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OF
GENERAL MEDICAL SCIENCES

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

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Report of program activities.

CONTENTS

	Page
THE DIRECTOR'S REPORT	1
Pharmacological Sciences	1
Shared Instrumentation	1
Research Training	1
Office of Review Activities	2
Staff Changes	2
National Advisory General Medical Sciences Council	2
Fiscal Tables:	
Research Support	3
Fellowship Support	4
Training Support	5
National Research Service Awards Support:	
Predoctoral and Postdoctoral Individual	6
Predoctoral and Postdoctoral Institutional	7
PBME Career Award and Research Grant Support	8
Report for the Office of the Assistant Director for Clinical Research	9
CELLULAR AND MOLECULAR BASIS OF DISEASE PROGRAM	13
Objectives	13
Program Scope	13
Program Organization: Sections and Subprogram Areas	16
Cell Regulation, Differentiation, and Growth	17
Cell Organization, Motility, and Division	18
Membrane Structure and Function	19
Enzyme Catalysis and Regulation	21
Glycoconjugates	22
Bioenergetics	22
Structural Studies of Proteins and Related Model Systems	22
Diffraction Analysis of Proteins and Related Model Systems	23
Research Highlights	25
Research Training	42

Page

GENETICS PROGRAM---------- 45

Narrative---------- 45
Staffing---------- 46
Workshops---------- 46
Contracts---------- 48
Research Highlights---------- 50

PHARMACOLOGICAL SCIENCES PROGRAM---------- 83

Research Training---------- 84
Organization and Management---------- 85
Research Highlights---------- 86

PHYSIOLOGY AND BIOMEDICAL ENGINEERING PROGRAM---------- 93

Scope and Objectives---------- 93

Research---------- 94
Biomedical Engineering Section---------- 94
Instrumentation Section---------- 94
Physiological Sciences Section---------- 96

Research Centers---------- 97
New Investigator Research Awards---------- 98
Research Training---------- 98
Research Highlights---------- 99
Contracts---------- 105

MINORITY ACCESS TO RESEARCH CAREERS PROGRAM---------- 107

Scope---------- 107
Objectives---------- 107
Research Training---------- 107
Highlights---------- 108

THE DIRECTOR'S REPORT

This has been a year during which the basic biomedical research activities supported by the National Institute of General Medical Sciences have resulted in important discoveries which are relevant to the goals of all of the Institutes which comprise the National Institutes of Health. These are outlined in the reports of the specific programs and serve to underscore the importance of non-categorical basic biomedical science, and the significance of the mission of our Institute. It has become increasingly clear in recent years that, contrary to some expectations, research advances of the most fundamental nature can often be swiftly transplanted into preventive, diagnostic, or therapeutic approaches to a variety of diseases, particularly chronic diseases.

Pharmacological Sciences: In order to reaffirm the commitment of its programs to fundamental research and to alter the direction of some of its activities, the new Director of the Program, Dr. Sara Gardner, recommended, and approval was given to rename the Pharmacology-Toxicology Program as the Pharmacological Sciences Program. As indicated in last year's annual report, the Institute was most fortunate to have Dr. Folke Sjöqvist, Chairman of the Department of Clinical Pharmacology, Karolinska Institute, as a consultant for a one-year period ending January 1980. During the year, Dr. Sjöqvist provided very valuable advice regarding pharmacology research, in particular in clinical pharmacology. Together with Dr. William Potter and Dr. Sara Gardner, he developed the clinical pharmacology option of the Pharmacology Research Associate Training (PRAT) Program and helped set up a series of clinical pharmacology seminars which provided a forum for interactions between the NIH intramural scientists and the NIGMS Pharmacological Sciences Program.

Shared Instrumentation: Last year, the Institute made a number of grant awards for the purchase of analytic instruments which will be shared among several investigators. The Institute staff will be reviewing the use of these instruments during the coming year and determine whether the "sharing concept" has been a viable one. Based on this review, the Institute may extend the program of instrumentation awards, which are badly needed, according to a study done by the Association of American Universities.

Research Training: Fiscal year 1980 marked the start of the second five-year period for many of the Institutional fellowship awards under the National Research Service Act. Many of these "training grants" were renewed, but some of them were not found to be meritorious based on the review. It is Institute policy that only the most meritorious applications (both institutional and individual) for research training will be awarded, since the purpose of such research training is to assure that, in the future, there will be a pool of highly qualified principal investigators who can maintain the momentum and progress which makes biological science such an exciting field at present.

The two special research training programs, namely the Medical Scientist Training Program and the Minority Access to Research Careers (MARC) Program, are currently being evaluated in order to determine whether any mid-course corrections are needed or whether they are operating in the best mode. In addition, the staff of the Institute and the National Advisory General Medical Sciences Council have been considering the need for other facets of these programs. In the case of the MARC Program, it is planned to implement a new individual predoctoral fellowship award as a logical extension of the Honors Undergraduate Research Training Program.

Regarding graduates of the Medical Scientist Training Program, it is clear that most of them elect to obtain clinical training in one of the medical specialties before settling on their ultimate careers and that, during such training it is difficult, if not impossible, to continue research. We are currently considering the need for special programs designed to permit these young medical scientists to continue research while obtaining clinical training.

Office of Review Activities

During Fiscal Year 1980, Dr. Vincent Price, among his many other duties, assumed the position of Acting Chief of this office. In this period, the relatively new Executive Secretaries gained much experience and Dr. Price provided excellent leadership. This office is now an integral and essential part of the Institute and assures appropriate separation of scientific merit review from program administration. It is expected that a new Chief will be appointed early in FY 1981.

The combined review load of the four chartered review committees and several ad hoc groups is shown below:

Category	Number of Applications	Requested Dollars
Training Grants (T32)	91	$ 70,071,326
Centers	8	20,590,291
Program Projects	6	10,425,343
MARC Fellowships	31	(dollars not available)

Staff Changes: The following persons have been added to the staff:

Dr. Anthony Rene´, Executive Secretary, Office of Review Activities (ORA)
Dr. Carl Rhodes, Executive Secretary, Cellular and Molecular Basis of Disease (CMBD) Review Committee (ORA)
Ms. Dolores Lowery, MARC Program
Dr. William Potter, Coordinator, Clinical Pharmacology, PRAT Program
Ms. Martha Pine, Administrative Officer
Ms. Fu Temple, Equal Employment Opportunity Coordinator
Ms. Sharon Posey, Personnel Specialist

In addition, Ms. Emily Johnson became Secretary to the Director

The following staff members have left the Institute:

Mr. Paul Deming
Dr. Robert Gulley
Dr. Edward Hampp (retired)
Dr. Winfred Harris
Dr. Robert Melville
Dr. Eugene Oliver
Dr. Prince Rivers

National Advisory General Medical Sciences Council

The term of service of Drs. John Burnum, Geza Jako, and George Palade expired at the end of FY 1980.

FY 1980 TOTAL SUPPORT - BY ACTIVITY/GRANT TYPE
AND SHOWING PROGRAM AREAS
(Dollars in thousands)

		CMBD		GEN		PS		PBME		MARC		TOTAL	
		NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
P01	TYPES 5 & 7	7	2,861	4	2,083	6	2,183	9	3,716			26	10,843
	TYPES 2 & 9			3	1,376			1	256			4	1,632
	TYPE 1	3	1,221	2	933	1	285					6	2,439
	TYPE 3	1	106	1	36							2	142
		11	4,189	10	4,429	7	2,467	10	3,972			38	15,057
P41	TYPE 5							60	858			60	858
R01	TYPES 5 & 7	708	50,796	687	55,044	261	18,313	195	16,039			1,851	140,193
	TYPES 2 & 9	126	12,325	142	15,770	44	4,029	27	2,685			339	34,810
	TYPE 1	169	11,851	198	15,532	65	4,562	66	5,301			498	37,246
	TYPE 3	26	1,585	30	1,100	4	142	4	65			64	2,892
		1,029	76,558	1,057	87,446	374	27,046	292	24,090			2,752	215,141
R23	TYPES 5 & 7							15	607			15	607
	TYPE 1							6	249			6	249
								21	855			21	855
RESEARCH PROJECTS		1,040	80,747	1,067	91,875	381	29,514	383	29,776			2,871	231,911
R13	TYPES 2 & 9	1	35									1	35
	TYPE 1	3	26					1	13			4	39
	TYPE 3					1	2					1	2
OTHER RESEARCH		4	61			1	2	1	13			6	76
P50	TYPES 5 & 7			5	4,704	7	4,176	11	7,155			23	16,035
	TYPES 2 & 9	1	589					3	1,384			4	1,972
	TYPE 1							1	412			1	412
CENTERS		1	589	5	4,704	7	4,176	15	8,951			28	18,419
TOTAL RESEARCH		1,045	81,397	1,072	96,579	389	33,692	399	38,739			2,905	250,407

NOTE: Discrepancies in totals due to rounding.

FY 1980 TOTAL SUPPORT BY ACTIVITY/GRANT TYPE
AND SHOWING PROGRAM AREAS
(Dollars in thousands)

		CMBD		GEN		PS		PBME		MARC		TOTAL	
		NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
K04	TYPES 5 & 7	20	769	22	808	3	122	14	505			59	2,204
	TYPE 1							2	70			2	70
	TYPE 3	2	6	2	6	1	2	2	9			7	22
		22	774	24	814	4	124	18	584			68	2,296
K06	TYPES 5 & 7	7	227	7	226	1	34	4	129			19	617
	TYPE 3	1	2									1	2
		8	230	7	226	1	34	4	129			20	619
TOTAL CAREER		30	1,004	31	1,040	5	158	22	713			88	2,915
F32	TYPES 5 & 7	74	1,354	91	1,675	13	248	2	39			180	3,316
	TYPES 2 & 9	2	39	5	87							7	127
	TYPE 1	52	977	98	1,811	18	332	3	62			171	3,182
	TYPE 3	111	163	136	245	20	16	9	18			276	442
		239	2,533	330	3,818	51	597	14	118			634	7,067
F33	TYPE 1	1	27	2	57							3	84
F34	TYPES 5 & 7									16	345	16	345
	TYPE 1									8	185	8	185
	TYPE 3									3	22	3	22
										27	552	27	552
TOTAL FELLOWSHIPS		240	2,560	332	3,875	51	597	14	118	27	552	664	7,703

(This table is continued below on page 5)

FY 1980 TOTAL SUPPORT BY ACTIVITY/GRANT TYPE
AND SHOWING PROGRAM AREAS
(Dollars in thousands)

		CMBD		GEN		PS		PBME		MARC		TOTAL	
		NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
T14	TYPE 1			1	30							1	30
T32	TYPES 5 & 7	40	6,935	35	3,783	34	3,012	28	2,804	26	2,526	163	19,061
	TYPES 2 & 9	40	12,868	22	3,021	18	1,653	12	1,245			92	18,787
	TYPE 1	4	209	2	123	1	23	5	242	13	1,250	25	1,847
	TYPE 3	1	30							3	77	4	106
T35	TYPE 1							5	130			5	130
TOTAL TRAINING		85	20,042	60	6,958	53	4,688	50	4,421	42	3,853	290	39,961
FINAL TOTAL GRANTS		1,400	105,002	1,495	108,453	498	39,134	485	43,992	69	4,405	3,947	300,985

NOTE: Discrepancies in totals due to rounding.

FY 1980 NATIONAL RESEARCH SERVICE AWARDS TOTAL SUPPORT
BY ACADEMIC LEVEL/ACTIVITY/ANNOUNCED AREA
(Dollars in thousands)

ANNOUNCED AREA	TYPES 5 & 7		TYPES 2 & 9		TYPE 1		TYPE 3		TOTAL	
	NO. OF AWARDS	DOLLARS	NO. OF AWARDS	DOLLARS	NO. OF AWARDS	DOLLARS	NO. OF AWARDS	DOLLARS	NO. OF AWARDS	DOLLARS
NRSA FOR SENIOR FELLOWS (F33)										
CELL-MOL					1	27			1	27
GEN					2	57			2	57
					3	84			3	84
PREDOCTORAL INDIVIDUAL (F34)										
MARC	11	230			5	116	1	17	17	363
MARC (Paid by other Insts.)	(6	110)			(2	46)	(1	9)	(9	164)
POSTDOCTORAL INDIVIDUAL (F32, F34, F36)										
PATH					1	22			1	22
CELL-MOL	74	1,354	2	39	51	955	111	163	238	2,511
GEN	91	1,675	5	87	98	1,811	136	245	330	3,818
CLIN P/T					1	20			1	20
PHARM-SCI	13	248			17	311	20	16	50	576
SYS-INT					1	20	5	9	6	29
TRAUMA	1	21			1	20	2	6	4	48
ANES					1	21	1	2	2	23
BEHAV-SCI	1	17					1	2	2	19
MARC	5	116			3	69	2	5	10	189
MARC (Paid by other Insts.)	(3	82)			(1	23)			(4	104)
TOTAL	185	3,431	7	127	174	3,252	278	446	644	7,256

NOTE: Discrepancies in totals due to rounding.

