events of steroid hormone action. These events include steroid binding to the intracellular receptor molecule, "activation" of the receptor-steroid complex to a DNA-binding and nuclear-binding species, and binding of the activated complex to those nuclear acceptor sites involved in the regulation of transcription of specific genes. One approach that has been used to examine these steps is to compare the properties of various steroids in different cell lines. Thus previous studies of the amount of induction of tyrosine aminotransferase (TAT) by several glucocorticoids in two rat hepatoma tissue culture lines (HTC and Fu5-5) revealed that the steroid concentration required for 50% of maximal TAT induction in HTC cells was about 7-fold higher than in Fu5-5 cells. The same difference is now seen in the induction of TAT enzyme and mRNA levels by the stable cAMP derivative, (8-[4-chlorophenylthio]cAMP). These data suggest that a common pre-translational event determines the different sensitivity of TAT induction by glucocorticoids and by cAMP in HTC and Fu5-5 cells.

A second approach has been to examine the properties of th irreversible antiglucocorticoid (and affinity label) dexamethasor 21-mesylate (Dex-Mes) at a molecular level. Dex-Mes specifically reacts with the cysteines of proteins in basic aqueous solutions. Dex- es reaction with the glucocorticoid receptor occurs uniquely at Cys-656 in the steroid binding site. This identification of the first amino acid associated with a biological property of the glucocorticoid receptor should facilitate future structure-function studies. (S.S. Simons, Jr., P.A.

Miller, P. Yen, G. Wasner, F. Sistare, A. Cavanaugh, N. Miller, and H. Oshima).

THE DEVELOPMENT OF METHODS AND MATERIALS FOR THE STUDY OF MEDICAL PROBLEMS:

The objective of this project is to make contributions to the investigation and solution of basic biological and medical problems by the application of chemical, physical and biological methods.

Lethality from cancer frequently results from metastases. Of tumor cells which enter the circulation less than one percent are successful in negotiating the steps of metastais. This vulnerability may afford opportunities for selectively inhibiting the process. The purposes of this study are to increase our knowledge of the biology and chemistry of metastasis and to study the effect of selected biologicals and chemicals on the process. Such studies will also contribute to the investigation of other biological and medical problems.

The stable phenotypes of many malignant cells suggest that there is a genetic basis for cancer. However, the population of malignant cells in a tumor is heterogeneous and the cells vary in metastatic potencies. Epidemiological studies and recent studies with oncogenes suggest that carcinogenesis is a multistep process. The cancer phenotype in metastasis does not represent the initial change in growth control leading to tumorigenicity. If one or more additional genetic events are required for the metastatic phenotype, they may provide approaches to the prevention or treatment of metastasis.

NIH 3T3 cells, non-tumorigenic maxine cells, have been transfected with a pBR322 plasmid bearing the src gene. Transformed cells bearing the src gene were invected into nude mice subcut and iv to test for tumorigenic, metastatic and lung-colonizing capabilities. NIH 3T3 cells were similarly transfected with constructs of the v-abl, c-mos, and v-mos onocogenes. Three cell lines developed with the src gene were tumorigenic but not metastatic and had poor lung colonizing potency. Three lines of transformed cells developed from v-mos transfected cells have also been tested in nude mice. Of cell lines derived from NIH 3T3 cells by transfections with src and v-mos oncogenes and tested in nude mice, all are tumorigenic, not metastatic, and weak in producing lung colonies. These cell lines appear to be good candidates for further transfections to determine whether greater metastatic and/or greater lung-colonizing capabilities can be developed in this way. Positive results might afford new routes for the prevention and treatment of metastases. Other means for interfering with metastasis at the various steps of the process are also being sought using murine tumor cells, such as the Lewis lung carcinoma and PMT fibrosarcoma cells. (C.M. Foltz, L.A. Liotta, R. Muschel).

SECTION ON BIOPHYSICAL HISTOLOGY

A RHODAMINE FOR INTRACELLULAR INJECTION

Studies on neuronal structure in isogenic snails, on the synthesis of a new rhodamine dye, and on the possible use of this dye as an intracellular tracer have been interrupted. This work will be resumed as resources become available.

GENETICS OF NERVE CELL SHAPE

Studies are continuing on the genetics of Biomphalaria glabrata, a snail well suited to examining the genetic determinants of neuron structure and function. We have produced over a hundred highly inbred stains and have found about ten morphologic markers with a simple genetic basis.

PROFESSIONAL PRACTICES OF A GROUP OF BIOMEDICAL SCIENTISTS

Studies are continuing on the professional practices of scientists and on the accuracy of the scientific literature. A study completed several years ago was recently published after obstacles to publication had been overcome. This study showed a high frequency of professional misconduct in a non-randomly chosen group of biomedical scientists. A more recent study bearing on the accuracy of an article in molecular biology has been completed but has not yet been submitted to a scientific journal. (N. Feder and S. Stewart).

SECTION ON BIOMEDICAL CHEMISTRY

APPLICATION OF ORGANIC CHEMISTRY TO THE UNDERSTANDING OF THE INTERFERON-INDUCED 2-5A SYSTEM

3'Deoxyadenosine and xylofuranosyl-adenine substituted analogs of 2-5A have been employed to determine that the 2',5'-phosphodresterase requires a 3'-hydroxyl group in the penultimate nucleotide residue to cleave the 2,5'-linked oligonucleotide. 8-Bromoadenosine sequence-specific analogs of 2-5A have led to the conclusion that changes in the base-sugar torsion angles of the composite nucleotides of 2-5A may modulate binding to and activation of RNase L. Stabilized toward degradation, seco-adenosine 2',5'-oligomers have been prepared and are under current study as activators of RNase L. A bis-3'-deoxyadenosine substituted analog of 2-5A has been prepared and used to verify earlier conclusions that the 3'-hydroxyl residue of the second (from the 5'-terminus) nucleotide unit of 2-5A is most critical for activation of RNase L. (P. Torrence, D. Alster, Y. Kitade, and D. Brozda).

SECTION ON MEDICINAL CHEMISTRY

COLCHICINOLIDS:

Partial synthesis of the therapeutically "less toxic" colchicinoids (-)-2,3-didemethylcolchicine and (-)-cornigerine has been accomplished from natural colchicine with conc. sulfuric acid and with boron tribromide, and conversion of the catechol into cornigerine by methylenation.

Both, allocolchicine and N-acetylcolchinol methylether, prepared by published procedures, showed potent binding to tubulin and their in vivo activity is presently being evaluated. An analysis of all data collected on colchicine and its analogs suggests that spectral changes in solution, and formation of conformationally stable isomers in the cases of 1-acetyldemethylcolchicine, can best be explained by atropisomerism. Phenyl-tropolone isomerism possibly assisted by solvent incorporation, forces the two aromatic rings out of plane.

The importance of the N-acyl groups, increasing potentcy of colchicinoids in tubulin binding assays in vitro considerably, and necessary for in vivo activity in assays measuring inhibition of tumors and antiinflammatory activity, is not fully understood. Reaction of phenolic colchicinoids with dihydrofluorescein diacetate (DADF) gave esters which can be detected by UV and on TLC plates in less than nanomolar quantities. (A. Brossi, R. Dumont, M. Chrzanowska, R. Alonso).

ANTIMALARIALS

Optical isomers of primaquine do not seem to represent superior drugs, suggesting that the antimalarial is involved in oxido-reduction processes not requiring enzymes. The basic side chain is an essential element in primaquine's structure and antimalarial activity is grately suppressed by N-acylation, or degradation to a carboxylic acid. Blue dyes obtained from N-acylprimaquines in chloroform solution in the presence of light, and from 5-hydroxydemethylprimaquine in methylenechloride solution in the presence of light, have been established in structure, The former is a bisquinolinyl methine with a carbon atom coming from chloroform, the other is the o-quinone tautomer of a structure originally proposed by Strother. Oxidation of N-acylprimaquines dispersed on silica gel surface in the presence of light afforded o-quinones. These oxidation products are presently being studied in assays measuring tissue schizontocidal activity and methaemoglobinemia. (A. Brossi, W. Gessner, B. Venugopalan).

MAMMALIAN ALKALOIDS

The most interesting alkaloids of this group are those which are formed in patients suffering from defects or lack in certain enzymes. Their detection by quantitative analysis, would provide useful diagnostic information. We now have synthesized optically active isomers of 3',4'-dideoxynorlaudano-soline-1-carboxylic acid formed in phenylketonurics, and so far only assayed as a racemic mixture.

The compounds were prepared by fragmentation of optically active ureas in refluxing butanol, a method which already has been used to prepare optically active salsolinol-1-carboxylic acids. The optically active compounds are now being compared with each other in several enzymic assays. The configurations are based on x-ray studies of appropriate intermediates. (A. Brossi, M.Chrzanowska).

ANALOGS OF THE ANTIVIRAL DRUG DIDEOXYCYTIDINE (DDC)

Reaction of DDC with acid anhydrides afforded a diacetate, diethoxyacetate and dipivalate. Reaction with methyl isocyanate afforded a N-methylcarbamoyl methylcarbamate. These prodrugs have different physical properties, have probably different bioavailability, and will now be compared with DDC as antiviral agents. (Brossi, Yu).

PHYSOSTIGMINE AND ANALOGS

Reaction of (-)-eseroline, now conveniently prepared from (-)-physostigmine, with a variety of commercially available isocyanates afforded a new series of carbamates to be compared as inhibitors of acetylcholinesterase with physostigmine. N1-norphysostigmine and (-)-eseramine, both alkaloids from Calabar beans were prepared by total synthesis. (A. Brossi, Q. Yu).

BETA-CARBOLINES:

Flazin and substance YS occuring in Japanese sake and soy sauce were synthesized. Several 6-oxygenated beta-carbolines were made from 5-methoxytryptamine and formaldehyde followed by O-demethylation, N-methylation and aromatization. Several 1-methyl substituted analogs were similarly prepared by condensation with acetaldehyde. Inhibition of MAO A and B will be assessed in comparison to harmine and harmaline. (A. Brossi, W. Gessner).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 58000-42LAC

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Service Functions and Instrumentation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.F. Johnson	Chief, Lab. Anal. Chem	LAC/NIDDK
OTHERS:	H.J.C. Yeh	Research Chemist	LAC/NIDDK
	N. Whittaker	Chemist	LAC/NIDDK
	W. White	Biologist	LAC/NIDDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Analytical Chemistry

SECTION

Instrumentation Section

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	4.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH and to a limited extent to personnel of other government agencies. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, infrared, nuclear magnetic resonance, ultraviolet and flame photometry. Assistance in interpretation of spectra is rendered on request. Samples for microanalysis are handled by external contracts.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58001-14LAC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Applications of NMR in Biochemical and Biological Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	H. Yeh	Research Chemist	LAC/NIDDK
Others:	D.M. Jerina	Sec. Chief	LBC/NIDDK
	A. Brossi	Sec. Chief	LC/NIDDK
	P. Kovac	Visiting Assoc.	LC/NIDDK

Cooperating Units
A.M. Acquaviva, E. Consiglio, S. Formisano, D. Liquoro, and A. Gallo (Center of Endocrinology and Oncology Experiments, Italy). R.M.E. Greene, N.D. Sharma, and D.R. Boyd (Queen's University of Belfast, Ireland),

D.R. Thakker (Bureau of Biologic), B.D. Hilton, M.A. Pigott, and A. Dipple (NCI-Frederick Cancer Research Facility). D.E. Ryan, P.E. Thomas, A.H. Conney and W. Levin (Roche Inst. of Molecular Biology)

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Instrumentation

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to develop and apply nuclear magnetic resonance for elucidating molecular structures and for studying the interactions within and between molecules in making contribution to the solution of various chemical problems.