FY 1980 NATIONAL RESEARCH SERVICE AWARDS TOTAL SUPPORT
BY ACADEMIC LEVEL/ACTIVITY/ANNOUNCED AREA
(Dollars in thousands)

ANNOUNCED AREA	TYPES 5 & 7			TYPES 2 & 9			TYPE 1			TYPE 3			TOTAL		
	AWDS.	POS.	DOLLARS	AWDS.	POS.	DOLLARS	AWDS.	POS.	DOLLARS	AWDS.	POS.	DOLLARS	AWDS.	POS.	DOLLARS
PREDOCTORAL INSTITUTIONAL (T32)															
CELL-MOL	22	332	3,262	27	649	7,189	2	10	107				51	991	10,558
GEN	17	208	2,126	18	263	2,645	1	4	60				36	475	4,831
PHARM-SCI	23	220	2,190	16	145	1,567							39	365	3,757
SYS-INT	19	213	2,195	9	90	939	2	5	62				30	308	3,197
MED-SCI	12	231	2,953	10	429	5,452	1	3	40	1	2	30	24	665	8,474
MARC	26	211	2,526				13	67	1,250	3	2	77	42	280	3,853
	119	1,415	15,252	80	1,576	17,792	19	89	1,518	4	4	106	222	60	34,669
PREDOCTORAL INSTITUTIONAL (T35)															
Short-Term Res. Trng.							5	60	130*				5	60	130
POSTDOCTORAL INSTITUTIONAL (T32)															
PATH	6	36	721	3	11	227	1	3	63				10	50	1,010
GEN	18	81	1,657	4	18	377	2	11	94				24	110	2,127
CLIN P/T	11	37	822	2	5	86	1	1	23				14	43	930
TRAUMA	5	13	274	3	14	306	2	7	158				10	34	737
[illegible]ES	4	14	335				1	1	22				5	15	357
	44	181	3,808	12	48	995	7	23	359				63	252	5,162

*$13 of the $130 funded by NEI; $117 funded by NIGMS

NOTE: Discrepancies in totals due to rounding.

FY 1980 PBME CAREER AWARD AND RESEARCH GRANT SUPPORT
BY PROGRAM/ACTIVITY/GRANT TYPE
(Dollars in thousands)

PROGRAM AREA	ACTIVITY	TYPES 5 & 7		TYPES 2 & 9		TYPE 1		TYPE 3		TOTAL	
		NO.	DOLLARS	NO.	DOLLARS	NO.	DOLLARS	NO.	DOLLARS	NO.	DOLLARS
ANES	K04	4	151			1	38			5	189
	P50	2	1,652							2	1,652
	R01	22	1,369	5	348	8	541			35	2,259
	R23	9	361			4	165			13	526
		37	3,533	5	348	13	744			55	4,625
BIOENGR	K04	5	196					1	2	6	198
	K06	3	96							3	96
	P01	5	2,457							5	2,457
	P41	60	858							60	858
	P50	3	2,436							3	2,436
	R01	120	10,041	14	1,180	39	3,105	3	55	176	14,381
	R13					1	13			1	13
		196	16,084	14	1,180	40	3,117	4	57	254	20,439
RADIOL	K04	2	78							2	78
	P01	1	294	1	256					2	549
	P50	1	591							1	591
	R01	12	1,123	4	679	7	632			23	2.435
		16	2,086	5	934	7	632			28	3,653
TRAUMA	K04	3	80			1	32	1	6	5	118
	K06	1	33							1	33
	P01	3	966							3	966
	P50	5	2,477	3	1,384	1	412			9	4,272
	R01	41	3,504	4	478	12	1,023	1	10	58	5,016
	R23	6	245			2	84			8	329
		59	7,306	7	1,862	16	1,550	2	17	84	10,734
TOTALS (PBME)		308	29,009	31	4,325	76	6,044	6	74	421	39,452

NOTE: Discrepancies in totals due to rounding.

REPORT FOR THE OFFICE OF THE ASSISTANT DIRECTOR FOR CLINICAL RESEARCH

The emphasis in FY 1980 by this Office was mainly placed on attempts to broaden activities in the area of trauma and burn research and research training. The following is an account of these attempts:

I. Consensus Development Conference:

The proceedings of the NIGMS Consensus Development Conference on Supportive Therapy in Burn Care were published as a supplement to the November 1979 issue of the Journal of Trauma. The Institute has widely distributed it to the medical profession, public, and press. It has been well received and, based on comments, it appears that it is referred to extensively by clinicians in the care of the severely burned patients. To determine the extent to which the burn consensus conference recommendations have influenced the practice of burn medicine, the Institute has awarded a contract to study the specific impact of the recommendations on the six model burn care demonstration sites (serving the six New England States, the Fingerlakes and Central Upstate New York, Virginia, Alabama, Northern Texas, and San Diego and Imperial County, California) supported by the Health Services Administration's (HSA) Division of Emergency Medical Services (EMS). Two of the directors of the six demonstration sites (New England and Texas) are also directors of NIGMS supported burn research centers in Boston and Dallas and thus can determine if the research performed in these two centers was important in the development of the recommendations. The HSA is extremely interested in coordinating the data collected under the NIGMS contract with its own review of the six burn demonstration sites. The results of the contract work should be available by November 1980.

A second smaller conference on supportive therapy in burn care planned for October 9-10, 1980, will have several goals. First, the participants will present new work in an effort to determine whether the previous recommendations should be modified. Second, the Institute staff will reiterate the opportunities available for support of research and research training and will seek suggestions on new avenues of approach to burn research and on ways to assure a sufficient group of new investigators for the future. Third, since the NIGMS is also interested in seeking ways to adequately disseminate the conference results and to measure their impact on the medical community, the proceedings of the conference will be published and distributed as before.

II. NIGMS Task Force on Technology Transfer and Assessment

In the Fall of 1979 a small group of consultants was asssembled to review the use of liposomes with particular emphasis on the appropriateness of the area as a topic for a consensus development conference. The consultants felt that such a conference could be oriented toward the use of liposomes as carriers in therapeutic application. Specific topics were suggested but in follow-up discussions it became apparent that these were only presented as items for information transfer and not evaluative modes for possible new technology dissemination. It was felt that this was not the opportune time for such a conference. Despite the limited role of the Institute in the participation of consensus development conferences, its Task Force will continue to consider other areas of research which ultimately may become appropriate for such conferences.

III. Presentations and Meetings

The Assistant Director for Clinical Research was a keynote speaker at the Denver, Colorado meeting on The Management of Patients with Burn Injuries held on December 13-15, 1979, sponsored by the International Society for Burn Injuries and the World Health Organization. Her speech, "The Role of NIH in Burn Care and Research," was presented to physicians, paramedical personnel, and scientists working in the area of burn care and research and served as an information exchange on the available mechanisms of support provided for research and research training. Numerous requests were made by the attendees for information on the NIGMS program. During the remainder of the meeting, there were panel discussions and educational sessions on research techniques and methods. A number of the NIGMS grantees participated in various scientific sessions.

On March 5-7, 1980, as part of an ongoing evaluation study, the Assistant Director accompanied the Deputy Director of the Division of Emergency Medical Services (EMS), HSA to the University of California at San Diego to visit one of the six EMS burn demonstration sites previously mentioned in this report.

The project area visited consists of the two-county region of San Diego and Imperial Counties in California which have a combined population of about 1.8 million, and a land area of almost 8,500 square miles. The data collecting process was viewed at one of the outlying hospitals and the data analyses, costs, and reimbursements were discussed at the University facility in San Diego.

One of the projects at San Diego is a comparison of the effects of various fluid resuscitation regimes (i.e. crystalloid resuscitation, Baxter; plasma, Muir and Barclay; and hypertonic saline, Monafo). Drs. Baxter (GM 21681) and Monafo (GM 22364) are both supported by NIGMS for research in this area. A protocol was presented for the study of interstitial pressure and composition changes which uses the Wick catheter technique (modified by Peters, GM 17284) during the fluid resuscitation. At the time of the visit, only a few patients had been studied and the number was too few to draw any conclusions. The importance of the findings emanating from this research in regard to patient care will be evaluated.

The 12th annual meeting of the American Burn Association was held on March 26-29, 1980. The papers presented at the meeting were divided into three groups and given at three concurrent sessions (clinical, research, and associate programs). This year a total of 106 papers were presented. Forty, or 38% of the papers were presented by NIGMS grantees and the following are descriptions of some of their work:

"The In Vitro Growth of Guinea Pig Epithelial Cells"
GM 26145 (Curreri) Cornell Medical Center, New York

The growth of epithelial cells in tissue culture is a promising solution to the problem of insufficient autogenous skin supply in the massively burned patient. At the 1978 annual meeting of the American Burn Association a new method was presented for growing epithelial cells from single cell suspensions directly on plastic surfaces. The technique does not support the growth of animal epithelial cells because they do not separate as easily as human epithelial cells from fibroblastic dermal elements. As a result, a number of modifications have been made in the technique for the basic growth of cells which have resulted in successful growth of multilayered sheets of guinea pig epithelial cells and have allowed

controlled laboratory investigations using inbred strains of animals. These sheets can be removed from the bottom of the tissue culture vessel and can be made to adhere to pigskin or Biobrane®, a synthetic covering.

Thus, a simple tissue culture system for the growth of human epidermal cells has been modified to allow the growth of guinea pig epithelial cells into confluent sheets which may easily be transplanted.

Pulmonary infection is a leading cause of death in patients with smoke inhalation. However, little research has been performed to determine its underlying effects. Several NIGMS grantees reported on their work in this area.

"Animal Model of Smoke Inhalation: Smoke Generation System"
GM 24990 (Carrico) University of Washington, Seattle

At the University of Washington NIGMS trauma research center, Dr. C. James Carrico and his group of investigators have developed an animal model of pulmonary injury due to smoke inhalation in which physiologic studies hopefully will provide information on the nature of the pathological and physiological abnormalities occurring in the rabbit as a result of smoke inhalation. Ultimately these findings should lead to improved clinical care.

Rabbits are exposed to smoke for a total of 40 minutes. Preliminary pathologic studies at 24 hours after exposure on the lungs of 10 exposed and 4 control rabbits show a distinctive and reproducible injury, concentrated in the proximal portion of the lower respiratory tract. It consists of sloughing of the ciliated and mucus-secreting epithelial cells lining the airways.

This smoke generation and exposure model allows reproducible conditions of exposure and results in reproducible lung injury in exposed rabbits. It should provide better predictability regarding patients who are at increased risk of developing pulmonary complications and permit the testing of therapeutic maneuvers including the use of drugs. It also should provide information on which products of combustion commonly encountered in fires are associated with lung injury.

"The Value of Spirometry and Chest Roentgenographic Findings in the Clinical Detection of Smoke Inhalation"
GM 21681 (Baxter) University of Texas, Dallas

The effects of smoke inhalation frequently are not clinically apparent during the early post-injury period, and insidious respiratory failure may ensue. The diagnosis has been based on clinical data and a constellation of sophisticated laboratory tests, but a simple, reliable method of detecting smoke inhalation has not been found. Thus, Dr. Baxter and his group in the NIGMS supported burn research center at Dallas have made serial spirometric measurements in patients with and without clinical evidence of smoke inhalation injury.

The measurements indicated that both restrictive and obstructive defects exist in inhalation injured patients on admission, but not in burned patients without inhalation injury. These defects resolved gradually over a five-month period of observation. More sophisticated tests that were simultaneously performed, including closing volumes, flow-volume loops on room air and helium, lung volumes, and arterial gas analysis, did not add to the predictive value of spirometry. It was concluded

that spirometry is one of only a few useful techniques for accurate evaluation of pulmonary injury in patients with suspected smoke inhalation.

In conjunction with the spirometry studies, Dr. Baxter has completed the first prospective study of the use of roentgenographic findings in the diagnosis of smoke inhalation. Clinical evidence of smoke inhalation did not correlate with the development of chest roentgenographic abnormalities. The investigators feel that considerably more research is needed to develop better methods for early detection of smoke inhalation.

"Loss of Beta-Adrenergic Receptors with Retention of Catecholamine-Stimulated Adenylate Cyclase Activity after Chronic Stress"
GM 21700 (Burke) Massachusetts General Hospital, Boston

Dr. John Burke and his team of investigators at the NIGMS burn center in Boston have found that acute exposure of cells to catecholamines causes those cells to become refractory to further catecholamine stimulation. Desensitization is thought to be mediated by a reduction in the number of and/or affinity of beta-adrenergic receptors in the plasma membrane. Therefore, it might be hypothesized that one of the effects of increased catecholamine secretion during stress might be a loss of catecholamine sensitivity in target tissues. Contrary to this expectation the investigators have found that adenylate cyclase in fat cells remains fully responsive to stimulation by catecholamine in two models of chronic stress, namely burn injury, and exposure to cold for three weeks.

It was concluded that, in spite of the loss of nearly half of the beta-adrenergic receptors during chronic stress, adenylate cyclase remained maximally responsive to catecholamines. This may be explained by the presence of betareceptors in excess of what is needed for interaction with all the available adenylate cyclase. Alternatively, chronic stress may evoke a mechanism to compensate for the loss of receptors. The results are consistent with the principal investigator's idea that the hypermetabolism associated with chronic stress may be mediated by catecholamines.

The Assistant Director for Clinical Research will continue to explore avenues to stimulate more research activities in the area of trauma and burns. One approach being discussed with the program directors of the Institute's Cellular and Molecular Basis of Disease and Pharmacological Sciences programs is the availability of training slots for use by the young clinicians interested in research careers. Another approach is an attempt to inform basic scientists of the problems encountered in trauma and burn research so that they may attempt to tackle some of these areas in collaboration with the trauma/burn clinician scientists or by submission of their own individual applications.

CELLULAR AND MOLECULAR BASIS OF DISEASE PROGRAM

OBJECTIVES

The Program's objective is to gain the fullest knowledge about human cells to assist in the prevention, treatment, and cure of diseases of man. Limitations of research on human cells per se prevent the most direct approach toward achieving this central goal. Consequently, the Cellular and Molecular Basis of Disease Program selects for support the most meritorious research on all manner of cell systems from the biotic world, within the accepted principle that living cells generally share fundamental biological and chemical properties. The supported research ranges from precise physical and mathematical approaches in the study of enzyme catalyzed reactions in the cell to descriptive studies of newly discovered cell forms and their biological behavior as possible model systems. Results from this broad range of research are reported continually in many scientific journals adding important general principles, techniques, and information which contribute directly or indirectly to our expanded knowledge about both normal and abnormal functions of human cells. A number of examples of the results of research sponsored by the Program during this fiscal year are presented in this report.

PROGRAM SCOPE

The diversity of research supported by the Cellular and Molecular Basis of Disease Program may be appreciated by some brief comments about the nature and function of cells. The living dynamic cell -- a highly organized array of ions and complex molecules -- is the fundamental biological unit of all living organisms. The living cell holds the capacity for generating from rather simple exogenous sources all of its enzymatic machinery and the energy required for the most complicated of biochemical syntheses, varied movement and motility, and for self-regulated propagation. Cells form selective cell-cell associations of many kinds, continually displaying highly specific intercommunication over considerable distances and interacting with and adapting to all manner of environmental factors. Programmed by thousands of internal computer-like memory banks called genes, millions of offspring from a single egg cell are transformed into hundreds of groups of very specialized cells in an orderly fashion. These developmental processes occur in all living forms ranging from slime molds to man.

Millions of cells in higher organisms function in concert to form specialized tissues or organs. Cells are the seat of all metabolic processes, skeletal and heart muscle contractions, reception and interpretation of light waves, memory, action and reaction with environmental factors and signals, growth and aging, general behavior of all organisms, photosynthesis in plants, and even biological evolution.

Cells are also the sites of infection and disease, and death may result from the disturbance of a relatively few cells in even the largest animal.

The variety of unicellular organisms and of cells from the tissues of higher organisms available for study number in the thousands. Further, the intricate membrane surface and highly organized internal structure of each cell type, even the simplest cell, is of such complexity that groups of individual scientists may spend much of their professional lives studying the chemical structure and function of a single component of the cell. These cell components or compartments, often called cell organelles, in turn, are collections of highly organized molecules with molecular weights ranging up to the millions.

Only a few examples of cell organelles currently being investigated in hundreds of laboratories over the nation with research grant support from the Cellular and Molecular Basis of Disease Program can be mentioned here. Mitochondria are discrete organelles in all nucleated cells, though highly variable in size and shape in different cell types and animal species. Mitochondria are the major sites for energy conversion and the source for all biochemical, electrical and mechanical activities of cells and their host organisms, including man. They are well-defined entities, microscopically and chemically. The numerous mitochondria of each cell contain at least one hundred distinct types of enzymes. These enzymes are arranged in an intricately organized fashion on a convoluted membranous envelope which can be readily observed by electron microscopy. A special group of 20 or more coupled iron and copper enzymes, together with numerous associated chemical co-factors in the mitochondria, are referred to as the cytochrome or electron transport system. These enzymes function collectively and in intimate association to generate high energy phosphate, ATP, for distribution and use throughout the cell for chemical synthesis, muscle contraction, etc. Several major laboratories throughout the world conduct intense research on components of this enzyme system alone, many with support from the CMBD Program.

Microtubules, hollow, microscopic, rod shaped structures constructed from the assembly of rings of proteins called tubulin, are ubiquitous cell organelles. Their role in cells seems to be multiple. They function as cytoskeletal structures contributing to cell form and shape. The contractile protein actomyosin may be attached to microtubules. The structure and arrangement of microtubules in parallel bundles, cross linked by a second protein, dynein, has been carefully worked out as the internal mechanism for motion of cilia and flagella, minute projections extending from the surfaces of many cells for locomotion. The fibrillar strands of the mitotic spindle on which chromosomes are pulled apart into each daughter cell in cell division are formed from microtubules. Tubulin molecules readily assemble into microtubules and disassemble under carefully regulated conditions within the cell. It is believed by some researchers that this may be the manner in which they move chromosomes and other cell components in cell division and possibly move selected sites in the cell surface membrane. Very recent evidence suggests that microtubules in the cell cytoplasm also may have a similar associated cross-linked protein like those of flagella. Brain cells and all nerve cells (neurons) are rich in microtubule content. Their special role in the brain is not fully understood; however, there is considerable evidence that microtubules serve to guide the transport of other molecules along the extremely lengthy neurons of the body.

Each cell contains thousands of microsomes which are tiny spherical protein bodies, requiring very high magnification to be discerned by electron microscopy. They are the sites of all protein synthesis and many are attached to an extensive, convoluted inner cellular membrane called the endoplasmic reticulum (ER). Protein/enzyme synthesis in these ribosomes is under the direct control of the cell's genes through messenger RNA molecules coded for the specific proteins to be manufactured. The ribosome itself is a complex molecular structure, and innumerable scientific papers have already reported a mass of data on its detailed chemical function. Quite recent findings from research supported by the CMBD Program have described the synthesis of proteins to be secreted from cells. Here, still another organelle, the Golgi apparatus, also a layered membrane structure, is involved. In a kind of assembly line fashion, prescribed amino acid chains, called signal peptides, from ribosomes pass into the ER membrane and are further coupled and folded into globular proteins. They are then transported to and modified in the Golgi apparatus and passed through membrane channels or vesicles to the cell surface for excretion into the extracellular environment, such as the blood stream or the lumen of an endocrine gland or the intestine. In microorganisms, similar mechanisms may be used for the excretion of digestive enzymes or of toxins.

A final example for mention is the outer plasma membrane of all cells which also may be considered as a distinct cell organelle. The plasma membrane is a transparent lipid bilayer, two molecule lengths in thickness of polar oriented fatty acid chains, with numerous kinds of protein molecules embedded throughout the lipid film. Many of the specific membrane proteins are known to be highly mobile within the surface membrane. Some are known to form channels through the membrane for transport of ions such as Na^+, K^+, Ca^{++} as well as sugars, amino acids and other compounds into and out of the cell. Still other membrane proteins, many ligated with known glycosides, function as specific receptor sites for the attachment of hormones and other cell regulators emanating from distant cells in an entirely different organ of the human body. Cell surface antigens are yet another class of proteins and carbohydrates in the plasma membrane which become attachment points of antibodies. A cell's surface is also the site of attachment and penetration of viruses and other pathogens which attack cells. In all neurons, the plasma membrane functions to propagate the action current, via ion exchange, along its length.

The Program's grantees conduct research focused on the plasma membrane using approaches and techniques employed in immunology, enzymology, endocrinology, virology, bacteriology, chemistry and physics. Instrumental analyses of all sorts are employed in research on the plasma membrane and the membranes of the cell organelles. Electron microscopy is used extensively in numerous laboratories in the study of membranes. The plasma membrane, only 60 to 100 angstroms thick, can be fractured by special freeze-etch techniques into its inner and outer layers and photographed by the electron microscope. Sophisticated instruments for the measurement of nuclear magnetic resonance and circular dichroism, developed initially for wide use in chemistry are routinely used to study in situ the biochemistry and biophysics of cells and cell organelles. In the last few years, and with major expansion in the current year, CMBD grantees are now

employing intense beam energies from high-energy physics synchrotron machines to achieve more refined physical-chemical measurements of the intricacies of the molecular components of cells.

The general classes of compounds that make up the cell's organelles, the proteins, carbohydrates, and lipids each comprise many molecular subsets. It is estimated that some 50,000 different enzymes have been identified or postulated for subsequent study, but only a fraction of these have been characterized in great detail. The complete chemical structure is known for a very few, but the precise mechanism of action in the fullest physical-chemical terms is known for none. Much remains to be learned of the precise mechanism of action of enzymes in order to fully understand cell function or cell dysfunction in many diseases. The same is true of many other classes of cellular molecules and associated trace metals and ions in terms of their structural, metabolic and regulatory role in the cell. To be sure, a great deal of valuable knowledge has been accumulated about enzymes and their specific catalytic role in metabolism, in synthetic reactions, in proteolysis, in other metabolic functions, and in cascading biochemical mechanisms such as blood coagulation and in immune phenomena. Much of this knowledge is now being applied clinically. A major goup of extracellular and body fluid proteins are enzymes. All such extra-cellular enzymes are synthesized and secreted by cells. The altered levels of these enzymes in disease states is under active study in many clinical laboratories. Many, if not most, of the separate functions and reactions of cells can now be attributed to specific classes or groups of compounds and ions within or attached to the surface of cells. We now recognize communication among molecules and information storage at many levels of sophistication in the molecules that comprise cells and serve to communicate between cells. This is so in bacteria and in man.

PROGRAM ORGANIZATION

The overall CMBD research program is administered by a Director and Deputy Director and nine other professional staff members. It is organized into two sections and with subprogram areas. Their size, structure, and content are outlined below.

CELLULAR BASIS OF DISEASE SECTION
approximately 432 grants, $23,500,000

The emphasis of the Cellular Basis of Disease Section is on research on the cell and its subcellular components including basic research on all types of cells, (i.e., prokaryotic and eukaryotic microorganisms, cells in tissue

or organ culture, isolated cells, such as blood cells, sperm and fertilized and unfertilized ova), as well as research focused on specific cells and their functions in excised and intact tissues and organisms. Questions and problems explored and pertaining to specific cell types or components are of a fundamental or general nature applicable to other cell types. This section includes all classes of basic cellular or subcellular research not expressly directed to the disease oriented mission of a single categorical Institute, as well as cell biology related to several disease or general pathological states. The full range of physical, chemical and biological (e.g., genetic and immunological) methods are employed throughout, and the development of new methods and techniques are sponsored.

CELL REGULATION, DIFFERENTIATION, AND GROWTH
approximately 90 grants, $4,600,000

The Cell Regulation, Differentiation, and Growth Subprogram supports a spectrum of investigations on normal cell function that encompass many active areas of cell biology. About two thirds of the research areas center on two major themes: cell differentiation, and the cell cycle. The other third supports a wide selection of research on diverse topics including cell adhesion and interaction, circadian rhythms, and the interaction of cell water with proteins and small molecules.

Cell Differentiation - 44 grants, $2,300,000

The largest group of research projects are those investigating the mechanisms of cell differentiation. Using a multiplicity of systems ranging from the cellular slime molds to the vertebrate cell in culture, from sea urchin eggs to multicellular algae, and from multicellular bacterial systems to nematode embryos, investigators are asking numerous questions concerning the process by which similar cells, during development, assume different functions. Subjects being investigated include: the role of external signals in the fertilization process; membrane and cell surface receptors; communication and interaction between cells; cellular compartmentalization; metabolic processes; and the mechanism of active hormones.