Various nmr techniques have been employed: 1) to study the conformation and atropisomerism of colchicinoids; (2) to study the binding of the D and L isomers of 5-fluoro-tryptophan and the 2,3-dihydro derivatives to tryptophan synthase; (3) to assign configurations of both the tetrahydro epoxide and K-region arene oxide derivatives of polycyclic aromatic hydrocarbons; (4) to elucidate the structures of principal adducts formed from the deoxyguanosine residues of DNA upon reaction in vitro with four configurationally isomeric benzo[c]phenanthrene 3,4-diol-1,2-epoxides; (5) to assign structures of several fluorinated carbohydrates; and (6) to demonstrate the presence and heterogeneity of the phosphate residues on thyroglobulin preparations.

PHS 6040 (Rev. 1/84)

GPO 814-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58002-12LAC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nature of Steroid-Receptor Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	S.S. Simons, Jr.,	Chief, Steroid Hormones Section	LAC/NIDDK
OTHERS:	P.A. Miller	Staff Fellow	LAC/NIDDK
	F.D. Sistare	PRAT Fellow/Staff Fellow	LAC/NIDDK
	P.M. Yen	Intramural NRSA Fellow	LAC/NIDDK
	A. Cavanaugh	Extramural NRSA/PRAT Fellow	LAC/NIDDK
	N.R. Miller	Special Expert	LAC/NIDDK
	H. Oshima	Visiting Fellow	LAC/NIDDK

COOPERATING UNITS *(if any)*
Stewart Rudikoff (NCI)
Howard J. Eisen (NICHHD)

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Steroid Hormones

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	5.1	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 objective of this project is to define the initial, intracellular events of steroid hormone action. These events include steroid binding to the intracellular receptor molecule, "activation" of the receptor-steroid complex to a DNA-binding and nuclear-binding species, and binding of the activated complex to those nuclear acceptor sites involved in the regulation of transcription of specific genes. One approach that has been used to examine these steps is to compare the properties of various steroids in different cell lines. Thus previous studies of the amount of induction of tyrosine aminotransferase (TAT) by several glucocorticoids in two rat hepatoma tissue culture lines (HTC and Fu5-5) revealed that the steroid concentration required for 50% of maximal TAT induction in HTC cells was about 6-fold higher than in Fu5-5 cells. The same difference is now seen in the induction of TAT enzyme and mRNA levels by the stable cAMP derivative, (8-[4-chlorophenylthio]cAMP). These data suggest that a common pre-translational event determines the different sensitivity of TAT induction by glucocorticoids and by cAMP in HTC and Fu5-5 cells.

A second approach has been to examine the properties of the irreversible antiglucocorticoid (and affinity label) dexamethasone 21-mesylate (Dex-Mes) at a molecular level. Dex-Mes specifically reacts with the cysteines of proteins in basic aqueous solutions. Dex-Mes reaction with the glucocorticoid receptor occurs uniquely at Cys-656 in the steroid binding site. This identification of the first amino acid associated with a biological property of the glucocorticoid receptor should facilitate future structure-function studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 DK 58003-14LAC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development of Methods and Materials for the Study of Medical Problems.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Foltz	Research Chemist	LAC/NIDDK
OTHERS:	B. Baer	Chemist	LAC/NIDDK

COOPERATING UNITS *(if any)*
Lance A. Liotta and Ruth Muschel, Pathologists, Laboratory of Pathology, NCI

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Steroid Hormones

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this work is to contribute to the investigation and solution of basic medical problems by the application of chemical, physical and biological methods. This goal is being pursued by studies of the biology and molecular biology of murine tumor cells with emphasis on cancer metastasis. Areas of special interest are organic chemistry, biochemistry, cell biology, tissue culture, cancer biology, cancer chemotherapy and recombinant DNA methodology.

Studies are being conducted to determine whether specific gene products confer on certain tumor cells the properties required for the formation of viable metastases. NIH 3T3 cells have been transfected with constructs of several oncogenes. Transformed cells have been selected and their tumorigenic and metastatic potencies determined by subcutaneous and tail vein injections in nude mice. The correlation of turmorigenic and metastatic potencies with the expression of the oncogene introduced is being determined.

Additional transfections of certain cell lines, e.g., those with tumorigenic but not spontaneous metastatic potency and with or without lung colonizing potency will be performed in an attempt to endow the cells with the properties necessary for spontaneous metastasis. Success in this would increase our knowledge of the genetic requirements for metastasis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58004-20LAC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Histochemistry: Principles, Methods and Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Feder	Medical Officer (Research)	LAC/NIDDK
Others:	W. Stewart	Research Physicist	LAC/NIDDK

COOPERATING UNITS *(if any)*

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Laboratory of Analytical Chemistry

SECTION
Section on Biophysical Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies have continued on the genetics of Biomphalaria glabrata.

Studies are continuing on the professional practices of biomedical scientists and on the accuracy of the scientific literature.

PHS 6040 (Rev. 1/84) GPO 814-018

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 58005-14 LAC
FORMERLY
Z01 DK 19402-14 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interferon Induction and Action. The Antiviral Activity of Nucleoside Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.F. Torrence	Research Chemist LAC/NIDDK
Others:	D. Alster	National Research Service Award Fellow LAC/NIDDK
	Y. Kitade	Visiting Fellow LAC/NIDDK
	D. Brozda	Visiting Fellow LAC/NIDDK

COOPERATING UNITS *(if any)*
FOREIGN: J. L. Imbach, U. Montpellier, France; C. Altona, Univ. Leiden, Netherlands; W. Pfleiderer, U. Konstanz, W. Germany, Domestic: F. Castora, U. of Maryland, Baltimore Co.

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SECTION
Section on Biomedical Chemistry

INSTITUTE AND LOCATION
NIH, NIDDK, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interferon-induced enzyme activities such as the oligo(2' 5') adenylate synthetase, the 67K dalton protein kinase and oligo(2' 5') A phosphodiesterase are investigated with a goal of understanding their role in the action of interferon, the induction of interferon by double-stranded RNA and, perhaps, control of cell growth and differentiation. Analogs of the mediator of interferon action, 2-5A, are synthesized in order to define the relationship between oligonucleotide structure and binding to and activation of the 2-5A dependent endonuclease with the eventual goal of designing useful chemotherapeutic agents based on this system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK 58006-04 LAC
FORMERLY
ZO1 DK 19250-04 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry and Metabolism of Qinghaosu, a Chinese Antimalarial Drug

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Brossi - Visiting Scientist, LAC/NIDDK
Others: L. Dominguez - Guest Scientist Gerpe, University, Spain
H. Yeh LAC/NIDDK

COOPERATING UNITS *(if any)*
Walter Reed Research Institute; P. Buchs, SAPEC, Lugano; Judith Flippen-Anderson, Laboratory of the Structure of Matter, Navy Research Dept.: P. Trigg, SWG-CHEMAL, WHO, Geneva, Switzerland

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A deoxyanalog of the Chinese antimalarial drug qinghaosu = artemisinin and deoxyanalogs of dihydroqinghaosu, arteether and its alfa isomer were prepared as analytical standards. Dihydroqinghaosu and deoxydihydroqinghaosu were characterized as highly fluorescent diacetyldihydrofluorescein esters, converted by exposure to ammonia vapors followed by iodine vapors, into red colored and highly visible dyes. Treatment of arteether and its alfa-isomer with hydrochloric acid in ethanol led to a new ethyl ether isomer with the methyl group at C-11 inverted as established by solid state X-ray diffraction analysis.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DK 58007-03 LAC
FORMERLY
ZO1 DK 19253-03 LC

PERIOD COVERED
October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physostigmine and Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	Q.-S. Yu	Visiting Fellow	LAC/NIDDK

COOPERATING UNITS *(if any)*
J. R. Attack, NIA, LN, NIH; E. X. Albuquerque, University of Maryland, Baltimore; R. Ray, Pharmacology Branch, US Army Medical Research, Aberdeen Proving Ground.

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Laboratory of Analytical Chemistry

SECTION
Section on Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New carbamate analogs of (-)-physostigmine were prepared from (-)-eseroline and various isocyanates, including optically active 1-phenylethyl isocyanates. N-Benzylation of (-)-N1-noreseroline O-methylether prepared by total synthesis afforded N1-benzylnor-physostigmine, after O-demethylation and reaction with N-methyliso-cyanate, and N1-norphysostigmine after catalytic debenzylation over Pd-catalyst in methanol. A larger quantity of (+)- physostigmine was prepared by total synthesis, using for purification of intermediates and end product fumarate and salicylate salts and avoiding column chromatography.

PHS 6040 (Rev. 1/84) GPO 914-818

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 58008-05 LAC
FORMERLY
Z01 DK 19243-05 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pyrrolidine Ant Toxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	W. Gessner	Visiting Associate	LAC/NIDDK
	R. Alonso	Visiting Fellow	LAC/NIDDK

COOPERATING UNITS *(if any)*
Dr. E. Costa, Fidia-Georgetown Research Inst. for Neurosciences; M. Kowalski and M. A. Kaliner, ILCI, NIAID.

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SECTION
Medicinal Chemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 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.)*

Several trans-substituted (±)-2,5-disubstituted pyrrolidines occuring in various ant species were synthesized. These ant alkaloids when tested for release of blue dye deposited in rat tissues had a distinct effect. The Lukes-Sorm dilactam on reaction with alcohols and amines in the presence of acid afforded pyrrolidine-2-one-3-propionic acid esters and amides to be tested in biochemical assays representative for geriatric dissorders such as Altzheimer disease.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 58009-03 LAC
FORMERLY
Z01 DK 19255-03 LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
8-Aminoquinoline Antimalarials

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	W. Gessner	Visiting Associate	LAC/NIDDK
	B. Venugopalan	Guest Scientist	LAC/NIDDK

COOPERATING UNITS *(if any)*
C. D. Hufford, University of Mississippi; I. Landau, Laboratoire de Zoology, CNRS, Paris: C. W. Abell, U. of Texas at Austin.

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structure of two blue dyes obtained from the antimalarial drug primaquine were investigated. N-Acylprimaquines are photooxidized in chloroform solution to diquinolyl methines present in solution as imine salts. This blue dye was correlated by reduction with N-acetyl-methylenebisprimaquine, a microbial metabolite. The second blue dye is conveniantly in prepared from 5-hydroxdemethylprimaquine by photooxidation in sunlight and obtained in 50% yield. Its hydrogen bonded o-quinone structure was established on the basis of spectral data and by chemical reactions. Photooxidation of N-acylprimaquines dispersed on silica gel afforded o-quinones which were isolated and characterized.
Antimalarial screening of these oxidation products of primaquine has been initiated. Optical isomers of primaquine

prepared by published procedures did not show significant differences in an in vitro screening measuring tissue schizontocidal activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 58010-02 LAC
FORMERLY
Z01 DK 19256-02 LC

PERIOD COVERED
October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mammalian Alkaloids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	M. Chrzanowska	Visiting Fellow	LAC/NIDDK
	C. Creveling, Lab. of Bio-Organic Chemistry, NIDDK		

COOPERATING UNITS *(if any)*

J. L. Flippen-Anderson, Naval Research Labs., Dept. of the Navy; W. Abell, College of Pharmacy, University of Texas at Austin,

LAB/BRANCH
~~Laboratory of Analytical Chemistry~~

SECTION
~~Medicinal Chemistry~~

INSTITUTE AND LOCATION
~~NIDDK, NIH~~ Bethesda, Maryland 20892

TOTAL MAN-YEARS: 0.6	PROFESSIONAL: 0.6	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.)*

Mammalian 3',4'-dideoxynorlaudanosoline-1-carboxylic acids were synthesized and obtained as optically pure S-(+)- and R-(-)-hydrobromides. Configuration was established by X-ray analysis of the (+)-rotating hydantoin obtained besides the S-(+)-methylester in the fragmentation of the less polar urea in refluxing butanol. Presence of the compounds in phenyketonurics has been established and enantiospecificity will now be determined.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58011-11 LAC
FORMERLY
Z01 DK 19216-11-LC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure-Activity Relationships of Colchicinoids Based on Tubulin Binding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	R. Dumont	Visiting Fellow	LAC/NIDDK
	M. Chrzanowska	Visiting Fellow	LAC/NIDDK
	R. Alonso	Visiting Fellow	LAC/NIDDK
	J. Wolff		NIDDK
	H. Yeh		LAC/NIDDK

COOPERATING UNITS *(if any)*
E. Hame., NCI, NIH; F. Quinn and M. Suffness, NCI;
C. F. Chignell, NIEH, Research Triangle Park

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Section on Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

2,3-Didemethylcolchicine and cornigerine showed potent antiinflammatory activity. Allocolchicine and N-acetylcolchinol methylether have potent affinity for tubulin. Reaction of 2-demethylcolchicine with DADF gave a fluorescent ester converted into red dyes and useful to study metabolic conversion of colchicinoids into phenolic congeners. Formation of conformationally stable isomers of colchicine is seen when the methoxy group at C-1 is replaced with bulkier acetoxy- or benzoyloxy groups. The importance of the methoxy group at C-10 in colchicine was demonstrated with a synthesis of pure colchicide and several colchicide analogs, which all showed poor affinity to tubulin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58012-01 LAC

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antiviral Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Brossi	Visiting Scientist	LAC/NIDDK
Others:	Q.-S. Yu	Visiting Fellow	LAC/NIDDK

COOPERATING UNITS *(if any)*
Dr. S. Broder, Clinical Oncology Program, NCI, Bethesda

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Medicinal Chemistry Section

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Reaction of 2',3'-dideoxycytidine (DDC) with acid anhydrides afforded the following analogs: Diacetate, diethoxyacetate, dipivalate. Reaction with methyl isocyanate afforded a N-methylcarbamoylmethylcarbamate. These prodrugs of DDC will be compared with DDC in antiviral screening.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58013-01 LAC

PERIOD COVERED
October 1, 1986 to September 31, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Beta-Carbolines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Brossi Visiting Scientist LAC/NIDDK
Others: W. Gessner Visiting Associate LAC/NIDDK
P. Skolnick, Laboratory of Neurosciences, NIDDK

COOPERATING UNITS *(if any)*
C. W. Abell, Department of Pharmacology, University of Texas at Austin

LAB/BRANCH
Laboratory of Analytical Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Beta carbolines flazin and substance YS occuring in Japanese sake and soy sauce have been prepared by efficient syntheses. Pictet-Spengler reaction of 5-methoxytryptamine with formaldehyde afforded a number of 6-oxygenated beta-carbolines related to serotonine. Reaction with acetaldehyde similarly afforded several 1-methyl substituted beta-carbolines. Optically active tetrahydroharmines were prepared from harmaline by reduction with sodium borohydride, urea fragmentation followed by purification as camphorsulfonates.

PHS 6040 (Rev. 1/84) GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF NEUROSCIENCE

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SECTION ON DRUG DESIGN AND SYNTHESIS

Design and Synthesis of Drugs Acting on Central and Peripheral Tissues

Opiates and Opiate Receptors. Opiate receptors are saturable, high affinity, stereospecific binding sites which are localized in well defined regions of the mammalian central nervous system and peripheral tissues. These receptors mediate the effects of narcotics, their antagonists, and the endogenous opioid peptides. The opiate receptor-endorphin system consists of the well defined mu, delta and kappa receptor subtypes and numerous endorphins, and it appears likely to control many aspects of the human perception of pain, pleasure, mood, as well as certain aspects of immune function. In order to gain further insight into the fundamental questions of receptor subtype structure and function, we are currently pursuing several lines of investigation. The design, synthesis and tritiation of SUPERFIT, a new affinity label based on the extremely potent opiate agonist cis-(+)-methylfentanyl, has made possible labeling and purification to homogeneity of the delta receptor subtype from NG-108-15 neuroblastoma x glioma hybrid cells [in collaboration with W. Klee (NIMH) and associates.] Data suggestive of a modulatory role of delta receptors on analgesia produced by the mu agonist morphine has been obtained by study of subanalgesic doses of highly selective mu and delta receptor selective drugs. In other investigations of the function of opiate receptors, positron emission tomography in primates has been done [in collaboration with S. Larson and associates (Clinical Center)], and studies in conscious human subjects will begin in the very near future. Such studies in humans are expected to provide insight into the function of the opiate receptor-endorphin system in mormal and diseased states. The NIH Opiate Total Syntheses has continued to be utilized to provide compound as research tools for study of the opiate receptor-endorphin and system and the mechanism of cough. Recent synthetic results in the N-nor-4,5-epoxymorphinan series have greatly extended the versatility of this route to both the natural and unnatural opiate series. Based on results by others with dextromethorphan and dextrorphan, it seems quite likely that unnatural opiate enantiomers available by the NIH synthesis will prove to enhance the action of nerve growth factor, show anticonvulsant and antischemic activity. We anticipate increasing our effort in the unnatural opiate area because of these exciting possibilities in the treatment of epilipsy and prevention of neurological deficit after stroke or hypoxic insult. In collaboration with E. L. May and associates (Medical College of Virginia), a new structural type of narcotic

antagonist has been identified which is nearly equipotent with nalorphine and can precipitate a complete abstinence syndrome in morphine addicted monkeys. The possibility that endogenous peptides function as neuroendrocrine modulators of immune responses in vivo has been suggested by the observation of naloxone reversible immunosuppression by natural, but not unnatural morphine.

Studies with Ligands for the Phencyclidine Receptor. As part of our study of the phencyclidine (PCP) receptor, we have examined the in vitro and in vivo activity of a number of PCP-like compounds, and the action of our affinity ligand, metaphit. A PCP-like compound, dexoxadrol, is unique among the four stereoisomers of this structure in that it, but not its stereoisomeric relatives, binds to PCP receptors with an affinity similar to PCP, and shows PCP-like activity in monkeys trained to discriminate ketamine, as well as in other behavioral paradigms. In order to determine the optimum chirality necessary for receptor binding and PCP-like activity in this series, the absolute configuration of dexoxadrol was determined by single crystal x-ray analysis as 4S, 6S. Based on these and other considerations, receptor active theoretical conformations of PCP and dexoxadrol have been proposed. The exquisite sensitivity of the PCP receptor to slight molecular modification of a substrate was indicated by the synthesis of molecules based on a recently discovered new class of PCP-like molecules, the 2-methyl-3,3-diphenyl-3-propanolamines (2-MDP), as well as dexoxadrol. We have discovered that even slight changes in the molecular pattern acceptable to the receptor leads to abrupt and dramatic changes in the ability of these molecules to interact with the PCP receptor.

Studies with Metaphit, An Affinity Ligand for the Phencyclidine Receptor. Metaphit (1-[3-isothocyanotophenyl) cyclohexzl] peperidine) has been used in vivo and in vitro in a number of studies to discern the effect of its irreversible interaction with the phencyclidine receptor, the relationship of the interaction of the PCP receptor with the receptor associated with the N-methyl-D-aspartate type of excitatory amino acid, and other receptors. An example of the latter is its interaction with cocaine and methylphenidate binding sites. Experiments in collaboration with M. E. A. Reith and associates (N. S. Kline Institute for Psychiatric Research, N.Y.) suggest that metaphit antagonizes cocaine-induced locomotor stimulation by acylating cocaine binding sites on dopaminergic nerve terminals. It also resembled its parent compound (PCP) in its ability to inhibit the binding of the stimulant drug [^{3}H]threo-(±)-methylphenidate to crude synaptosomal membranes from rat striatal tissue [in collaboration with M. Schweri (Mercer University School of Medicine, GA). Unlike PCP, metaphit appears to inhibit binding of the radiolabeled stimulant in an irreversible manner, as the degree of inhibition of binding of the stimulant does not diminish when the metaphit-treated tissue is subjected to repeated washings before determination of the binding of

[^{3}H]threo-(±)-methylphenidate. These data suggest that metaphit may be a useful tool in the study of the molecular basis of stimulant action. The pharmacological specificity of the electrophysiological effects of PCP on cerebellar Purkinje neurons was also examined in collaboration with B. Hoffer and associates (University of Colorado Medical School). Metaphit selectively antagonizes the indirect catecholamine agonist effects of PCP on cerebellar Purkinje neurons. In a study using hippocampal neurons, it was found that two mechanisms of action of PCP were possible. The mechanisms of action of PCP in the hippocampus may be localized in part to different cell types. While PCP has been found to specifically antagonize NMDA effects in spinal cord, PCP actions in cerebellum do not appear to involve an NMDA mechanism. PCP-like compounds have multiple mechanisms of action in the CNS. Kappa opioid receptors may mediate the actions of the benzomorphan or morphinan classes of analgesics that are sensitive to high doses of naloxone, while the psychotomimetic side effects of these compounds are probably mediated through PCP receptors. Metaphit-insensitive, lower-dose effects of PCP derivatives, such as catatonia or anesthesia, may be mediated by the antagonism of central NMDA receptor mechanisms. The PCP-like catalepsy in pigeons that is produced by the excitatory amino acid antagonists, [in collaboration with J. Woods and associates (University of Michigan Medical School)] may result from a reduction of excitatory neurotransmission at NMDA-preferring receptors that are distinct from, but related to, PCP receptors.

Non-project Activity. Dr. Kenner Rice was a recipient of the 1987 Research Achievement Award in Pharmaceutical and Medicinal Chemistry, a national award given on March 29, 1987 by the American Pharmaceutical Society (APhA) at the annual meeting of the APhA held in Chicago, IL. Dr. Rice was also elected as a member of the Executive Committee of the Organic Chemistry Division of the American Chemical Society. Dr. Arthur E. Jacobson was reappointed as Chairman of the Drug Testing Program of the Committee on Problems of Drug Dependence for 1987-1988.

SECTION ON NEUROBIOLOGY

Studies on the benzodiazepine/GABA receptor chloride channel complex

Physiological role and implications in disease. The benzodiazepine/GABA receptor chloride channel complex ("supramolecular complex") is an oligomeric group of proteins that contain recognition sites (receptors) for many psychopharmacological agents including benzodiazepines, ß-carbolines, barbiturates, and "cage" convulsants (such as picrotoxin). The proteins comprising this complex act in concert to regulate the activity of chloride channels that are controlled ("gated) by γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter of the vertebrate central nervous system. Recent

studies have focussed on the physiological role and regulation of the various components of this supramolecular complex. It has been demonstrated that a brief, ambient temperature swim stress elicits robust changes in the apparent affinity and number of binding sites for [^{35}S]t-butylbicyclophosphorothionate (TBPS), a "cage" convulsant that binds to GABA-gated chloride channels. This effect has been shown to occur only in specific regions of the central nervous system and is mimicked by occupation of benzodiazepine receptors in vitro. The rapidity of stress-induced changes in GABA-gated chloride channels was underscored by the finding of a left/right asymmetry in the number and apparent affinity of [^{35}S]TBPS binding sites in rat cerebral cortex. That this asymmetry was functional rather than anatomical was demonstrated by the observation that swim stress abolished this asymmetry which indicates that changes in GABA-gated chloride channels between cerebral hemispheres can be reliably measured in <8 sec, prior to complete activation of the hypothalamic-pituitary-adrenal axis. These findings suggest the supramolecular complex may play an essential role in the regulation of stress and/or anxiety. Perturbation of the supramolecular complex may also be involved in a number of pathophysiological conditions. Thus, it has been shown that treatment of rodents with benzodiazepine receptor inverse agonists (previously shown to produce a syndrome resembling anxiety in rodents and primates, including man) will result in a significant reduction in both mitogen stimulated T-cell proliferation and cytotoxic T-cell (CTL) activity. This effect has been shown to be long lived, dose-dependent, and blocked by administration of the specific benzodiazepine receptor antagonist Ro 15-1788. These findings suggest that the supramolecular complex may play an important role in modulation of immune function. The supramolecular complex has also been shown to be involved in the pathogenesis of an experimental model of hepatic encephalopathy (HE). These studies (in collaboration with A. Jones and S. Gammal, Liver Unit, NIDDK) have demonstrated an increased sensitivity to both GABAmimetics and benzodiazepines in cerebellar Purkinje neurons of rabbits with HE due to galactosamine-induced fulminant hepatic failure. Further, qualitative differences in the response to benzodiazepine receptor antagonists (e.g. Ro 15-1788) were found in Purkinje neurons from vehicle and galactosamine treated rabbits. These findings are consistent with the involvement of the supramolecular complex in the pathogenesis of HE, and provide a potential explanation for the reported efficacy of benzodiazepine receptor antagonists in ameliorating this syndrome.