Cell Cycle and Control of Growth and Division - 25 grants, $1,200,000

The cell cycle of a growing cell is the period from its formation by fission from its mother cell to the time when it divides to form two daughter cells. Two separate classes of events occur in the normal cell cycle. One includes the physical events of mitosis and cell cleavage. The other includes the biochemical synthetic events which double the quantity of the organic components of the cell. This program section is concerned primarily with the latter. Scientists are investigating the various control points within the cell cycle; regulation

of synthesis of key components for initiation of genome segregation and cell division; control of gene expression during the cell cycle; and mechanisms of interaction of mitogens, hormones, polyamines, cyclic AMP, and external stimuli such as light in the control of cell growth and division. Such research has significance not only for understanding how embryonic development takes place, and how normal cell turnover and replacement is controlled in the adult, but also for an understanding of cell multiplication following tissue damage, immune response to infection, and the excessive production of abnormal cells which have escaped from normal regulatory controls, as in cancer.

Uncategorized - 21 grants, $ 1,000,000

The projects in this section represent many diverse topics including some research areas with only a small number of research workers, new and highly specialized areas of exploration which may expand, and some fields where the less basic aspect is supported by other Institutes. Some topics supported include biochemical studies on silicon, mechanisms of cell fusion, regulation of leukocyte function, studies of cell adhesion and interaction, circadian rhythms, and the structure of water in cells.

CELL ORGANIZATION, MOTILITY, AND DIVISION
approximately 84 grants, $4,300,000

This group of grants involves studies of movement in cells ranging from gross cellular movements such as protoplasmic streaming to the movement of cilia and flagella. Emphasis is on movement of and within non-muscle cells; however, a limited number of grants involving contractile mechanisms of smooth muscle cells are supported. Organisms used represent both procaryotic and eucaryotic cell systems. Investigations in this program area draw upon a wide range of disciplines including biochemistry, immunology, physiology, electron microscopy, and cytochemistry at both the light and electron optical level.

The larger portion of the work supported here involves studies of cell structures such as microtubules and microfilaments, and the proteins associated with motile structures, including microtubular associated proteins, tubulin, myosin, paramyosin, meromyosin, actomysin, actin, and various protein kinases. Motility studies involve control of shape associated with normal as well as transformed cells. Studies of the role of ions, principally Ca^{++} ions and sulfhydryl groups in cell movement and cell division are included. Many of the projects emphasize the recurring theme of Ca^{++} involvement in the regulation of motility and more recently by the function of calmodulin referred to as activator protein, modulator protein, Ca^{++} dependent regulator protein (CDR) and troponin C-like protein) in their various biological or model systems. This has included the role of Ca^{++} and/or calmodulin in microtubular initiation and elongation; regulation of microtubules and microfilaments; actomyosin interactions; ciliary motion, mitotic spindle assembly and function, as well as the biological role of intracellular Ca^{++} *per se*. There has also been an increase in researchers in this area who are applying microinjection and fluorescent

techniques along with other aspects of immunobiology to explore the role of specific proteins in these biological systems.

Cell division studies which center around the mitotic spindle, the movement of chromosomes and associated cellular proteins involved in the division process, are also included in this program area. Research focusing on the mitotic spindle includes studies relating to the composition of this structure, its isolation, the movement of related structures with this apparatus and its relationship to the cell membrane. Finally, an area of research which began to show growth in Fiscal Year 1978 and has continued through 1980 is that whose subject matter concerns the interaction of cytoskeleton structures and the cellular membrane during movement and division.

MEMBRANE STRUCTURE AND FUNCTION
approximately 258 grants, $14,600,000

This program area deals with all aspects of the biology and chemistry of membranes. The emphasis is on the plasma membrane of cells, but a significant fraction of the effort is devoted to intracellular membranes. An overwhelming majority of the research is at the cellular or subcellular level. The principal investigators include biochemists, biophysicists, cell biologists, chemists, neurobiologists and physiologists. This is a reflection of the present multi-disciplinary approach to the study of cellular membranes and the increasing awareness of the pervasive and important role of membranes in the many different functions of all cells. The research grants are categorized in the following way.

Active Transport - 48 grants, $2,300,000

It is apparent that since membranes separate the inside of the cell from the outside and are generally impermeable to most species of molecules, there must be specific transport mechanisms. Much of the research has focused on selected systems of broad importance in eukaryotes: sodium/potassium ATPase and amino acid transport. Some success has been achieved in the isolation of transport proteins and protein complexes. Their reincorporation into reconstituted membrane systems is just beginning to show results. General advances in the manipulation of bacterial mutants and the isolation of selected plasmids have been used to isolate and synthesize bacterial transport proteins. One emerging area is the cellular control of active transport. Inter- and intracellular signaling involving calcium and/or cyclic nucleotides is a rapidly growing subject of considerable interest.

Membrane Structure and Protein Composition - 102 grants, $6,200,000

This is the largest group of grants in this program area. This group contains highly sophisticated biophysical research. Questions of the organization and orientation of membrane proteins and multi-unit complexes require elaborate instrumentation extending from purely microscopic observation to biochemical and biophysical approaches. Many investigators are now employing multipronged

attacks, locating a protein by microscopic techniques and then isolating it for purification, sequencing, and reinsertion into lipid for diffraction studies.

Membrane Biogenesis - 28 grants, $1,450,000

Some of this work is solely biochemical, looking at the insertion of lipid or protein into membrane and the location, pathway, and timing of attachment of the carbohydrate moiety. The most exciting work is an extension of molecular biology from the already well-established studies of the synthesis of cytoplasmic proteins to those of membrane proteins.

The cycling of membrane, particularly between the plasma membrane and the cytoplasmic vesicles, ER, Golgi apparatus and lysosomes, is an expanding field. Only recently have some of the technical difficulties begun to be overcome by ingenious use of labels, clever selection of tissues and organisms, and development of ultrafast freezing techniques for electron microscopy.

Lipids and Lipid Properties - 35 grants $1,900,000

Although NIGMS has long supported work on lipid monolayers and bilayers, it is only recently that there has been a literal explosion of interest in lipid chemistry, particularly in the area of physical chemistry. With improvements in nuclear magnetic resonance and electron spin resonance techniques, a great deal of effort has been expended in describing the microenvironment of proteins floating in the "lipid sea" of the membrane. There has been much excitement generated by the recent discoveries and measurements of the lateral mobility of proteins and lipids in the bilayer. Much work has been devoted to measuring the apparent viscosity of membrane lipids and determining the physicochemical state of the various lipid components of the membrane. Recently, attention has been focused on mechanisms of lipid-lipid fusion, particularly with a view to possible use of artificial lipid vesicles to introduce enzymes and drugs into specfic cells.

Passive Transport - 25 grants, $1,400,000

It is now clear that some intrinsic membrane proteins, singly or in combination, form channels through natural membranes. There has been some success isolating these complexes and incorporating them in artificial membranes. Other work has been devoted to the study of the kinetics and selectivity of channels. There has been some success at chemical modification of natural channels in vitro.

Research employing synthetic ionophores continues to grow rapidly. Some ionophores are being used as antibiotics and there is some evidence that some naturally occurring insecticides are acting as ionophores. Excellent organic chemistry is being devoted to the synthesis of new ionophores and the modification of existing ones.

Other Membrane Enzymes - 20 grants, $1,000,000

These grants are devoted to membrane-bound enzymes, other than those involved in active transport. Although this is the smallest category of grants in the

program, it includes some of the most interesting work in the entire membrane field. In some cases, such as in the study of some membrane-bound glycolytic enzymes, one of the main questions relates to the significance and advantage of a specific subcellular localization for these enzymes. In other cases, such as the study of NADH cytochrome b_5 reductase and cytochrome b_5 in artificial membranes, the questions include the orientation of the individual enzymes and the relationship of their various intermediates during catalysis. Some of the most elegant studies of tertiary protein structure and active site analysis have been carried out on the cytochrome b_5 system, which is involved in the metabolism of drugs.

MOLECULAR BASIS OF DISEASE SECTION
approximately 550 grants, $30,200,000

The Molecular Basis of Disease Section supports basic research on enzymology and regulatory processes in intermediary metabolism; structure and function of the enzyme active site, including the involvement of coenzymes and metal ions; characterization of the energy transducing apparatus; physical and chemical studies on the ordered structure of protein and model systems; and theoretical studies of systems of biological importance. Support is provided for many studies aimed at basic principles, regardless of the particular experimental system.

ENZYME CATALYSIS AND REGULATION
approximately 212 grants, $11,000,000

The major emphasis of this program area is the analysis of intermediary metabolism on a molecular level. This ranges from a very narrow focus such as probes of enzyme active sites to broader constructs such as the overall regulation of metabolic pathways. In general, the grants in this area concern themselves with events at the enzyme level.

Active site studies of a variety of different kinds represent a major portion of the activity in this program. An area of particular interest in recent years involves the use of compounds which mimic the transition state during the catalytic process. Transition state analogs can be designed which resemble activated forms of the substrate but are in fact inhibitors. Such compounds can be useful as powerful enzyme-specific antimetabolites, and as probes for distinguishing between mechanistic alternatives. A related area of study involves the use of "suicide inhibitors." These compounds act as substrates for the enzyme, and are transformed in the catalytic process to a structure which covalently couples with a group at the enzyme active site. This site-specific labelling can be used to identify and analyze the active site.

Another important sub-area involves the analysis of metabolic regulation by covalent modification of enzymes. The most widely studied phenomena continues to be the cAMP-dependent phosphorylation reactions. However, other forms of

covalent modification, including limited proteolysis and linkage with groups other than inorganic phosphate, are becoming of increasing interest.

Beyond these specific categories, the bulk of the grants in this program are in the area of enzyme mechanism. A wide variety of techniques are applied to these studies, which represent a central problem area of emphasis in biochemistry.

GLYCOCONJUGATES
approximately 17 grants, $1,200,000

This small but important program centers on the structure, function, and metabolism of carbohydrate-containing macromolecules. Included in this class are glycoprotein hormones, antigenic determinants, and cell-surface receptors.

Developments in this area have come very slowly, in part because of the extreme difficulty of accurately determining the structure of oligosaccharides covalently bound to proteins. Thus, the problem of developing appropriate methodologies is a recurring one. In this regard, a recently funded study which focusses on the application of mass spectroscopy to a variety of structural problems involving glycoconjugates is of considerable interest. These studies, of course, go hand-in-hand with the determination of glycoconjugate synthesis, and the role of the oligosaccarides in protein function.

BIOENERGETICS
approximately 71 grants, $4,400,000

The grants in this area support research on oxidation and on energy-producing systems central to providing energy for all cellular processes. A major focus of this program is the study of the mechanism of oxidative phosphorylation and electron transport, and the energetics of ion and substrate transport across membranes. In particular, the very large number of components now recognized to be part of the electron transport chain poses a formidable challenge to understanding the spatial organization of the complex involved in the synthesis of ATP. This problem is being studied by the use of a wide variety of physical and chemical tools, with the hope that the three-dimensional structure of some of the enzyme components will be available in the near future. On the other hand, the roles of additional proteins in the electron transport chain are still being resolved. An example is the recent purification of the iron-sulfur protein of the $\underline{b}$-$\underline{c}_1$ complex from heart mitochondria, and the postulation of its participation in the oxidation-reduction of ubiquinone.

STRUCTURAL STUDIES OF PROTEINS AND RELATED MODEL SYSTEMS
approximately 172 grants, $9,300,000

This sub-program area represents physical and chemical studies on the structure of proteins, peptides, and synthetic polymers.

Almost one-half of the grants in this area deal with spectroscopic measurements of protein structure. Included are techniques such as nuclear and electron spin resonance, fluorescence, Mossbauer spectroscopy, circular dichroism and magnetic circular dichroism, and Raman spectroscopy.

Important developments continue to emerge as a consequence of the innovative application of powerful new methodologies. Examples are the application of Mossbauer spectroscopy to identify the structural details of iron-sulfur proteins, and the use of nuclear magnetic resonance and Raman spectroscopy to determine the conformation and structural transitions of membrane lipids.

Another major sub-area, comprising about 10 percent of the grants in this program, deals with determination of protein sequences. Such analyses provide data that are used for refinement of X-ray structures, evolution of structure-function relationships near an active or allosteric site, and determination of homologies between different proteins for the purpose of defining genetic and evolutionary relationships. Many of the remaining grants in the program are concerned with experimental or theoretical considerations of protein conformation. A major area of interest continues to be the forces controlling the folding of proteins and the acquisition of stable folded conformations.

DIFFRACTION ANALYSIS OF PROTEINS AND RELATED MODEL SYSTEMS

approximately 59 grants, $3,400,000

This section of the CMBD Program supports diffraction experiments on proteins and other macromolecules. During the last 25 years, X-ray crystallography has made a tremendous contribution to our knowledge of the structure and function of proteins and other biological molecules. It is the unique method that reveals precise atomic positions, and is thus crucial to other research in the field since the catalytic or functional behavior of all biological macromolecules is closely related to the details of molecular structure. The research projects in this section can be classified according to molecular size and method.