Studies on "peripheral" benzodiazepine receptors

Receptor regulation and purification Recognition sites for benzodiazepines have been described in extraneuronal tissues. These sites, referred to as "peripheral-type" benzodiazepine receptors (PBR) are physically and pharmacologically distinct from benzodiazepine receptors that comprise the supramolecular complex. Thus, PBR are not associated with a GABA-gated chloride

channel, and are not regulated by GABA or barbiturates. There is evidence, however, that anions can regulate the apparent affinity of compounds (e.g. 4′-chlorodiazepam, Ro 5-4864) that bind to PBR. A high correlation was obtained between the permeability of ions (relative to chloride) and their efficacies to inhibit [^{3}H]Ro 5-4864 binding in kidney membranes. Moreover, the density of PBR in kidney can be modulated by administration of diuretics that are ion transport/exchange inhibitors. The synthesis of an irreversible derivative of Ro 5-4864 (AHN-086) (synthesized by A.H. Newman, Section on Drug Design and Synthesis, LN) has permitted the purification of PBR from kidney membranes. Biochemical and physiological studies are in progress to elucidate the physiological role of these sites.

Non-project activities. Dr. Phil Skolnick received a Grass Travelling Scientist Award, and was the recipient of a Wellcome Professorship in Pharmacology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DK 58,501-01 LNS
formerly
Z01 DK 31103-09 LBC2

PERIOD COVERED
October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Receptors for Neurotransmitters and Drugs in Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: P. Skolnick Chief LN,NIDDK

Others: R.O. Trullas Visiting Fellow LN, NIDDK J.C. Marvizon Guest Worker
H.W. Lueddens Guest Worker LN,NIDDK E.J. Moody LN,NIDDK Guest Worker
T.D. McIntyre Staff Fellow LN,NIDDK A.H. Lewin LN,NIDDK Guest Worker
A.S. Basile Staff Fellow LN,NIDDK R.H. Havunjian LN,NIDDK Guest Worker
G.E. Evoniuk Guest Worker LN,NIDDK P.K. Arora LN,NIDDK Guest Worker
J.M. Petitto Guest Worker LN,NIDDK E. Kempner LPB, NIDDK
E.A. Jones DDB, NIDDK, S. Gammal DDB, NIDDK.

COOPERATING UNITS *(if any)*
S. Paul, J. Crawley, R. Drugan, P. Sudzak, CNB, NIMH, N. Ostrowski, CPB, NIMH, E. Hanna, LMG, NICHD; D. Klein, LDN, NICHD; J. Cook, M. Trudell, T. Hagen, M. Allen, Univ. Wisconsin; J. Barrett, USUHS

LAB/BRANCH
Laboratory of Neuroscience

SECTION
Section on Neurobiology

INSTITUTE AND LOCATION
NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	8	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.)*

High affinity, stereospecific recognition sites (receptors) for neurotransmitters, neuromodulators, and many clinically useful drugs have been identified in both peripheral tissues and the central nervous system. The interaction of a neurotransmitter, neuromodulator or drug with a specific recognition site initiates a series of events (for example, the opening of an ion channel or activation of an enzyme) resulting in either a physiological/behavioral response (in the case of a neurotransmitter or neuromodulator) or pharmacological effect in the case of a drug). Furthermore, the presence of recognition sites for synthetic compounds suggests that endogenous substances may also be present that mimic (or antagonize) the effects of exogenously applied substances. Studies are in progress to characterize "recognition-effector" systems, and to link novel recognition sites to effector systems under study include: a) the benzodiazepine/GABA receptor chloride ionophore complex; b) the glycine-gated chloride ionophore; c) "peripheral-type benzodiazepine receptors (in both peripheral tissues and the central nervous system); d) receptors for central stimulants (e.g. amphetamine, methylphenidate); e) recognition sites for hallucinogens (phencyclidine), and f) recognition sites for compounds that regulate voltage -sensitive calcium channels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58,502-01LNS

PERIOD COVERED: October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Design, Synthesis and Drugs Acting on Central and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Rice	Section Chief	LN-NIDDK	P. Skolnick
	A. E. Jacobson	Research Chemist	LN-NIDDK	Laboratory Chief
OTHERS:	A. Newman	Guest Worker	LN-NIDDK	
	B. deCosta	Fogarty Fellow	LN-NIDDK	
	C.-H. Kim	Staff Fellow	LN-NIDDK	
	N. A. Grayson	IRTA	LN-NIDDK	
	J. A. Monn	NIH Special Volunteer	LN-NIDDK	
	M. Mattson	Biologist	LN-NIDDK	

COOPERATING UNITS *(if any)* J. V. Silverton, V. Manganiello (NIHLB), W. Klee, R. Rothman, A. Pert, C. Pert, M. Herkenham (NIMH), R. Weber (NINCDS), T. Mercado (NIAID), J. Daly, A. Brossi (NIDDK) C. Klee (NCI), J. Holaday (WRAIR), E. May, L. Harris, M. Aceto (Med. Col. VA), J. Woods (U. MI), W. Bowen (Brown U.).

LAB/BRANCH: Laboratory of Neuroscience

SECTION: Drug Design and Synthesis

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	5.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Formerly projects: Z01 DK 19226-08LC, Z01 DK 19202-13LC, Z01 DK 19237-05LC, Z01 AN 19239-04LC, Z01 DK 19246-04LC Z01 DK 19233-07LC, and Z01 DK 19235-05LC.

Recent recognition that the effects of diverse classes of pharmacological agents are mediated by discrete cell receptors, which normally function in concert with their endogenous ligands, has presented unique opportunities for dramatic advances in the understanding of many central and peripheral regulatory systems in animals and humans. Optimal exploitation of such opportunities requires design and synthesis of highly selective drugs as probes for study of these systems and a collaborative, multidisciplinary approach in such studies. Elucidation of the exact molecular structure and mechanism of action of these endogenous ligand-receptor systems, and the molecular mechanism of action of endogenous ligand-mimetic drugs and their antagonists will provide new opportunities for therapeutic intervention in many clinical situations and for the design of superior drugs, particularly for disorders which are now little-understood. Studies in progress are currently aimed at identification, purification, and elucidation of the structure and function of opiate, benzodiazepine and phencyclidine receptor subpopulations in the overall modulation of the CNS. These studies require synthesis of new receptor ligands for several lines of investigation utilizing: (1) irreversible ligands specific for receptor subpopulations; (2) high specific activity radiolabeled ligands for autoradiographic visualization of receptors; (3) conformationally restricted analogs of potent receptor ligands as topological probes; (4) position emission transaxial tomographic visualization of receptor patterns in living brains. Studies designed to identify a clinically useful antagonist of PCP are underway. Synthesis of previously inaccessible unnatural opiate enantiomers using the recently developed NIH Opiate Total Synthesis is also in progress with the goals of identification of new pharmacological agents and effects of opiates mediated through nonclassical opiate receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 58,503-01 LNS

PERIOD COVERED: October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design, Synthesis, and Evaluation of Medicinal Agents and Research Tools.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. E. Jacobson	Research Chemist	LN-NIDDK
Others:	K. C. Rice	Section Chief	LN-NIDDK
	A. Thurkauf	Guest Worker	LN-NIDDK
	M. V. Mattson	Biologist	LN-NIDDK

COOPERATING UNITS (if any): J. H. Woods, W. Koek, G. D. Winger, F. Medzhidradsky, C. B. Smith, (U. Mich); M. D. Aceto, E. R. Bowman, L. S. Harris, E. L. May, G. Patrick, L. Powell, (Med. Col. VA); R. Griffiths, N. Ator, J. Brady, (Johns Hopkins), (continued on pg 2)

LAB/BRANCH: Laboratory of Neuroscience

SECTION: Drug Design and Synthesis

INSTITUTE AND LOCATION: NIDDK, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This new project was formerly projects Z01 DK 19200-36LC, Z01 DK 19236-05LC and Z01DK 19241-05LC.

The design and synthesis of drugs, and their evaluation in several paradigms, is essential for the elucidation of the function and mechanism of action of CNS receptors. Phencyclidine receptors have recently been implicated as allosteric sites which interact with glutamate receptors of the N-methyl-D-aspartate (NMDA) type. NMDA is an excitatory amino acid which acts to open certain ion channels. Some phencyclidine-like compounds have recently been reported to exert a protective effect against neuronal degeneration in ischaemia; they supposedly act as antagonists against the depolarizing action of NMDA in animal brain. Studies are in progress towards the design and synthesis of new phencyclidine-like compounds which might exert protection against neuronal degeneration. One of our affinity ligands for the phencyclidine receptor, metaphit, has been used in several studies to study the function of the phencyclidine receptor and its interaction with the excitatory amino acids. The design and synthesis of new affinity ligands for the phencyclidine receptor is in progress. The affinity of various opioids for their receptors, their pharmacological study in a number of assays, as well as the pharmacological evaluation of compounds from the stimulant and depressant classes of CNS agents, has been studied under the auspices of the Committee on Problems of Drug Dependence with the goal of identifying new types of ligands which interact with specific receptors as agonists or antagonists, and which might have fewer undesirable side-effects. Data from these studies have been utilized by the Expert Committee of the World Health Organization who have been examining the scheduling of these drugs under the Psychotropic Substances Convention.

ANNUAL REPORT OF PHOENIX EPIDEMIOLOGY AND CLINICAL RESEARCH BRANCH
NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

Summary of Activities

Overview

The Phoenix Epidemiology and Clinical Research Branch performs research in diabetes, obesity, lipoprotein metabolism, digestive and kidney diseases. Many of the branch activities are focused on conditions which are particularly prevalent among the American Indians of the southwest. The majority of the investigations are related to diseases which are particularly prevalent among the Pima Indian population of the Gila River Indian Community in Arizona. This population has the highest reported frequency of non-insulin dependent diabetes in the world, and a very high prevalence of obesity and gallbladder disease. The specific vascular and metabolic complications of diabetes, including kidney disease of diabetes mellitus, as well as the other typical vascular complications, such as retinopathy and neuropathy, are associated with the diabetes. As a result of longitudinal population based epidemiological studies of the Gila River Indian Community conducted for the past 22 years, a comprehensive investigation of the occurrence and determinants of these diseases and their complications is being made. As a result of the long-term followup it is possible to examine the genetic and environmental determinants of these diseases in the population, and because of the availability of a large clinical research facility, to perform detailed metabolic studies to further elucidate the pathogenesis and mechanisms of these diseases and related complications.

The branch activities, therefore, fall into two main categories: epidemiological studies of the total population and clinical research studies directed at well-defined subsets of the population. The major goals of the branch relate to the elucidation of the determinants and pathogenesis of the diseases which occur in high frequency in the population.

The epidemiological studies are conducted by the Diabetes and Arthritis Epidemiology Section, the clinical and laboratory studies are performed in the Clinical Diabetes and Nutrition Section of the branch. The Biostatistics and Data Management Section, the third component of the branch, is responsible for maintaining the extensive data bases that the ongoing longitudinal studies have generated and providing support to investigators in the other sections in matters relating to data processing, data management and biostatistics.

During the past year the Diabetes and Arthritis Epidemiology Section has continued to assemble an increasing body of evidence that suggests that the susceptibility to non-insulin dependent diabetes is determined by a major gene. As a result of these findings, in conjunction with work by others in extending the known location of genetic markers in the human genome, it should now be possible to identify the locus of the gene responsible for susceptibility to non-insulin dependent diabetes. Because of the duration of the epidemiological studies and the well-defined and closed nature of the Gila River Indian Community, large kindreds are available in which to search for the gene locus by linkage analysis.

Knowledge of other determinants of diabetes in the population has been expanded by detailed analyses of the contributions of maternal and paternal diabetes to the occurrence of the disease among offspring. Increasing evidence of

strong intrauterine environmental effect on the expression of non-insulin dependent diabetes has been assembled. In addition, further efforts to clarify the possible roles of physical activity and diet on the expression of non-insulin dependent diabetes have been initiated.

Epidemiological studies of the determinants of some of the specific complications of non-insulin dependent diabetes have been continued. As the epidemiological studies have been continued for a period of over 20 years, it is now possible to examine the incidence of complications that have developed in persons in whom it was possible to document the onset of non-insulin dependent diabetes. Because the examinations of the population have been carried out at approximately 2-yearly intervals, the time sequence of the appearance of factors such as increasing blood pressure, increasing degrees of proteinuria, and changes in other variables such as weight, biochemical determinants, and treatment, the contribution of such factors to the development of the end-stage complications of the disease has been examined. During the past year particular emphasis has focused on the determinants of end-stage renal disease and the occurrence of amputation as late complications of the disease.

Evidence of the contribution of the severity of diabetes and the potential importance of blood pressure in the development and pathogenesis of diabetic nephropathy have been obtained. Because this complication have a high incidence in the population, a specific initiative to extend knowledge of the determinants and natural history of the kidney disease in diabetes mellitus has been started. This initiative will involve further epidemiological investigations as well as detailed studies of the natural history of kidney disease in diabetes mellitus and exploration of possible therapeutic modalities to delay or prevent the onset of this complication.

The Diabetes and Arthritis Epidemiology Section has performed additional analyses of the longitudinal data relating to the occurrence of rheumatoid arthritis, and the significance of rheumatoid factor in the development of rheumatoid arthritis in the population.

The Clinical Diabetes and Nutrition Section is conducting a major longitudinal study of the pathogenetic mechanisms which relate to the development of non-insulin dependent diabetes. A prospective clinical research study is being conducted among subjects who, by virtue of a known family history of diabetes and the presence of obesity, could be predicted to have a high risk of developing the disease. This investigation has indicated that insulin resistance (or impaired insulin-mediated glucose disposal) appears to be a critical component in the pathogenesis of impaired glucose tolerance and non-insulin dependent diabetes.

The section has also made major contributions to the knowledge of mechanisms underlying impaired insulin-mediated glucose disposal. These studies have shown that impaired insulin-mediated glucose disposal is a familial characteristic, suggesting that the genetic factors predisposing to diabetes susceptibility may operate through this mechanism, and that impaired insulin mediated glucose disposal is associated with different distributions of fiber types in muscle. The importance of glucose disposal in muscle in this process has been suggested by demonstrating evidence of abnormal processing of glucose in biopsies of human muscle. These studies strongly suggest that a post-binding defect in insulin action during the intracellular processing of glucose may be the underlying defect in abnormal insulin-mediated glucose disposal.

The Clinical Diabetes and Nutrition Section has also made major contributions to the understanding of obesity. The availability of an environmental chamber has permitted detailed studies and analysis of the components of energy balance. These have shown that there are large individual variations in 24-hour energy expenditure and that there is familial aggregation of both resting and 24-hour energy expenditure. The studies have also indicated that the amount of spontaneous physical activity, which also shows familial aggregation, is a major contributor to 24 hour energy expenditure. Prospective studies of energy balance have indicated that there is a relationship between energy expenditure and weight gain such that those with a lower than expected energy expenditure are those most likely to gain weight.

Further studies of lipoprotein metabolism, with particular emphasis on difference between those with and without non-insulin dependent diabetes, have pointed to differential rates of metabolism of lipoprotein subfractions. The contributions of obesity and diabetes to these disturbances has been examined by performing perturbations of diet and control of hyperglycemia to further understanding of the complex factors that are responsible for abnormal lipoprotein metabolism in diabetes. The purpose of these investigations has been to attempt to understand possible determinants of the increased rates of cardiovascular disease that occur in non-insulin dependent diabetes.

Other Professional Activities

Members of the branch play an active role in both national and international activities in relation to obesity and diabetes. All of the tenured staff have been invited to give invited lectures and seminars to national and international organizations, as well as participating in the membership of editorial boards of major scientific journals.

Members of the staff have been active in providing assistance to the National Center for Health Statistics in the planning of major national studies. Several have served as office holders in major scientific societies during the past year, as well as being asked to serve as advisors and consultants for other organizations and universities.

The specific scientific contributions of the individual sections are described below.

Diabetes and Arthritis Epidemiology Section

The Diabetes and Arthritis Epidemiology Section has continued its 22-year longitudinal studies of genetic and environmental risk factors for diabetes and vascular complications of diabetes in the Pima Indians, as well as continuing data collection for epidemiological studies of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, cholelithiasis, mortality rates and causes of death. The long follow-up provided by this study is yielding increasingly valuable data on late complications of diabetes and the transmission of risk factors for diabetes from one generation to the next. Susceptibility to diabetes appears to be transmitted by a major autosomal gene, the location of which will be sought by means of linkage analysis. Maternal diabetes in pregnancy also increases the risk

of diabetes at an early age in the offspring, an effect which has no simple genetic explanation, but appears to be due to metabolic abnormalities in utero. New factors which will be studied include physical activity and diet.

Diabetes complications are being documented and their risk factors determined. Major complications of diabetes under study are nephropathy, end stage renal disease, retinopathy, peripheral vascular disease, and periodontal disease, all of which are related to the duration and severity of hyperglycemia and appear to develop at least as frequently in this population with non-insulin dependent diabetes as in people with insulin-dependent diabetes.

The adverse affects of diabetes in pregnancy, both for the mother and offspring, are being studied. Offspring of diabetic women are at increased risk of obesity and glucose intolerance during childhood and young adulthood. The study of the causes of these adverse outcomes is being expanded by measuring proinsulin, insulin, and glycosylated hemoglobin in cord blood and glycosylated hemoglobin in maternal blood. The diabetes status of the fathers of these offspring is also determined so that familial and genetic factors can be evaluated as well as the effects of the intrauterine environment.

The incidence and prevalence of rheumatoid arthritis (RA) is determined using clinical, serologic and x-ray data. RA is diagnosed by ARA criteria as modified for use in population studies. Age-specific incidence rates are appreciably higher than reported in Caucasian and Japanese populations. Rates generally increased with age, but age-specific incidence rates were stable over the 20 year period.

Collection and analysis of tissues form autopsied subjects and of data on cause of death on all deceased subjects continue, although the collection of autopsy data is seriously hampered due to the low autopsy rate.

Section staff continue to be active in medical research and education beyond the projects described here. Staff collaborate extensively in research projects conducted by the Clinical Diabetes and Nutrition Section of the branch and the National Center for Health Statistics as well as contributing to national and international meetings and workshops.

Clinical Diabetes and Nutrition Section

Research in the Clinical Diabetes and Nutrition Section is in three major areas: non-insulin dependent diabetes mellitus, obesity and energy balance, and lipoprotein metabolism.

Pathogenesis of Non-Insulin Dependent Diabetes Mellitus

The Pima Indians of the Gila River Indian Community have the highest reported prevalence and incidence of non-insulin dependent diabetes mellitus (NIDDM). Diabetes occurs more often in the offspring of diabetic mothers than in the offspring of nondiabetic parents. The major effort of the Clinical Diabetes and Nutrition Section continues to be a longitudinal study of the offspring of these two parental types. Obese offspring of diabetic mothers and of nondiabetic parents are admitted yearly to the clinical unit for detailed studies of many

aspects of in vivo and in vitro carbohydrate metabolism. Based on previously collected epidemiologic data, approximately 30% of the obese offspring of diabetic mothers will develop NIDDM within five years, such that it will be possible to carefully document the sequence of metabolic events that occurs as subjects with normal glucose tolerance develop diabetes. This will enable determination of which metabolic parameter, specifically an abnormality of insulin secretion or of insulin action, is predictive of the development of NIDDM.

To date nearly 300 subjects have entered this study, approximately 175 subjects have been studied a second time, 100 subjects have been studied three times, 50 subjects have been studied four times, and 15 subjects have been studied as many as five times. Analyses of cross sectional data from this large data base have lead to a number of important observations. Most recently the major observation has been that insulin resistance is a strongly familial characteristic, even after adjusting for familial differences in degree of obesity and physical fitness. Thus it appears that insulin resistance may have a genetic or a congenital basis, and that these insulin resistant and insulin sensitive families can form the nucleus for future genetic studies.

We have begun analyses of the longitudinal data. Results demonstrate that the development of impaired glucose tolerance is associated with increasing insulin resistance and increasing plasma insulin concentrations. This suggested that the development of impaired glucose tolerance is not due to insulin deficiency, but rather to worsening of insulin resistance. Eighteen of the 175 subjects who have returned for at least one examination have developed diabetes mellitus. Preliminary analyses suggest that insulin resistance is predictive of the development of this disease, independent of the degree of obesity. Other predictors included insulin resistance at the level of the adipocytes as well as hyperinsulinemia.

Because of the key role that insulin resistance appears to play in the development of NIDDM some of our attention has been focused on the mechanism of this insulin resistance. Since skeletal muscle is the site of uptake for much of an intravenous or oral glucose load, we have examined the relationship between skeletal muscle morphology and insulin action. A significant correlation was observed between capillary density in skeletal muscle and in vivo insulin action. This suggests that increased diffusion distances created by muscle cell enlargement are part of the mechanism by which obesity is associated with insulin resistance. Since muscle fiber type appears to be genetically determined, this may provide a mechanism for the familial dependence of in vivo insulin action. We have also found a relationship between muscle fiber type and body fat distribution which suggests that central obesity may be part of a more generalized syndrome.

Indirect calorimetric data collected during the measurement of insulin action in vivo have demonstrated that non-oxidative pathways are the major routes for glucose disposal during insulin infusion. In vitro studies to determine the possible mechanisms of non-oxidative insulin-mediated glucose disposal have shown that the rate of insulin-mediated glucose storage is well correlated with insulin activation of the human skeletal muscle enzyme glycogen synthase. Subjects with low glucose storage rates were found to have reduced glycogen synthase activity and reduced rates of glycogen synthesis to levels one quarter of those observed in subjects with high glucose storage rates. These data suggest that glycogen synthase may even be a rate limiting step for insulin action in vivo in insulin resistant man. To explore this further we have measured the levels of glucose-6-

phosphate (G6P) in insulin resistant and sensitive subjects. Elevated G6P was observed in muscle from insulin resistant subjects, suggesting that a significant reduction in their glucose metabolism occurs after G6P, and that abnormal regulation of glycogen synthase is not secondary to abnormal glucose transport. Further support for a regulatory site at the level of glycogen synthesis comes from the observation that there is reduced glycogen synthase phosphatase activity in human muscle tissue isolated from individuals with reduced insulin-mediated glucose disposal.