Low Molecular Weight Structures - 7 grants, $260,000

X-ray crystallographic studies in this area are concentrated on small biomolecules, such as vitamins, complex lipids found in membranes, polypeptides, antibiotics, and carbohydrates found in a variety of biological systems. With smaller molecules, the crystallographic results are very accurate and can form the basis for stereochemical studies of larger systems. This is especially true for many of the refinement methods, where the macromolecule is constructed from small molecular components to fit the data.

Moderate Weight Proteins - 32 grants, $1,200,000

A decade ago few protein structures had been solved, and most of those were small, monomeric, extracellular proteins involved in hydrolysis. Today, a wide variety of protein structures have been determined. With advanced instru-

mentation and methodology, it is no longer essential to obtain large well-ordered crystals. Proteins are now usually chosen for their biological or medical relevance. Many of the research projects in this group examine such topics as enzyme mechanisms, energy transport, substrate binding and active site stereochemistry, protein folding, protein dynamics, and protein interactions.

High Molecular Weight Structures - 13 grants, $900,000

This section of research projects is characterized by an assortment of both experimental techniques and biological systems. Most of the research utilizes X-ray crystallography, but other methods include low angle diffraction, neutron and electron diffraction, and three dimensional reconstruction. The structure of large regulatory enzymes, enzyme complexes, and viruses can now be solved by X-ray crystallography. Such work would be impossible without recent advances in instrumentation. Knowledge of subunit interactions, quaternary structures, and macromolecular assemblies is needed for investigations of the function of enzyme complexes. A few projects are aimed at large oriented macromolecular assemblies, such as membranes and polysaccharides. The primary tool for this research is low angle diffraction, with results centering on the location of components within a larger biological system.

Refinements and Methods - 7 grants, $260,000

Accurate atomic positions are essential for analyses of protein structure and function, such as substrate binding, active site stereochemistry, and intermolecular interactions. However, crystallographic data from macromolecules is seldom of sufficient quality to permit the determination of accurate atomic positions by traditional crystallographic means. The development of constrained refinements that utilize small biomolecular structures has greatly extended the accuracy of many protein structures. Much of the research in this section involves such refinements. Several other computation methods are also included.

THEORETICAL STUDIES OF PROTEINS AND RELATED MODEL SYSTEMS

approximately 20 grants, $800,000

This small program supports theoretical and mathematical research on a variety of biological topics. The vast complexity of living organisms and biological systems makes relevant theoretical studies difficult in conception, execution, and interpretation. In biology, as in all scientific fields, interesting theoretical calculations are often carried out on simplified and well-defined systems. Theoretical work is crucial to the overall biomedical research program, in that it provides the stimulus for many experiments. In addition, theoretical studies of simplified systems provide the basis for interpretations of experimental data from more complex biological systems.

Structural and Physical Properties of Water and Aqueous Solutions - 12 grants, $560,000

This subprogram focuses primarily on water and its interaction with biological macromolecules. Modern techniques in the fields of statistical mechanics and

molecular quantum mechanics are utilized by the researchers in this section of grants. Research on the structure, molecular dynamics, and physical properties of water and related research (e.g., on topics such as hydrophobic interactions, hydrogen bonds, and electrolytes) lead to an understanding of the vital role water plays in determining the structure and biological function of proteins, nucleic acids, membranes, and other biological molecules.

Molecular Interactions and Potential Energy - 8 grants, $240,000

Various aspects of biomolecular interactions are examined through calculations of potential energy functions utilizing advanced methods of quantum chemistry. In most of these theoretical studies, results are linked to experimental data, and the resulting formulations are then useful in the interpretation and prediction of the molecular properties of proteins and other biomolecules. Problems examined include: protein folding, antibody-antigen interactions, drug-receptor interactions, enzymatic reactions, electronic structures, and spectroscopic, thermal, and lattice vibration analyses. Three other grants include theoretical work on optical rotation theory, microwave effects on biological systems, and isotope effects in biochemistry.

RESEARCH HIGHLIGHTS

Presented below are examples of recent accomplishments from a variety of cell and molecular systems being investigated by grantees of the CMBD Program. These highlights reflect the broad range and kinds of activities currently being supported.

1. Structure and Function of an Acetylcholine Receptor
GM 24485-04 (Robert Stroud), University of California, San Francisco

Dr. Robert M. Stroud is examining the structure of the acetylcholine cell-surface receptor (AcChR), which is fundamental to neuromuscular transmission in humans as well as mammals and fish. It is also a prototype for other kinds of cell surface receptors, such as hormone receptors which are involved in cell-cell, or plasma-cell communication. Dr. Stroud's work has focused on the electric fish Torpedo californica, where the electric organ is essentially a battery stack of electrically excitable cells, from which acetylcholine receptors can be isolated in large quantities.

An initial structural problem was simply to identify the AcChR in electron microscopic images. This was achieved using antibodies made against the purified AcChR, coupled to an electron dense label that can be clearly visualized. Colloidal gold spheres (about 200 Å) were coupled ionically to antibodies and thence specifically to AcChR. In other work, X-ray scattering from purified noncrystalline AcChR membranes, highly enriched in AcChR, revealed that the AcChR protrudes from the membrane by 55 Å on one side of the membrane and very little on the other side. These experiments showed that the AcChR protruded like a funnel on the outside of the electrocyte, the side where the neurotransmitter (AcCh) binds to evoke AcChR response. The funnel shaped molecule has a large central "well" that reaches down to the membrane surface

where the gated ion channel begins. Ultimately Dr. Stroud and his co-workers were able to reconstruct images of AcChR using procedures similar to those used to clean up astronomical images, but also taking advantage of the many hundreds of identically oriented molecular images in two-dimensional crystalline arrays within some of the membrane sheets. The result is a three-dimensional model of AcChR in the membrane sheet, to a resolution of about 20 Å.

Location of the ligand binding site for AcCh is vital for an understanding of the function of AcChR, which changes conformation upon binding AcCh. This is a difficult problem because the receptor, composed of five homologous glycoprotein subunits ($\alpha_2\beta\gamma\delta$) has a large total molecular weight of 250,000 daltons, while the AcCh is small, 132 daltons. In the low resolution image, or even in high resolution views, this would be impossible, especially with the evoked conformational changes in AcChR. Snake neurotoxins provide part of the answer, and reveal much about how they function in preventing any conformational change. Many kinds of neurotoxins bind tightly to the acetylcholine receptor and "lock" it in the closed state. It can no longer become a transmembrane ion channel gated by action of the small neurotransmitter molecule acetylcholine. In fact, neurotoxins also provide the means to further purify the receptor.

Since neurotoxins are such an important tool for studying AcChR, Dr. Stroud has been working on the atomic structure of α-bungarotoxin, a soluble, highly stable small protein of 8,000 daltons (72 amino acids), which is isolated from the Formosan Banded Krait Bungarus multicinctus. Crystals of the protein were grown and many heavy atom containing derivatives made in order to provide necessary phase factors to go with the diffraction amplitudes. The problem was technically difficult, but was recently solved using all the original data in conjunction with a new crystal form of the protein.

In this structure, the polymer chain is folded in three extended loops like three fingers anchored together at the "hand" by four disulfide bonds. The molecule has two almost flat surfaces on either side. Published results on chemical modifications and many neurotoxin amino acid sequences enabled Dr. Stroud to identify many groups which all seem to contribute almost equally to AcChR binding. These are all located on the more concave 20x30 Å surface of the molecule. Most antibodies made against α-bungarotoxin bind tightly to the other more convex surface. The clear indication is that the large concave 20x30 Å surface of α-bungarotoxin folds over the AcChR surface rather than binding at a small area of contact as seen in more usual enzyme-substrate interactions.

The toxin structures available now include two others, a sea snake toxin determined by Dr. B. Low at Columbia, Drs. Tsernoglou and Petsko at Wayne State University and an α-cobratoxin by Dr. Saenger in Gottingen, Sweden. Comparison shows them all to be of the same general structure, but they are surprisingly different around the crucial, evolutionary conserved binding sites.

Further, the toxins like α-bungarotoxin contain a site that mimics AcCh. It seems that this site blocks the AcCh site on AcChR, but the remainder of the binding over an extended area serves to "lock" the receptor in its closed

state. X-ray studies on the toxin-AcChR complex in membranes now begin to show that the toxin is bound to the very outside crest of the funnel shaped AcChR, and indeed anti-toxin antibodies all bind at this point in this complex. The clear indication is that neurotransmitters bind at this same site, some 55 Å-70 Å away from the gated channel. X-ray scattering also shows that AcChR contains 80 Å long α-helical rods perpendicular to the membrane plane and these could play a key structural role in relaying the message from the binding site to the channel.

Dr. Stroud is now attempting to locate the five subunits and to determine the change in their structure induced by AcCh binding. He is using antibody F_{ab} fragments made against each subunit, visualized in the two-dimensional crystalline AcChR arrays. The ion channel is normally opened for only 1 millisecond, but Dr. A. Karlin at Columbia discovered a way in which the channel could be locked open using bromo-acetylcholine, an analog of the physiological effector. Analysis of this form should determine the conformational change which corresponds to conduction of ions and "short circuiting" the membrane from its normal 65 mV voltage gradient. Could the change be like the untwisting of a rope, to generate space through the center, or is it a more subtle set of relays and gates? Most probably the former, but in the final analysis this receptor will provide a basis for understanding cell to cell communication so crucial to the function of higher organisms.

2. Computer Graphics in Protein Conformation Studies
GM 25664-02 (F. Salemme), University of Arizona

With the development and utilization of computer graphic systems, the structures of proteins can be determined more quickly and accurately. These systems, which produce graphical displays of complicated maps and models, are now being used by protein crystallographers at many stages of structural research investigations. Previously, much of this work was done by hand, and was laborious, tedious, and imprecise. Electron density maps were hand-drawn, metal or plastic skeleton models were constructed to fit these maps, atomic coordinates were estimated, and then the process was repeated as the protein structure was refined. In contrast to these difficulties, atomic models generated on computer graphics systems are more accurate and can be easily changed or analyzed. Comparison of this model with the maps is accomplished with various sophisticated computer-controlled display techniques that permit the optimal interaction of the researcher's experience and the system's computational capability.

In addition to their general role in the determination of a protein structure, the computer graphics system can be used as a scientific instrument in the theoretical analysis of protein structure, function, and interaction. Work of this type is being done by Dr. Francis Salemme and his colleagues at the University of Arizona. They are attempting to understand the specific origins of the forces and interactions that are responsible for the attainment of the particular spatial configurations that are observed in proteins of known structure. Many spatially extended arrangements appear as common features among proteins which otherwise bear little similarity in amino acid sequence or function. Although many of these recurrent structural patterns were originally thought to be manifestations of previously unexpected evolutionary relationships,

subsequent interpretations have emphasized that the recurrence of such patterns probably reflects the existence of kinetic or thermodynamic requirements which govern the folding of a protein into a stable close-packed structure.

Dr. Salemme is studying the forces and interactions of various structural arrangements using computational methods aimed at establishing the accessible conformational (and geometrical) degrees of freedom of extended protein structural arrangements. The results of these computations are examined using interactive computer graphics, without which it would be virtually impossible to understand the inter-relationship among various structural arrangements. Thus far, his efforts have focussed upon the conformational and long-range geometrical properties of protein β-sheets and extended packed arrays of α-helices.

In the case of twisted protein β-sheets, it has been possible to show that the long range geometrical properties of these structures reflect the equilibration between: (1) tendencies of the individual polypeptides chains to twist in order to minimize their local conformational potential energy, and (2) the requirements for the optimal interchain hydrogen bonding (which generally tend to resist the introduction of twist into the sheet). The structures which result are therefore isotropically stressed surfaces (similar in general to soap films stretched on twisted wire frames), which, however, may have quite different geometrical properties depending upon both the hydrogen-bonded connectivity and symmetry properties of the particular sheet. The various computationally generated β-sheet structures typically approximate crystallographically observed structures with a precision (less than 1 Å) which gives essentially atom for atom correspondence between computed and observed structures.

Work on α-helical proteins has focussed on establishing the packing constraints and geometrical parameters of extended arrays of α-helices. The results derived from this study explain many of the structural and aggregate properties of proteins as diverse as hemerythrin and tobacco mosaic virus coat protein, and are of likely relevance to the helical structural arrangement found in membrane proteins such as bacteriorhodopsin.

An unanticipated result of these computer graphical/conformational studies carried out on both β-sheets and α-helical arrays is that there appears to exist, in virtually all cases, uniquely defined and continuous pathways for the interconversion of various structural configurations. For example, there are defined pathways by which beta sheets are most readily twisted, or by which one symmetrical helix packing array may be converted to another. These transformations correspond to coupled oscillations in these structures, and so are of potential relevance to an understanding of the thermally excited dynamical behavior in proteins. Since these oscillations involve systems of high effective mass, such cooperative motions may have special relevance to protein catalytic mechanisms, which in many cases clearly appear to require the ordering of chemical and structural events both in space and time.