The exploration of possible sites of rate limiting steps of insulin action has also been extended to in vivo studies, where euglycemic clamps and forearm perfusions were performed at four different glucose concentrations and four different insulin levels. At the lowest insulin levels the Michaelis constants for glucose disposal in the whole body and across the forearm were compatible with the constants determined in vitro for the glucose transport system. At higher insulin levels however, the apparent Ks increased significantly both in whole body and across the forearm, suggesting that there might be a shift in the rate limiting step from glucose transport to some step beyond transport. Measurements of glycogen synthase activity in biopsies from these subjects showed that activation of glycogen synthase by insulin was highly correlated with stimulation of whole body glucose disposal, especially at high rates of glucose disposal when glucose storage rather than oxidation predominates. Glucose had no effect of glycogen synthase activity. These results support the concept that post transport processes, possibly at the level of glycogen synthesis, determine the rate of glucose disposal during insulin stimulation in normal subjects.

Obesity and Energy Balance

It has been proposed that the high prevalence of obesity among the Pima Indians may be due to genetic selection of a "thrifty" gene. To determine if differences in metabolic rate exist between Indians and Caucasians, or among individuals within the Indian population, we have measured rates of energy expenditure in the resting condition using indirect calorimetry. In addition, a human respiratory chamber has been constructed which allows measurements of rates of energy expenditure over 24-hour periods. In addition, the chamber can be used to measure substrate utilization and therefore substrate balance and short-term changes in body composition.

Data collected on approximately 150 siblings showed that both resting and 24-hour energy expenditure are familial traits, independent of family differences in metabolic size, age and sex. It appears, therefore, that there are individual variations in 24-hour energy expenditure. Another source of individual variation is in spontaneous physical activity or "fidgeting" which represents 100-800 kcal per day of the total energy expenditure depending on the level of activity and body weight. This degree of spontaneous physical activity within the chamber is also a familial characteristic, and thus, may predispose to obesity in the less active subjects.

A prospective study was performed on 122 subjects who underwent measures of resting metabolic rate and another 71 who underwent measures of 24-hour energy expenditure to determine the relationship between energy expenditure and weight gain. The data indicate that a low metabolic rate for a given age, sex, weight, and body composition is a predictor of significant weight gain in the population.

Thus a low rate of energy expenditure may contribute to the development of obesity, and weight gain may be a regulatory mechanism compensating for a reduced resting metabolic rate.

Lipoprotein Metabolism

Despite their obesity, sedentary life style and high fat diets, nondiabetic Pimas have surprisingly low plasma cholesterol concentrations and low prevalence of cardiovascular disease. Lipoprotein composition and metabolism are therefore being investigated in the Pima Indians to further understand control of lipoprotein metabolism and also how lipoproteins are related to obesity, insulin resistance and diabetes. To examine these issues in detail a number of methods have been developed including kinetic methods for the simultaneous study of VLDL, IDL and LDL metabolism, kinetic methods for the short-term study of VLDL triglyceride turnover, and in vitro systems for the evaluation of the binding properties of LDL.

We have recently examined the coordination of VLDL apoB and VLDL triglyceride metabolism in 53 subjects who underwent simultaneous metabolic studies. Increases in VLDL apoB and triglyceride production were coordinated in obese subjects, on the other hand a dissociation of VLDL apoB and triglyceride metabolism is observed in NIDDM, where VLDL triglyceride production appears to be simulated through increasing plasma free fatty acids or glucose. Studies on factors relating to HDL suggested that HDL concentrations are related to both sex hormones and also to measures of insulin resistance, and that men and women may differ with respect to the relative importance to these various factors which control HDL.

Studies on lipoprotein metabolism in diabetics have focused both on metabolic differences in diabetics and on the influence of various modes of therapy. Diabetics have large TG rich VLDL which are cleared more slowly; a lower proportion of these is converted to LDL. LDL concentrations in diabetics are influenced by two opposing changes, increased direct removal of VLDL but decrease in clearance. Improvement of glycemic control with oral sulfonylurea therapy is followed by reversal of abnormalities of VLDL composition, VLDL triglyceride production, lipase activities, and HDL subfractions. Transfer of non-insulin dependent diabetics to a high carbohydrate low saturated fat diet is associated with decreases in LDL and no changes in HDL. In most diabetics there were no elevations in VLDL on the high carbohydrate diet. Metabolic studies indicate that the LDL decreases on the low fat diet are due to increased clearance. The data suggest that both sulfonylurea therapy and high carbohydrate low fat diets can result in less atherogenic lipoproteins in many non-insulin dependent diabetics.

Finally, we have performed free fatty acid turnover measurements combined with lipid oxidation rate to investigate mechanisms of regulation of fatty acid metabolism and their inter-relationship with carbohydrate utilization and lipoprotein production. Our studies have shown that free fatty mobilization is less per gram of fat in obese subjects; we also showed that a significant component of fatty turnover is non-oxidative disposal. We have recently conducted studies of free fatty turnover under two metabolic perturbations, the infusion of propranolol and the transfer of individuals to a low fat high carbohydrate diet. The data suggest that the sympathetic nervous system plays a role in regulating the output of fatty acids from adipose stores, and that obese individuals differ

in their sensitivity to this regulation. Finally, the non-oxidative component of fatty acid metabolism was lower in situations where lipid oxidation was increased; this may reflect inhibition of glyceride synthesis in sites other than adipose tissue.

Biostatistics and Data Management Section

The BDMS has been engaged in data management and support activities for the research operations of the PECRB as a whole. A major activity is supporting the updating, error checking, storage, and retrieval of datasets for the extensive epidemiological study, as well as assistance with many smaller datasets from the studies conducted by CDNS. This has included work on the Phoenix Clinical Information System (PCIS) which is being programmed by the Data Management Branch, DCRT, from documentation provided by the BDMS. Progress is being made toward completing this system, with much BDMS staff time during this year being spent on verification of the accuracy of data and of the data checking routines of PCIS.

Staff of the section have also been involved in analysis and organization of other complex data systems, such as that supporting the indirect calorimeter (chamber) in CDNS. Other major activities include support of laboratory instrument-computer interfacing, and extensive support of personal computers.

Consulting on statistical methods and data management for specific scientific projects has been the other major activity of the Section, which has resulted in increased productivity of the direct research activities of the Branch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69000-22 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	R.G. Nelson	Staff Fellow	DAES, NIDDK
	D. Mott	Research Chemist	CDNS, NIDDK
	W.J. Butler	Computer Systems Analysist	BDMS, NIDDK
	H.R. Baird	Mathematician	BDMS, NIDDK

COOPERATING UNITS *(if any)*

Biostat. and Data Management Sec., Clinical Diabetes and Nutrition Sec., PECRB, NIDDK; Indian Health Service; Ariz. State U.; State U. of New York at Buffalo; U. of Missouri, Columbia; U. of New Mexico, Albuquerque.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.0	2.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to identify the determinants of non-insulin-dependent diabetes, various types of arthritis, and gallbladder disease, and elucidate the natural history of the diseases and their complications. Genetic and environmental risk factors for non-insulin-dependent diabetes and vascular complications of diabetes have been studied in the Pima Indians. The residents of the study area, currently numbering approximately 5000 people, have participated in a longitudinal population study for the last 22 years, allowing observations of the natural history of diabetes mellitus and its complications. Risk factors for obesity, hypertension, and cholelithiasis are also studied, along with the relationships of these diseases to diabetes. The genetics of diabetes is studied by means of family studies and relationships of genetic markers to disease. The roles of obesity, serum insulin concentrations, impaired glucose tolerance, and diabetes in relatives are assessed. Assessments of occupational and leisure-time physical activity and diet will be added. Risk factors for the major complications of diabetes, retinopathy, nephropathy, coronary artery disease, and peripheral vascular disease are determined by longitudinal followup of diabetic subjects. Methods for ascertainment of these complications include fundus photography, measurement of urine albumin and serum creatinine concentrations, electrocardiography, and documentation of lower extremity amputations secondary to gangrene. The severity of abnormality of glucose homeostasis is assessed be measurement of plasma glucose and serum insulin concentrations during glucose tolerance tests and measurement of glycosylated hemoglobin. The effects of these diseases and their complications on mortality are assessed by analysis of death certificates and examination of medical records for the classification of causes of death. This study has shown diabetes to be a serious and common disease with both genetic and environmental components. The complications, especially when involving the kidney, are an important cause of increased mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 60001-18 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications and Outcome of Diabetic and Prediabetic Pregnancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
Others:	P.H. Bennett	Chief	PECRB, NIDDK
	H.R. Baird	Mathematician	BDMS, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	R.G. Nelson	Staff Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service; Biostatistics and Data Management Section, PECRB. Karolinska Institut, Stockholm, Sweden (Foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.6	1.8	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diabetes during pregnancy affects the pregnant woman and her offspring. Toxemia and cesarean section are both more common in women with diabetes during pregnancy, and malformations, macrosomia, prematurity and perinatal mortality are more common in infants of diabetic mothers. Also, offspring of diabetic women are at an increased risk of developing obesity and glucose intolerance during childhood and young adulthood. The purposes of the project are to identify diabetes and impaired glucose tolerance during pregnancy in women in the Gila River Indian Community, to determine the effects of abnormal glucose tolerance on outcome of the pregnancy, and to determine long term prognosis for the women and their offspring. The diabetes status of every woman is determined at two-yearly intervals and during the third trimester of each pregnancy. The characteristics of women who have diabetes or impaired glucose tolerance during the pregnancy are compared to those of women who are normal during the pregnancy and subsequently develop diabetes and to those of women who remain normal. At birth, cord blood is collected for determination of glycosylated fetal hemoglobin and proinsulin, and maternal blood is also collected for glycosylated hemoglobin. These women and their offspring are followed at two-yearly intervals. It has been previously reported that offspring of diabetic women have more diabetes and more obesity than offspring of nondiabetic and prediabetic women. Diabetes mellitus during pregnancy in Pima Indian women results in offspring who have a higher prevalence of diabetes, 45% at age 20-24 years, than offspring of nondiabetic women, 1.4%, or offspring of prediabetic women, defined as women who developed diabetes only subsequent to the pregnancy, 8.6%. These differences persist after taking into account paternal diabetes, the age of onset of diabetes in the parents, and the offspring's relative weight for height. The findings suggest that the intrauterine environment is an important determinant of the development of diabetes, and that its effect is in addition to those of genetic factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69003-14 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle Capillary Basement Membrane Thickness Prior to Onset of Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	C. Bogardus	Chief	CDNS, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK

COOPERATING UNITS *(if any)*

Department of Biology, Case Western Reserve Univ., Cleveland, Ohio (N.B. Rushforth) and Department of Medicine, Univ. of California, San Francisco, California (M.D. Siperstein)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several metabolic and morphologic changes have been claimed to precede the onset of diabetes, including changes in the pattern and quantity of insulin secretion and alteration in the thickness of capillary basement membranes. This study will determine if muscle capillary basement membrane thickening is a characteristic of the prediabetic state, and if so whether the thickening is present many years before the onset of diabetes, and therefore can be considered a prediabetic marker, or whether it develops pari passu with metabolic abnormalities that occur prior to the onset of diabetic hyperglycemia. Some 12 years ago, Pima Indians with two diabetic parents, and with neither patient diabetic received oral and intravemous glucose tolerance tests, and a biopsy of the quadriceps muscle from which quantitative determinations of the thickness of the capillary basement membrane were made. The same subjects are being reexamined to determine if there was differential thickening of the muscle capillary basement membrane with increasing age in those with diabetic parents compared to those without. The results will help to determine if vascular lesions at the level of the capillary are present before hyperglycemia develops.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69006-17 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charactars or less. Title must fit on one line between the bordars.)*

Gila River Indian Community Autopsy and Mortality Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	W.C. Knowler	Chief	DAES, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	M.L. Sievers	Guest Researcher	DAES, NIDDK

COOPERATING UNITS *(if any)*

Pathology Department, Phoenix Indian Medical Center, Phoenix, Arizona;

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

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.)*

The postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects. Medical records are reviewed for the determination of cause of death and for the occurrence of certain serious diseases or complications of diabetes.

The purpose of the study is to relate the outcome and cause of death to events or risk factors measured in life among Pima Indian residents of the Gila River Indian Community, particularly in relation to diabetes, cardiovascular diseases and gallbladder disease. Post-mortem examinations are obtained whenever possible on members of the Gila River Indian Community to ascertain conditions present at the time of death and ascertain cause of death as precisely as possible. In addition, death certificates and all available medical records pertaining to the subjects are obtained and reviewed in a standardized way for evidence of the complications of diabetes, vascular disease, neoplasms and other conditions, which may have been recognized prior to death. The records of the occurrence of such conditions, together with conditions recognized at autopsy, are used to determine the causes of death and incidence of complications associated with diabetes and other conditions identified initially during life by the longitudinal epidemiologic studies in the population.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69009-22 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Arthritis and Rheumatism in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Others:	D.J. Pettitt	Assistant Chief	DAES, NIDDK
	W.C. Knowler	Chief	DAES, NIDDK
	K.R. Slaine	Staff Fellow	DAES, NIDDK
	R. Nelson	Staff Fellow	DAES, NIDDK
	H.R. Baird	Mathematician	BDMS, NIDDK
	A. Del Puente	Visiting Fellow	DAES, NIDDK

COOPERATING UNITS *(if any)*

Biostatistics and Data Management Section, PECRB

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.9	1.3	1.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of osteoarthrosis, rheumatoid arthritis and ankylosing spondylitis are being determined by means of clinical, radiographic and serological examinations carried out prospectively at two-yearly intervals among adults of the Gila River Indian Community (Pima Indians) in Arizona, in conjunction with epidemiological studies of diabetes in the same community. The purpose of this investigation is to ascertain the determinants of these diseases in the population, and to identify factors which alter the natural history of progression of the disease. Host factors such as age, sex, and various gene markers including HLA and Gm, together with various potential environmental determinants, such as obesity and evidence of exposure to infectious agents, will be studied prospectively to determine their relationship to the development of these diseases. Longitudinal data have now been collected over a 22year period in the population and represent a unique data set for epidemiological studies of arthritis.

The longitudinal data have been used to determine the incidence of rheumatoid arthritis in the population and comparisons have been made with similar data from other ethnic groups and countries. The prognostic significance of rheumatoid factor as a predictive factor for the development of rheumatoid arthritis has been determined. The relationship between the risk of developing rheumatoid arthritis and prognostic significance of rheumatoid factor in relation to HLA-DR typing is being examined

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69013-06 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes, Arthritis and Other Metabolic Diseases in the Pacific Region

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P.H. Bennett Chief PECRB, NIDDK

COOPERATING UNITS *(if any)*

WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus (P. Zimmet) (Foreign)
South Pacific Commission (R. Taylor) (Foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	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 prevalence of diabetes and its associated vascular complications have been assessed in several Pacific Island populations, including Polynesians and Melanesians, living in traditional ways as well as in urbanized communities.

In general, much higher prevalences of diabetes and the associated complications were found in the urbanized populations, and attempts to determine the reasons for these differences are being pursued. Increased obesity, reduced physical activity, changes in dietary composition and intake appear to contribute to these differences in frequency, but genetic factors also are likely important in determining the frequency of the diabetes, itself, and possibly the type and frequency of associated complications. Identification of the relative importance of environmental determinants of diabetes is a prerequisite to formulating preventive measures for this disease in developing countries.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69014-10 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lipoprotein Composition and Metabolism in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS, NIDDK
Others:	W. Abbott	Visiting Scientist	CDNS, NIDDK
	B. Swinburn	Visiting Associate	CDNS, NIDDK
	G. Ruotolo	Visiting Fellow	CDNS, NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, Dept of Med,Dept of Molecular Genetics, Univ. of TX, SW Med. School, Dallas, TX; Dept. of Med., Univ. of Hiroshima Med. School (foreign); Dept. of Med.,Univ. CA, San Diego, Med. School, La Jolla, CA

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.6	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipoprotein composition and metabolism in Pima Indians are being investigated to understand control of lipoprotein metabolism and how lipoproteins are related to obesity, insulin resistance and CVD. Kinetic methods have been developed for the simultaneous study of VLDL, IDL, and LDL metabolism, for the short-term study of VLDL triglyceride metabolism, and for the in vitro evaluation of binding properties of LDL. Studies of the relationships between lipoproteins and insulin-mediated glucose disposal indicated that there is a significant positive correlation between VLDL and insulin resistance, and a significant negative correlation between HDL concentrations and insulin resistance. These correlations were stronger in men than in women and were independent of each other. When the relationships between VLDL triglyceride and VLDL apoB metabolism were examined, the data suggested that in obese subjects hyperinsulinemia or insulin resistance induces overproduction of both VLDL apoB and triglyceride, whereas in diabetes VLDL triglyceride production is stimulated through increases in plasma free fatty acids or glucose. Obesity in the Pimas had a stronger influence on HDL in women, and the changes in HDL in obese women were associated with decreases in plasma estradiol and increases in hepatic lipase activities. Our studies on HDL suggest that HDL concentrations are related to both sex hormones and also to measures of insulin resistance and that men and women may differ with respect to the relative importance of these factors.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69015-05 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-sectional and longitudinal study of "prediabetes" in the Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS/NIDDK
Others:	B.V. Howard	Associate Chief	CDNS/NIDDK
	D.M. Mott	Research Chemist	CDNS/NIDDK
Visiting Associates:	H. Yki-Jarvinen, S. Lillioja, B. Nyomba, B. Swinburn		CDNS/NIDDK
Visiting Fellows:	L. Christin, F. Zurlo, D. Freymond, G. Ruotolo, J. Zawadzki		CDNS/NIDDK
Visiting Scientist:	W. Abbott		CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, AZ, 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.4	4.6	2.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indians have the highest reported prevalence and incidence rate of non-insulin dependent diabetes mellitus (NIDDM) of any population in the world. In this study we are longitudinally examining Pima Indians to determine the sequence of metabolic events that occur with the development of NIDDM to isolate the metabolic predictor of this debilitating disease. Studies are done on our clinical research ward on obese, adult, Pima Indians, who are at the highest risk of the development of NIDDM. They are admitted to the clinical research ward for 7-10 days to characterize their insulin and carbohydrate metabolism both in vivo and in vitro. The results to date have shown that the Pima Indians are hyperinsulinemic compared to Caucasians, as well as more insulin resistant. The insulin resistance cannot be attributed solely to degree of obesity. A substantial portion of the variability in insulin resistance is familial in nature, and therefore likely to be genetically determined. The development of impaired glucose tolerance is associated with a worsening of insulin resistance and not any deficit in insulin secretion. Insulin resistance also appears to be a predictor of the development of the disease among the 18 subjects who have developed diabetes out of the initial 165 who entered the study. The insulin resistance was predictive of the disease independent of the effect of obesity.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69016-04 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Rate-limiting Steps For Insulin-mediated Glucose Uptake in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief, CDNS/NIDDK	
OTHERS:	H. Yki-Jarvinen	Visiting Associate	CDNS/NIDDK
	D. Mott	Research Chemist	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service
Sandoz Research Institute, E. Hanover, NJ

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	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.)*

We previously demonstrated that under hyperinsulinemic or hyperglycemic conditions glucose transport is not rate-limiting for glucose disposal in the rat hindlimb. In the present study we evaluated whether a similar limitation of the capacity of muscle to metabolize glucose exists in man. We also measured the relative contributions of increasing glucose and insulin concentrations on glycogen synthase activity, an enzyme potentially important in determining rates of glucose storage into muscle. A total of 88 separate studies were performed in 22 Caucasian males. Glucose uptake rates were measured at 4 different glucose concentrations at 4 insulin levels. At the lowest insulin level, the Michaelis constants (Ks) for glucose disposal in whole body and across the forearm were compatible with a Ks determined in vitro for the transport system. At higher insulin levels, the apparent Ks increased significantly in whole body and across the forearm. We interpret the apparent increase of Ks by insulin to reflect a shift in the rate-limiting step from glucose transport to some step beyond transport. Activation of glycogen synthase by insulin was highly correlated with stimulation of whole body glucose disposal by insulin, especially at high rates of glucose disposal where glucose storage rather than oxidation predominates. Glucose had no effect on glycogen synthase activity. Taken together, these results suggest that post transport processes determine the rate of glucose disposal during insulin stimulation in normal subjects.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69017-04 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic Effects of Weight Loss in Non-insulin Dependent Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Bogardus Chief CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, Burns Institute, Galveston, Texas; Sandoz Research Institute, East Hanover, New Jersey; Dept. of Endocrinology, Georgetown University Hospital, Washington, D.C.

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study was terminated in October of 1986.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69018-04 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lipoprotein Metabolism in Diabetes and the Effects of Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS/NIDDK
Others:	W. Abbott	Visiting Scientist	CDNS/NIDDK
	B. Swinburn	Visiting Associate	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service, 2nd Dept of Med,Univ of Helsinki School of Med,Helsinki,Finland (foreign); Dept of Med,Institute San Raffaele,Univ of Milan,Italy (foreign); Dept of Med,Univ of Naples,Naples,Italy (foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	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 increased VLDL and decreased HDL commonly associated with non-insulin dependent diabetes are of concern because of their possible role in the etiology of the greatly increased cardiovascular disease in this disorder. This study compares VLDL and LDL metabolism in non-insulin dependent diabetics and in age and weight-matched nondiabetics. Studies were conducted in diabetics before and after therapy with sulfonylureas; also diabetics were compared on high and low fat diets. The data suggest that diabetics have abnormal VLDL and that diabetes influences VLDL-TG production independent of that of apoB. LDL concentrations in diabetics are influenced by two opposing changes - increase in direct removal of VLDL, but decrease in FCR for VLDL. Improvement of glycemic control with oral hypoglycemic therapy is followed by significant falls in VLDL-TG and LDL cholesterol and reversal of abnormalities of VLDL composition, VLDL triglyceride productions, lipase activities, and HDL subfractions. Transfer of the diabetics to a high carbohydrate, low saturated fat diet is associated with decreases in LDL, no change in HDL, and no change in VLDL in most diabetics. Metabolic studies on the two diets indicate that VLDL decreases upon removal of dietary saturated fat are due to increased clearance. The larger triglyceride rich VLDL in diabetics on a low fat, high carbohydrate diet are less efficiently converted to LDL, but clearance of both VLDL apoB and VLDL triglyceride are lower. The results indicate that high carbohydrate low fat diets can result in less atherogenic lipoproteins in most subjects with non-insulin dependent diabetes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69019-04 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Free-fatty Acid Metabolism and Obesity

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B.V. Howard	Associate Chief	CDNS/NIDDK
Others:	C. Bogardus	Chief	CDNS/NIDDK
	S. Lillioja	Visiting Associate	CDNS/NIDDK
	W. Abbott	Visiting Scientist	CDNS/NIDDK
	L. Christin	Visiting Fellow	CDNS/NIDDK
	B. Swinburn	Visiting Associate	CDNS/NIDDK
	G. Ruotolo	Visiting Fellow	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