3. Enzymes in Crystalline State, Structure and Activity
GM 16429-10 (Martha L. Ludwig), University of Michigan

Dr. Martha Ludwig and her colleagues at the University of Michigan have determined the three-dimensional structures of several proteins containing flavin and heme groups. This work has provided insights into some of the processes by which the energy available from the oxidation of metabolites, or from the trapping of light in photosynthesis, is utilized in chemical reactions. The oxidation of intermediates like NADH or succinate involves a series of electron transfers between specialized prosthetic groups such as hemes, flavins and iron-sulfur centers; each of these groups is attached to proteins which are capable of modulating the rate and direction of electron transfer. The direction of electron transfer is controlled by the relative oxidation-reduction potentials of electron donor and acceptor groups, and these potentials can be profoundly affected by the apoproteins to which they are attached. For example, the one-electron reduction of flavin mononucleotide (FMN) from the semiquinone (intermediate state) to the fully reduced state has a potential of -0.18V at pH 7 in the absence of protein, whereas FMN bound to flavodoxin has a corresponding potential of -0.40V. This shift corresponds to a free energy difference of about 5 kcal/ mole. The thermodynamics implies that the stabilities of the semiquinone and reduced flavin are differentially affected by combination with their protein partner, but direct examination of the structures is essential in attempting to understand the potential shifts in terms of bonded and nonbonded chemical interactions.

Dr. Ludwig and her co-workers have analyzed the structures of the three accessible oxidation states of the flavodoxin from Clostridium MP. This work suggested that the very low potential for the equilibrium between semiquinone and reduced forms results partly from destabilization of the reduced state. In the reduced flavoprotein, the aromatic isoalloxazine ring of FMN is forced to be almost planar, whereas in free reduced FMN the equilibrium conformation shows appreciable folding. In addition, the butterfly motion of the ring when free is inhibited upon binding to the apoprotein. On the other hand, the potential for the oxidized/semiquinone equilibrium reflects preferential stabilization of the semiquinone form by formation of a hydrogen bond between the flavin molecule and the protein. No corresponding interaction is found in the oxidized molecule, and a conformation change in the protein is necessary to produce the new hydrogen bond in the semiquinone state.

The generality of these mechanisms can be tested by comparisons with flavodoxins from other species. In addition to the clostridial structures, Dr. Ludwig has determined the structure of the oxidized form of flavodoxin from the blue-green alga (or cyanobacterium) Anacystis nidulans. The orientation of the isoalloxazine portion of FMN, involved in oxidation-reduction, and the course of the chain adjoining the FMN, are very different in algal and clostridial flavodoxins, whereas algal and D. vulgaris flavodoxins (a structure determined by Jensen and co-workers, GM 13366-15) are more nearly superimposable in three dimensions. Preliminary data at low resolution indicate that the reduced flavin in algal flavodoxin must be constrained to a nearly planar conformation, just as is the ring in reduced clostridial flavodoxin. Unlike D vulgaris or clostridial flavodoxins, algal flavodoxin has a potential for the oxidized/semiquinone equilibrium

which is close to the value for free FMN; hence there should not be any preferential stabilizing interactions between FMN and protein residues when these higher oxidation states are compared. The close isomorphism of the semiquinone and oxidized crystals of algal flavodoxin suggests that, in contrast to the other species, algal flavodoxin does not undergo a conformation change on reduction to the semiquinone state. Analysis at higher resolution will be necessary to verify the similarity of the oxidized and semiquinone conformations.

Similar comparisons can be made with the cytochromes, which contain hemes as prosthetic groups. Soluble c-type cytochromes serve as electron carriers in both respiration and photosynthesis. The structures of several cytochromes c from very diverse procaryotic and eucaryotic organisms have previously been determined (Dickerson and co-workers, GM 12121-15, and Salemme and co-workers, GM 21534-06). From their results, it is clear that in cytochromes with redox potentials in the range 0.15 to 0.35V, the heme resides in an environment of low dielectric constant, with the few polar or charged groups (such as the heme propionate) compensated by local non-covalent interactions. Oxidation of these cytochromes, which produces a charge on the iron, is energetically unfavorable in the low dielectric environment, and hence the redox potentials are rather high on the biological scale. Dr. Ludwig's studies of cytochrome c_{554} from cyanobacterium, A. nidulans, show further structural variations which can be accommodated in the heme environment of a functional cytochrome c. In particular, the interaction between tryptophan and one heme propionate group, found in all other cytochrome structures, appears to be absent in algal cytochrome. Instead, alternative mechanisms of local hydrogen bonding are employed to stabilize the charged heme propionate in the hydrophobic heme pocket.

The picture which emerges from these structural studies of the control of redox potentials is that proteins exploit local microenvironment, steric hindrance, and non-covalent interactions to control the potential for electron transfer. The conformational states and stability of prosthetic groups are thus altered by proteins in ways which are analogous to the stabilization of transition states in enzymatic reactions.

4. Evaluation of Protein Structural Dynamics
GM 18051-08 (H. Frauenfelder) U. of Illinois, Urbana-Champaign

In all areas of research certain unexpressed assumptions influence the design of experiments and the interpretation of results. A "paradigm" of this type (as Kuhn has defined it) in the area of protein structure is that proteins are essentially rock-like crystals, with very little flexibility in their "native" state. This concept has been slowly modified over the years, with the acceptance of induced changes on substrate binding and the evolution of the allosteric models in regulatory proteins. However, it appears that a striking change in the general concept of protein structure has occurred recently. This current paradigm states that proteins are fluctuating systems, which can exist in a very large number of conformational substates. An even more striking description is that globular proteins have "a fluid-like interior, atoms displaced from their equilibrium position by up to 2A, and fluctuations on a picosecond scale."

Much of the impetus for this current concept comes from the theoretical studies of Karplus and his coworkers, and from the experimental work of Frauenfelder and his colleagues. A major focus of both these groups has been the study of O_2 and CO binding to hemoglobin and myoglobin. X-ray studies of both proteins have indicated that entry from the solvent to the heme site is blocked by certain amino acid side chains. Theoretical calculations carried out by Case and Karplus demonstrated that protein conformational fluctuations involving the concerted motion of a number of residues can reduce the barriers along two well-defined paths to values that make the heme pocket accessible at ambient temperature.

An essential basis for the theoretical work was the kinetic studies of Frauenfelder and his team. These experiments involved the rebinding of CO and O_2 to myoglobin (Mb) following photodissociation. The bound Mb is placed in a cryostat and the ligand photodissociated by a laser flash. Subsequent rebinding is followed optically from 100ns to 1 ks. For O_2 binding, the results suggest a pathway involving three sequential barriers, i.e.,

$$A \rightleftharpoons B \rightleftharpoons C \rightleftharpoons E$$

A represents Mb-O_2, and during photo dissociation the Fe-O_2 bond is broken and the O_2 moves to substate B. At low temperatures, O_2 cannot leave the protein, and returns directly to state A. With progressively higher temperatures, the probability of further diffusion through the protein matrix increases, until at the highest temperatures the ligand is capable of being released to the solvent (state E.)

These barrier properties revealed by the kinetics are presumably consequences of "substates" in the protein configuration, which are themselves generated by local fluctuations in its structure. Frauenfelder's studies on CO binding to myoglobin suggests the presence of four barriers. The innermost is a result of binding to the heme, and the outermost is due to the transition movement of ligand from the solvent to the protein matrix. The two intermediate barriers correspond to those calculated by Case and Karplus. The activation energies obtained experimentally are crude, but of the order of those predicted theoretically.

Recent developments have refined and advanced these studies. Frauenfelder et al. recognized that their assumption of static and temperature independent barriers was inconsistent with the model of a protein as a dynamically fluctuating system. They were able to show that the dynamic aspects of barriers in the binding of ligand to heme protein could be treated by introducing solvents of different viscosities as a new variable. The improved description of rate constants which resulted can be interpreted by a dynamic model in which transitions into and within heme proteins are governed by fluctuations between conformational substates.

The theoretical and experimental studies described buttress the model of fluctuating protein structure. Further work will surely provide new insights into the linkage of structure and function in proteins.

5. Photoaffinity Probes for Nucleotide Binding Sites
GM 21998-06 (B. Haley), U. of Wyoming

A standard approach to identifying binding and active sites of proteins is to attempt some form of chemical modification. Many of the reagents classically used were non-specific, and directed to a specific functional group (or groups) on the protein, regardless of location. Examples are the many sulfhydryl-directed reagents, and those which form covalent linkages with amino groups. More recently, specific active site-directed approaches have been developed. These are affinity labeling and "suicide inhibition." "Affinity" reagents generally contain most of the structural properties of the naturally occurring substrates plus an added reactive group which has the potential to form an irreversible covalent bond with an amino acid residue located within the active site. "Suicide inhibitors" make use of the catalytic function of enzymes to produce a compound from the substrate which irreversibly combines with the active site of the enzyme. Both of these approaches involve reactant groups that require an electrophilic or nucleophilic acceptor at the active site. Additional shortcomings are that affinity labels can also react at regions other than the active site, while suicide inhibitors require a catalytic event to be effective, and thus cannot be used to label allosteric sites or noncatalytic binding sites.

Photoaffinity probes have several advantages as labelling reagents: 1) They are analogs of natural substrates for catalysis or binding, and therefore are highly selective; 2) the reactive group is not generated until exposed to light; this allows activation in situ with less possibility of non-specific labelling; and 3) the reactive groups on the substrate are usually diazo or azido derivatives. When exposed to light, these generate carbenes or nitrenes which can react with C-H bonds, and thus do not require the presence of an especially reactive group in this protein. (Much of this work has been developed by F.H. Westheimer, GM 04712.)

Dr. Haley has developed a very effective nucleotide derivative which can act as a photoaffinity probe. This may be an 8-azido derivative of cAMP, cGMP, GTP, ATP, or deoxy ATP. Given the very large number of biological systems which utilize one or another of these compounds, the problem is which systems not to study. A sample of the systems studied by Haley and his collaborators include tubulin, DNA polymerase, acyl CoA transferase, protein kinase, and adenylate and guanylate cyclase.

A particularly good example of the use of such photoaffinity probes is in the study of tubulin polymerization. Geahlen and Haley examined several disputed points in the role of guanosine nucleotides in the polymerization process, using 8-azidoguanosine derivatives. The first question involved the location of GTP binding sites on tubulin. It is known that the tubulin heterodimer has both nonexchangeable and exchangeable GTP binding sites. The exchangeable (E) site was examined using [γ ^{32}P]8-N_3GTP. Incubation of tubulin with the derivative followed by photolysis showed that label incorporated into the α monomers was characteristic of non-specific labelling, while the β monomer labelling followed a pattern indicating a mixture of both specific and non-specific incorporation. Subtraction of α incorporation from β incorporation resulted in a typical saturation curve. In a confirmatory experiment, cold GTP competed with labelling by [γ ^{32}P]8-N_3GTP of the β monomer, but not of the α monomer, as would be expected for specific and non-specific sites, respectively.

A second question was the involvement of E-site GTP in the polymerization process. It is known that such GTP is hydrolyzed in the polymerization process, but the functional significance of the hydrolysis is not clear, i.e., does GTP hydrolysis occur as a result of polymerization, following polymerization, or unrelated to polymerization. A series of studies suggested that hydrolysis occurred in situ at a rate comparable to the rate of polymerization. This indicated that polymerization and hydrolysis of E-site GTP were tightly coupled. Studies of this kind demonstrate the power of photoaffinity reagents in probing protein-ligand interactions.

6. Recent Developments in Understanding the Structure and Function of Iron-Sulfur Proteins
GM 12394-16 (H. Beinert), GM 17170-08 (W. Orme-Johnson), U. Wisconsin; GM 12176-16 (R. Sands), GM 11106-17 (V. Massey) U. Michigan; GM 21337-05 (G. Palmer) Rice U.; GM 16406-12 (P. Debrunner) U. Illinois, Urbana-Champaign; GM 12202-16 (D. Wilson) U. Pennsylvania; GM 22352-05 (R. Holm) Stanford U.; GM 13366-15 (L. Jensen) U. Washington

One of the striking developments in biology in the last decade has been the identification of a totally new class of biological materials, the iron-sulfur (Fe-S) proteins. What is particularly significant is that at least some of these proteins appear to be major constituents of what was thought to be among the best-understood biological systems, the electrontransport chain. The development of this field also demonstrates how sophisticated physical techniques, applied originally to non-mammalian systems, generated results of great importance to the understanding of fundamental biological processes.

Many, perhaps most, of the investigators whose names are associated with the recent development of this field are supported by NIGMS. These include those mentioned above: Drs. Beinert, Sands, Palmer, Orme-Johnson, Wilson, Massey, Debrunner, Holm, and Jensen.

Iron-sulfur proteins are defined as non-heme containing proteins where the iron is coordinated to sulfur, from either cysteine or inorganic sulfur. It is now clear that, in general, these proteins act as electron carriers in such diverse processes as carbon metabolism, nitrogen fixation, photosynthesis, and steroid hydroxylation. More recently, they have been found to be components of mitochondria, and implicated in the oxidation-reduction process. The Fe-S proteins have been found in a wide range of organisms, from the primitive anaerobic bacteria to higher plants and animals.

The first Fe-S protein was discovered in C. pasteurianum, and was soon found to be interchangeable with a similar class of proteins which had been identified in plants. Both plant and bacterial proteins catalyze the photoreduction of NADP. This is a striking observation, since it means that the ferredoxins have extremely negative oxidation-reduction potentials. In fact, the spinach ferredoxin was shown to have a potential of -420 mV at pH 7, equivalent to that of the hydrogen electrode.

The ferredoxins are small proteins (6 -12000 MW) with a rather featureless absorption spectrum. They were recognized as non-heme containing iron proteins at the time of their discovery. Not long after, it was recognized that they contained equivalent amounts of non-heme iron and "acid-labile" or "inorganic"

sulfide. This was the result of experiments which demonstrated the release of H_2S upon acidification. The plant-type ferredoxins contain two iron and two acid-labile sulfur atoms per molecule, while the bacterial ferredoxins have eight irons and inorganic sulfurs, and the so-called "high-potential iron proteins" (HiPIP) have four. Fe-S proteins are now commonly classed as containing 2,4, or 8 iron atoms.

At about the same time as the purification and chemical characterization was underway, Beinert and Sands were reporting an ubiquitous and unusual EPR signal which they had observed at low temperatures (about 80°K). They showed that an asymmetric EPR signal with a major component at g=1.93-1.95 can be detected at low temperatures under reducing conditions in many organisms and biological preparations. The source of the g=1.94 signal was identified as iron, and it was only observed under strong reducing conditions. Further investigations strongly suggested that the iron was liganded both to cysteine and to inorganic sulfur. More recently, application of Mossbauer, NMR, and ENDOR spectroscopy has established in the 2-iron, 2-sulfur ferredoxins that a) the oxidized forms contain two high-spin ferric iron atoms; b) the reduced forms contain one ferric site and one high-spin ferrous site; and c) the iron bonding at all sites is covalent.

Since the EPR studies showed that the iron and the acid-labile sulfur are at the active site where the electron transfer takes place, it is important to know the details of active site structure. X-ray crystallography has provided accurate structures for HiPIP and P aerogenes Fe-S proteins. These both contain 4Fe-4S active sites. (Good crystals are not yet available for 2Fe-2S proteins.) The structure of the active site is shown in Fig. 1.

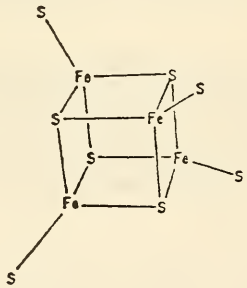


Fig. 1

The structure of the 2Fe-2S proteins has been inferred from spectroscopic and chemical data, and is shown in Figure 2.

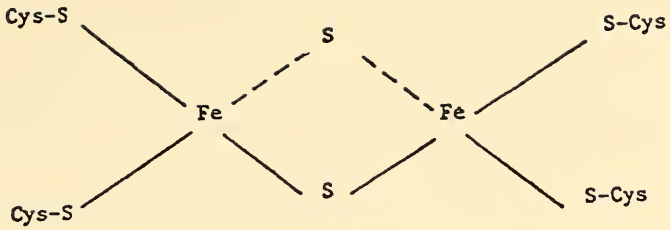


Fig. 2

The primary structures of a number of ferredoxin's, as well of the HiPIP from Chromatium, have also been determined.

What is truly remarkable in this relatively new research area is that in the last 15 years an entirely new class of proteins have been isolated and characterized, and that these are ubiquitous and abundant in biological systems. The key question, of course, is to understand their function. Our information on this point is still quite spotty. In the anaerobic bacteria, the ferredoxins play several roles. These include acting as a redox protein in the fermentation of organic compounds, nitrogen fixation and H_2 metabolism. In contrast, the function of the high-potential iron protein in photosynthetic bacteria is virtually unknown. Most exciting, however, has been the finding of numerous Fe-S centers in mitochrondria. It has been over 40 years since the contributions of Keilin laid the foundation for the construction of the electron transport chain. Until the early 1950's, the composition of this chain was generally considered to be clearly understood, even if its operation was still unclear. However, the make-up of the electron transport system was determined principally by the tools used to define it. This was largely absorption spectroscopy. Of course, NADH- and NADPH- linked dehydrogenases, flavins, and cytochromes all have prominent absorption characteristics in the near-UV and visible regions of the spectrometer. In contrast, the Fe-S proteins lack such optical features, and required EPR spectroscopy for their identification. (Parenthetically, one may wonder just how many other "well-understood" biological systems may be limited by the tools used to examine them.)

The existence of Fe-S proteins adds new uncertainty to the structure of the electron transport system, and the research is still too incomplete to provide a coherent picture. For example, NAPH dehydrogenase has been shown to be a Fe-S protein involved in oxidoreduction. There has been some evidence relating this system to ATP formation at site I, but this remains controversial. Even more surprising, aconitase has been shown to be a 2Fe-2S protein with an oxidation-reduction potential similar to HiPIP. Now, aconitase is a well-known and, presumably, well-understood component of the citric acid cycle. The dehydrogenation which it catalyzes does not involve an electron transfer. Either aconitase also catalyzes some unknown reaction, or Fe-S proteins may play some unknown role in non-oxidative reactions.

It is clear that this area is still in flux, and research is actively progressing in both the biological and biophysical aspects of the Fe-S systems. The history of this problem provides striking evidence of how new techniques, (in this case EPR), can revolutionize even an apparently established area of biology.

7. Molecular Events in Fertilization
GM 23910-03 (B. Shapiro), University of Washington

Among the many similarities in the events of fertilization between invertebrates and mammals, the involvement of Ca^{++} is striking. Calcium ion has been implicated in the activation of the sperm in the acrosome reaction and in the activation of the egg in the cortical reaction. In an analysis of ion movements involved with triggering the acrosome reaction in sea urchin sperm, Dr. Bennett M. Shapiro of the University of Washington found that calcium ion uptake occurs during the reaction and also that two inhibitors of ion movements, verapamil (a blocker of Ca^{++} channels) and tetraethylammonium (TEA; a blocker of K^{+} channels) block the acrosome reaction. These observations suggested that agents like verapamil and TEA, directed toward inhibition of specific ion movements required for fertilization, might have a similar effect in mammals and thus might block mammalian fertilization, for which the acrosome reaction is a necessary prerequisite. To investigate this possibility further Dr. Shapiro and his group explored the effects of these drugs on in vitro fertilization of mouse eggs.

They reported, in a recent paper in Gamete Research that a high level of fertilization can be obtained in vitro with gametes from C57BL/6 mice in a modified Krebs-Ringer-bicarbonate medium. Secondly, they demonstrated that calcium ion is required for in vitro fertilization in mice. And finally, that verapamil and TEA inhibit mouse fertilization in vitro.

These results suggest that Ca^{++} and K^{+} movements are required during fertilization in the mouse, and thus that studies of ion movements in the sea urchin may be a valid model of mammalian fertilization. In order to overcome a concern in interpreting these studies, which is that the inhibitors used may have effects on processes other than ion flow that account for their inhibitory effect, a somewhat more direct test was devised. TMA, a quaternary amine closely related to TEA but far less effective in blocking K^{+} channels, was used in the in vitro fertilization system. In these experiments TEA had an effect substantially greater than TMA, even at the high concentrations employed.

This demonstration of specific ion movements required for in vitro fertilization in the mouse complements the work of others and is part of the growing demonstration of a generalized role of calcium ions in fertilization, from sea urchins to mammals. The idea that ion movements play an important role in the acrosome reaction and, therefore, fertilization, and the inferred existence of ion channels in sea urchin sperm by using agents that block them serve as models for further studies of fertilization in mammaliam systems. Further, while additional work is needed to determine whether the drugs verapamil and TEA are actually blocking ion channels in the mouse system, such a confirmation would suggest consideration of compounds with similar actions as possible agents in contraceptives.

8. Differentiated Surface Domains in Hepatocytes
GM 23669-03 (A. Hubbard), Yale University

Dr. Hubbard's work is based on the apparent paradox between the "fluid mosaic model" of membrane structure and the requirements of specialized differentiated cells. She asks "How can there be fluidity and random mobility of molecules in a membrane where specialization of well-defined areas is essential for accurate contacts with other cells and for polarized transport?" She has been studying liver parenchymal cells, in which one domain is specialized for exchange of metabolites with blood, one domain for bile secretion and one for cell attachments and cell-cell communication. Each domain has a distinctive membrane composition.

Dr. Hubbard has succeeded in isolating plasma membrane from hepatocytes. Through some clever modifications of standard isolation procedures, she has succeeded in obtaining membrane proteins from all three domains. One example from the blood exchange domain is the asialo-glycoprotein (ASGP) receptor. The marker leucine aminopeptidase is from the bile front, while gap junctions, desmosomes and associated protein complexes are from the attachment zone. Dr. Hubbard has reported some success in separating the membrane proteins into three fractions, each enriched in the proteins of one front. She has also prepared EM cytochemical markers, such as lactosaminated-ferritin (which binds specifically to ASGP receptors) to localize the ASGP receptor both in the plasma membrane and within the possible subcellular compartments which might lie along its pathway of synthesis and insertion.

Much of the groundwork has now been laid to answer the following questions. 1. To what extent are the functionally and topographically distinct domains of the hepatocyte cell surface also distinct in molecular composition? 2. To what extent are the "life cycles" of glycoproteins among and within the different cell surface domains similar or distinct? 3. To what extent are the cell surface distributions and "life cycles" of membrane glycoproteins altered when cell polarity is disturbed and then re-established?

One of the central questions of development biology is how does a cell with one nucleus, and apparently one set of protein synthetic machinery, apportion specific proteins to specific cell locations. In the case of membrane proteins, this is compounded by the problem of the maintenance of membrane regionalization despite the fluidity of lipid bilayers. While this work of Dr. Hubbard is still somewhat preliminary, it holds good potential for making an important contribution toward subsequent solution of these problems.

9. Membrane, Vesicle and Clathrin Cytoskeletal Activity
GM 26829-02 (W.J. Schook), Mt. Sinai School of Medicine, New York

The nature of cellular endocytosis and exocytosis is of great interest. Intracellular vesicles are secreted (exocytosis) and in some cases, as at the neuromuscular synapse, the vesicle membrane and associated proteins have been shown to be recycled. Thus there is little or no addition of the vesicle membrane to the plasma membrane during synaptic secretion and many other types

of exocytosis. Endocytosis has recently been shown to be a significant process in the internalization of some extracellular messengers, such as the hormone EGF or the cholesterol transport complex, LDL. In both cases the membranous vesicles are maintained in their spherical form by a complex characterized in the EM as "coated vesicles." The spherical lipid shell is surrounded by a basket of protein displaying some observable order. This basket material appears to resemble the substance underlying the coated pits. These pits are the loci of the internalized receptors, such as that for LDL. In 1974-75, Pearse isolated a protein from porcine brain and adrenal medulla coated vesicles which she named clathrin. The isolated protein has been shown to form vesicle-sized baskets in vitro.

Dr. William Schook has been investigating the conditions for in vitro assembly of clathrin, and has perfected a purification procedure. He has been looking at: 1) the regulatory proteins involved in lattice assembly and disassembly; 2) the conditions by which the various lattice and filamentous forms are formed and interconverted; and 3) the binding of clathrin and its associated proteins to both synthetic vesicle and authentic synaptosomal fragments. In the past year Dr. Schook has demonstrated that two polypeptides which copurify with clathrin are the and subunits of cellular tropomyosin. Identification involved column comigration, antibody crossactivity, comparison of digest fragments, and the formation of appropriate paracrystalline structures. Removal of the tropomyosin from clathrin prevents basket formation. It appears that clathrin and tropomyosin interact to form a stable complex and this complex is necessary for basket formation. Clathrin has also been shown to have binding affinity for actin and -actinin. This affinity may be important in the mechanism of coated vesicle formation.