FFA turnover measurements combined with lipid oxidation rate and assessment of body composition were used to investigate possible mechanisms of regulation of in vivo FFA metabolism and the inter-relationships between the metabolisms of FFA, carbohydrate, and lipoproteins. Our previous studies had shown that FFA mobilization was less per gram of fat in obese subjects. We also showed that a significant component of fatty acid turnover was non-oxidative disposal. To further examine the mechanisms of control of free-fatty acids, we have conducted studies of free-fatty acid turnover under two metabolic perturbations. One was the infusion of propranolol and the other was comparing individuals on a high-saturated fat and a low-fat, high carbohydrate diet. Propranolol infusion decreased the turnover of free-fatty acid, and this effect was greater in more obese individuals. During propranolol infusion, lipid oxidation was also increased, resulting therefore in a greatly decreased proportion of non-oxidative fatty acid disposal. During transfer to a high saturated fat diet, fatty acid production rates also declined and lipid oxidation increased, thus resulting in a significantly lower proportion of non-oxidative fatty acid disposal. The data suggest that the sympathetic nervous system plays a role in regulating the output of fatty acids from adipose stores and that obese individuals may differ in their sensitivity to this regulation. Finally, the non-oxidative component of fatty acid metabolism was lower in situations where lipid oxidation was increased. This may reflect inhibition of triglyceride re-esterification in sites other than adipose tissue.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 69020-04 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle Glycogen Synthase Activity and Insulin-Mediated Glucose Disposal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS/NIDDK
Others:	D. Mott	Research Chemist	CDNS/NIDDK
	A. Young	Visiting Associate	CDNS/NIDDK
	S. Lillioja	Visiting Associate	CDNS/NIDDK
	H. Yki-Jarvinen	Visiting Associate	CDNS/NIDDK
	D. Freymond	Visiting Fellow	CDNS/NIDDK
	M. Okubo	Special Volunteer	CDNS/NIDDK
	A. Katz	Special Volunteer	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To clarify the importance of the regulation of muscle glycogen synthase to the regulation of insulin-mediated glucose storage, we have established the following: insulin activation of glucose storage and glycogen synthase have similar ED50 values. Subjects with low insulin-mediated glucose disposal rates have dose-response curves for both glycogen synthase and glucose storage which are shifted to the right (lower sensitivity) and have reduced capacity. In subjects with low insulin-mediated glucose storage rates, both the glycogen synthase activity and the glycogen synthesis rates are reduced to one quarter of the level observed in high storage rate subjects. These results suggest that alterations in the regulation of glycogen synthase activity coincide with the altered glucose storage observed in subjects with low insulin-mediated glucose storage rates. In an effort to identify the source of this lesion we have observed the following: The elevated G6P content in muscle from subjects with low insulin-mediated glucose disposal rates indicates that the most significant reduction in their glucose metabolism occurs post-G6P. This result suggests that abnormal regulation of glycogen synthase is not secondary to abnormal glucose transport. This concept is supported by our observation that in normal subjects, increases in plasma insulin, not glucose, lead to activation of human muscle glycogen synthase. In addition, preliminary results suggest that the abnormal synthase activity is secondary to reduced glycogen synthase phosphatase activity in human muscle tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69021-07 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Energy expenditure in Pima Indians: possible cause for obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS/NIDDK
Others:	E. Ravussin	Visiting Scientist	CDNS/NIDDK
	L. Christin	Visiting Fellow	CDNS/NIDDK
	W. Abbott	Visiting Associate	CDNS/NIDDK
	F. Zurlo	Visiting Fellow	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service
Metabolic Unit, Dept. of Medicine, Univ. of VT, Burlington, VT

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, AZ 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.65	1.35	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 Pima Indian population of Arizona has one of the highest reported prevalence rates of obesity in the world. To determine whether a "thrifty" gene is the genetic defect predisposing the Pima Indians to obesity, we have investigated different components of the daily energy expenditure in both Pima Indians and Caucasians. Basal metabolic rate have been measured by an open circuit hood system indirect calorimeter, whereas the other components of 24-hour energy expenditure, i.e. the cost of physical activity and the thermic effect of meals, have been measured using a respiratory chamber. The cross-sectional and longitudinal results to date have shown that: 1) the rate of resting or 24-hour energy metabolism is a familial trait independent of individual differences in body size, age, and sex, 2) reduced resting or 24-hour energy expenditure rates are associated with an increased risk of weight gain, 3) the thermic effect of food is independent of the degree of obesity, 4) part of the differences in energy expenditure observed between people can be attributed to differences in the level of sympathetic nervous system activity, 5) spontaneous physical activity or "fidgeting" represents 100-800 kcal/day of the total energy expenditure, and may therefore predispose to obesity in the least active subjects. We are continuing to use the respiratory chamber to investigate the short-term energy metabolism response to over- and underfeeding in adults as well as the response to mild overfeeding in children from families with or without a history of obesity.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69023-02 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Skeletal Muscle Morphology as a Determinant of In Vivo "Insulin Resistance" in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS/NIDDK
Others:	S. Lillioja	Visiting Associate	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service
Dept. of Physical Education, University of Texas, Austin, TX

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK/NIH, Phoenix, AZ, 85016

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.)*

In vivo insulin resistance to the action of insulin on glucose disposal is commonly found in obese subjects. Since skeletal muscle is the site of uptake of much of an oral or intravenous glucose load, we have assessed the role of obesity associated changes in skeletal muscle morphology on in vivo "insulin resistance," in Pima Indian and Caucasian men. We have found a significant correlation between capillary density (capillaries/square mm cross-section) in skeletal muscle and in vivo insulin action. This may suggest that the increased diffusion distances created by muscle cell enlargement are part of the mechanism by which obesity is associated with "insulin resistance." Since increased distances for insulin to diffuse will delay its onset of action, these findings also explain why there is a delayed onset of insulin action in the obese. We have also found a correlation between the proportions of muscle fiber types and in vivo insulin action. This confirms the relevance to humans of animal studies that have directly shown that oxidative fibers are more insulin sensitive than glycolytic fibers. Since muscle fiber type proportions appear to be genetically determined, these findings may provide a mechanism for the familial dependence of in vivo insulin action. Since the primary control of muscle fiber type proportions appears to be muscle innervation, the central nervous system is implicated as a determinant of in vivo insulin action. We have also found a correlation of muscle fiber type and body fat distribution which suggests that central obesity may be part of a more generalized syndrome.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DK 69024-01 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* WHO Collaborating Center for Epidemiological and Clinical Investigations in Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	PECRB, NIDDK
Other:	W.C. Knowler	Chief	DAES, PECRB, NIDDK
	C. Bogardus	Chief	CDNS, PECRB, NIDDK
	D.J. Pettitt	Assistant Chief	DAES, PECRB, NIDDK
	B.V. Howard	Associate Chief	CDNS, PECRB, NIDDK

COOPERATING UNITS *(if any)*

World Health Organization, Non-Communicable Diseases Program, Geneva, Switzerland (F), Other World Health Organization Collaborating Centers for Diabetes (F), China-Japanese Friendship Hospital, Beijing, China (F)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, AZ 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The WHO Collaborating Center for Design, Methodology and Analysis of Epidemiological and Clinical Investigations in Diabetes was designated in 1986. The purposes of the Center are to collaborate with the World Health Organization in the implementation of the WHO/IDF action program to provide advice, consultation and collaboration with other investigators in the design, methodology and analysis of epidemiology and clinical investigations relating to the etiology and pathogenesis of non-insulin dependent diabetes (NIDDM) and its complications. The center will assist in the development and application of standardized methods for epidemiological and clinical investigations, and data analysis relating to diabetes and collaborate with those interested in applying such techniques elsewhere. The Center will advise and help in the design of new studies, including onsite assistance when necessary.

The center serves as a central laboratory for the WHO Multicenter Study of Vascular Disease in Diabetes, as well as being a participating study center for this study which is examining the mortality and incidence of vascular complications of diabetes among different ethnic groups in different countries. In addition the center has initiated a collaborative study of impaired glucose tolerance in China, is collaborating in the preparation of a survey manual for diabetes mellitus on behalf of WHO Center personnel are participating in teaching a WHO sponsored course on Clinical Epidemiology and Public Health Aspects of Diabetes.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 69025-01 PECR

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Treatment of Impaired Glucose Tolerance in Malmohus County, Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W.C. Knowler Chief DAES, NIDDK

COOPERATING UNITS *(if any)*

Lund University, Dalby, Sweden (foreign)

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Diabetes and Arthritis Epidemiology Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mortality according to glucose tolerance was studied to determine the prognosis of impaired glucose tolerance. In 1962-65, 228,833 subjects were screened for glycosuria. Of 2477 with glycosuria, 2180 were given oral glucose tolerance tests and grouped according to normal tolerance, impaired glucose tolerance, or diabetes by World Health Organization criteria. Among subjects at least 25 years old with normal tolerance, impaired glucose tolerance, or diabetes, age-sex-adjusted mortality through 1983 was 39±2, 49±4, and 71±4 deaths/1000 person-years (± standard error) for all causes ($p<.001$ for difference in 3 groups), and 24±2, 25±3, nd 40±3 for vascular causes (cardiovascular, cerebrovascular, or renal disease) ($p<.001$). 206 men with abnormal tolerance by local, but not World Health Organization, criteria were randomly assigned to diet with tolbutamide, diet only, or no treatment, which was continued through 1975. Age-adjusted all-cause mortality through 1983 did not differ significantly among treatment groups (34±9, 52±10, 45±19), but vascular mortality was 10±5, 31±8, and 38±19 in those assigned to tolbutamide, diet only, or no treatment ($p<.05$). Thus compared with persons with normal tolerance, diabetic subjects had higher all-cause and vascular mortality, and those with impaired glucose tolerance had higher all-cause but similar vascular mortality. Treatment of abnormal glucose tolerance apparently reduced vascular but not total mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 DK 69026-01 PECR

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Glycolysis in Human Skeletal Muscle

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	CDNS/NIDDK
Others:	A. Katz	Special Volunteer	CDNS/NIDDK
	B. Nyomba	Visiting Associate	CDNS/NIDDK
	D. Mott	Research Chemist	CDNS/NIDDK

COOPERATING UNITS *(if any)*

Indian Health Service
Dept. of Physical Education, Arizona State University, Tempe, AZ

LAB/BRANCH

Phoenix Epidemiology and Clinical Research Branch

SECTION

Clinical Diabetes and Nutrition Section

INSTITUTE AND LOCATION

NIDDK, NIH, Phoenix, Arizona, 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	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.)*

Phosphofructokinase (PFK) is the rate-limiting enzyme for glycolysis, although its regulation is poorly understood. The understanding of PFK regulation may be of particular importance in abnormal states such as diabetes and insulin resistance, since under physiological conditions (i.e. plasma insulin < 100 uU/ml) a substantial portion of a glucose load (orally ingested or infused) is oxidized by skeletal muscle. To increase the oxidation of glucose, glycolysis must be accelerated. Glucose 1,6-bisphosphate (GP2) is a potent activator of PFK in vitro and could thus be a potentially important regulator of PFK in vivo. We have studied a number of regulators of PFK underconditions of anoxia, isometric contraction, and during euglycemic, hyperinsulinemia, in human skeletal muscle. Biopsies were obtained from the quadriceps femoris muscle with the needle biopsy technique. During anoxia, increases in ADP, AMP, Pi, fructose 1,6-bisphosphate (FP2), and F6P and decreases in phosphocreatine (PCr) are responsible for activation of glycolysis. During isometric contraction, increases in GP2 were observed in all subjects and the increase in GP2 was well correlated with the in vivo glycolytic rate. During euglycemic-hyperinsulinemia in insulin-sensitive men, GP2 increased markedly in all subjects. However, there were no changes in any of the other measured modulators of PFK (i.e. PCr, ATP, ADP, AMP, FP2, F6P, and citrate). Hence it is possible that adequate increases in GP2 are a prerequisite for insulin-mediated glucose disposal in skeletal muscle. We are currently investigating the changes in PFK regulators in subjects with impaired glucose tolerance.

PHS 6040 (Rev. 1/84)

GPO 914-818