The antibodies obtained from bovine brain clathrin have been used to detect the antigen in other cell types via immunofluorescence. A pattern of densely stained spots throughout the cytoplasm can be seen in fibroblasts, endothelial cells, glial cells and platelets among others. It appears that clathrin is a widely occurring molecule, probably playing an important role in many cells, interacting with contractile and structural proteins known to be central in the biochemistry of cell movement.

10. Membranes in Hypertension and Other Syndromes
GM 25686-02 (D. Tosteson/M. Canessa), Harvard University

Sodium intake has long been known to influence the course of essential hypertension. Furthermore, there is good evidence that the predisposition to essential hypertension is, at least in part, inherited. Recent investigations into the relationships of membrane transport and hypertension have produced some significant and unexpected results.

Sodium is transported through several pathways across the human erythrocyte membrane. The best known is the Na/K ATPase. This path is specifically blocked by ouabain. Several other pathways have been described that are ouabain-insensitive but can be distinguished by the sensitivity to other ions, their selectivity and kinetics. One of these is the sodium-lithium countertransport. This system facilitates the exchange of sodium for sodium, lithium for sodium,

or lithium for lithium. It prefers lithium to sodium and will not accept any other monovalent or divalent cations. This sodium-lithium countertransport system has been studied for several years by Dr. Daniel Tosteson, now of Harvard Medical School. As he has noted, in vivo it is not exposed to lithium and so serves as a sodium-sodium exchanger.

Dr. Tosteson has expanded this biophysical work in collaboration with Dr. Mitzy Canessa. Dr. Canessa has been particularly interested in the possibility of a relationship between essential hypertension and sodium transporters. These investigators, and other co-workers, measured the maximum rate of lithium-sodium countertransport in 62 subjects, 36 with essential hypertension and 26 normal controls. The values for countertransport, in nmol lithium/liter of cells/hr was 0.24 ± 0.02 (S.E. of mean) in controls and 0.55 ± 0.02 in hypertensives. This difference was significant in both men and women. Data from five patients with secondary hypertension, including estrogen-induced, or those with polycystic kidney or renovascular problems, were all within the normal range. First degree relatives of hypertensive subjects had lithium transport values in the hypertensive range, 0.54 ± 0.05. Treatment of hypertensive subjects with hydrochlorothiazide, a diuretic, failed to reduce Na/Li countertransport even when effectively controlling blood pressure.

Although this work does not resolve the mechanism of essential hypertension, it suggests several new avenues of approach. It does hold immediate promise of development of a major diagnostic tool to distinguish essential from secondary hypertension. In many cases this would obviate the need for extensive tests in attempting to determine the causes of hypertension. Moreover, the discovery of a parameter which is not changed by treatment, and is high in all essential hypertensives, and in close relatives as well, opens up a major avenue of research into the genetics of hypertension. Dr. Canessa has outlined a proposal to that effect. This is a textbook case of the extension of basic, in this case biophysical and biochemical membrane studies, to an area of great clinical significance.

11. Biologic Studies with Liver Peroxisome Proliferators
GM 23750-04 (J. Reddy), Northwestern University

Peroxisomes are ubiquitous cytoplasmic organelles containing catalase, several hydrogen peroxide-generating oxidases, carnitine acetyltransferase, as well as enzymes involved in the β-oxidation of long-chain fatty acids. Although the exact cellular function of peroxisomes is not known, they undergo rapid proliferation in response to certain agents. Dr. J.K. Reddy and his coworkers at Northwestern University have been studying this proliferative process, with support from GM 23750, in order to determine its mechanisms and consequences.

Among the agents which cause peroxisome proliferation is clofibrate, the drug most widely used for the treatment of hyperlipidemias in Europe and the U.S. Like other potent hypolipidemic agents, it causes hepatomegaly when administered to rats, mice, or hamsters. This hepatomegaly is characteristically associated with a marked increase in peroxisomes in the liver cells of all three species. The activities of the characteristic peroxisome enzymes are also elevated in association with drug induced peroxisome proliferation in liver. Since hepatomegaly and peroxisome proliferation persist for

as long as the drugs are administered in animals, Dr. Reddy and his coworkers initiated chronic toxicity studies with selected hepatic peroxisome proliferators.

Five structurally unrelated hypolipidemic compounds, clofibrate, nafenopin, Wy-14643, BR-931, and tibric acid induced hepatocellular carcinomas when administered to mice and/or rats. This observation supports Dr. Reddy's hypothesis that potent hepatic peroxisome proliferators are, as a class, carcinogenic. Further substantiation of this theory is important not only because hypolipidemic drugs are used extensively and on a long-term basis in clinical medicine, but because other peroxisomal proliferators such as plasticizers are ubiquitous contaminants of our environment.

While not statistically significant, an usually high number of deaths among clofibrate treated hypercholesterolemic men due to malignant neoplasms of the liver, gall bladder, and intestine was demonstrated in a recent report from the World Health Organization. The mean duration of clofibrate therapy in this trial was 5.3 years, and individual subjects were followed for only one year after leaving the trial. Since a 20 to 25 year latent period generally exists between initial carcinogen exposure and appearance of cancer in human populations, longer observation periods are clearly needed.

Since the ability to damage DNA is a property shared by a wide variety of physical and chemical carcinogens, Dr. Reddy tested a number of these hypolipidemic drugs in a lymphocyte ^{3}H-thymidine incorporation assay and the Ames Salmonella/microsome assay. Although the hypolipidemic drugs did inhibit ^{3}H-thymidine incorporation into replicating DNA of proliferating lymphocytes, this inhibition was reversed by a 3-hour incubation of drug-treated lymphocytes in fresh drug-free culture medium. The drugs behaved, therefore, as chemical agents which inhibit DNA replicative synthesis by metabolic means rather than like agents capable of interaction and damage of cellular DNA. The apparent absence of hypolipidemic drug reactivity toward DNA, indicated by the results with the lymphocyte assay, was confirmed by results with the Salmonella/microsome assay, since none of the drugs were mutagenic.

Dr. Reddy has identified an additional consequence of the administration of hypolipidemic drugs, or industrial plasticizers such as di-(2 ethylhexyl) phthalate, to rats and mice. These chemicals cause, concomitant with peroxisome proliferation, an increase in the amount of an 80,000 molecular weight polypeptide in the liver. The synthesis of this polypeptide, whose localization is consistent with its being in peroxisomes (although not definitely established as confined to this organelle) is not induced by drugs which cause proliferation of the smooth endoplasmic reticulum. This polypeptide is a normal constituent of the liver, and all peroxisome proliferators tested appear to stimulate the same polypeptide as determined by molecular weight and antigenic properties. Dr. Reddy has established that the polypeptide is <u>not</u> catalase, a major peroxisome constituent. Other workers in the field have tentatively identified this polypeptide as a multi-functional protein displaying enoyl-Co-A hydratase and hydroxyacyl Co-A dehydrogenase activities. Confirmation of this identification will open the way to understanding the role of peroxisomes in lipid metabolism, but the link to carcinogenicity demands much additional investigation.

12. Immunoadsorbents for Specific Immunotherapy
GM 23517-03 (D. Terman), Baylor College of Medicine

There is now considerable evidence that anti-DNA antibodies and immune complexes are important in the immunopathogenesis of systemic lupus erythematosus (SLE). In SLE, the kidney is involved in 50 to 70 percent of cases, and among the glomerular lesions, granular subendothelial immune deposits (presumably DNA-anti-DNA antibodies) occur. Patients with diffuse proliferative glomerulonephritis are usually treated with non-specific immunosuppressive agents such as prednisone. An alternate therapeutic approach, being developed by Dr. Terman and his colleagues, is to extract pathogenic circulating immune reactants by means of an extracorporeal system of specific immunoadsorption and plasma filtration.

A patient with known lupus erythematosus was admitted for evaluation of nephrotic syndrome. Her serum showed abnormal values for immune complexes, single stranded DNA (ssDNA) binding antibody, and native DNA (nDNA) binding antibody; serum creatinine and urinary protein excretion were abnormally high. Prednisone, an anti-inflammatory steroid, was administered throughout the case study. A renal biopsy specimen taken on the 14th day revealed severe membranoproliferative glomerulonephritis with extensive subendothelial deposits. By the 27th day of the prednisone treatment, nDNA binding levels were in the normal range. Although ssDNA binding levels, immune complexes, and creatinine had also declined, they remained stabilized at abnormally high values.

On day 35, an extracorporeal circuit was set up in which plasma was separated, filtered, passed through a DNA collodion charcoal column, and finally passed through another filter. Plasma was then remixed with the formed elements of the blood and returned to the femoral vein. Anticoagulants were used. As the result of the treatment of 4000 ml of plasma, ssDNA binding in serum declined from 42 percent to 6.5 percent; that is, to within the normal range, nDNA levels were not affected. No significant changes in other blood parameters were immediately evident following the treatment. Following the perfusion, for the duration of the case study (28 days) the ssDNA binding did not rebound substantially, remaining in the normal range. Immune complexes declined further, and there was a progressive reduction of serum creatinine and considerable diminution in urinary protein excretion. The patient died of other SLE related consequences on day 28 following the perfusion. A post-mortem examination of both kidneys revealed that the electron-dense sub-endothelial glomerular deposits were much reduced compared with those in the initial biopsy.

This is the first reported attempt to extract anti-ssDNA antibodies and immune complexes selectively from the circulation of a patient by extracorporeal immunoadsorption and filtration. It appears that the anti ssDNA antibodies were removed by the DNA column, while the immune complexes were removed by the filters. Few clinical ill-effects were observed. Shortly after the extracorporeal procedure, renal function improved considerably; the decrease in the subendothelial deposits in the kidney is consistent with this. The improvement in renal function and ultrastructure in the days that followed the reduction in ssDNA binding and immune complexes in plasma by the perfusion suggests that these substances were, in part, contributing to the glomerulonephritic process. While the reported findings on this single patient are extremely interesting, further work is needed to provide stronger correlations between the perfusion and the observed results of improved kidney function.

RESEARCH TRAINING

The Cellular and Molecular Basis of Disease Program supports predoctoral and postdoctoral research training in the broad range of fields encompassing cellular and molecular biology. Approximately one-half of the total NIGMS training effort is administered by the CMBD Program. In turn, the Institute accounts for two-thirds of the predoctoral trainees supported by all of the NIH. Research training at NIGMS, as well as at the whole of NIH, is supported under the authority of the National Research Service Award Act (NRSA) of 1974. With the advent of that legislation, predoctoral research training at the NIGMS has been in the form of interdisciplinary or interdepartmental training grants.

Postdoctoral support is provided both through training grants and individual fellowships. The research training program is essential for the long term mission of the CMBD Program since it fosters the development of a cadre of first class young scientists in the areas of cellular and molecular biology. These scientists will be among the leaders in research supported throughout the NIH over the next several decades.

Predoctoral training in the CMBD Program is provided in two areas: the Cellular and Molecular Biology (CMB) area and the Medical Scientist Program (MSTP) area. CMBD training support for postdoctorals is provided through training grants of its Basic Pathobiology area and through Cellular and Molecular Biology individual fellowships.

For the first time since the inception of the NRSA, stipends were increased this year (from $3900 to $5040 for predoctoral trainees and from $13,180 to $18,780 for postdoctorals). The NIGMS research training budget increased only slightly, and substantial reductions in trainees were necessary. The number of trainees in the MSTP was not reduced, in deference to concerns about the decreasing number of young clinical scientists. However, the numbers of other CMBD training programs were reduced to accommodate the reduced budget and the increases in stipend and tuition.

1. Cellular and Molecular Biology Institutional Predoctoral Training Program (51 grants, 991 trainee positions, $10,560,000)

These multidisciplinary training grants are designed to provide broad, fundamental research training in the biomedical sciences. The training faculty are generally drawn from several departments, and are chosen on the basis of their excellence in research and research training. A great deal of variation in composition, emphasis, and objectives exists among the various programs. The NIGMS training guidelines are not designed to discourage these differences, but rather to provide trainees access to course work and thesis research opportunities in an array of different research areas. The Cellular and Molecular Biology training grants are unique in their broad coverage of the biomedical sciences, with faculty and students from many disciplines, such as biochemistry, biophysics, microbiology, immunology, biology, cell biology, molecular biology, genetics, anatomy, pathology, physics, chemistry, physiology, pharmacology, and neurology.

A majority of these training programs have ended their final year of support under the initial five year awards. The Cellular and Molecular Basis of Disease Review Committee evaluated these applications, with overriding emphasis on quality -- of the faculty, the trainees, and the program.

Faced with a reduction of over 20 percent in total number of trainees, the CMBD staff chose to reduce the number of trainees recommended for each award made: new, renewal, and ongoing, though the committed dollar amounts were maintained for the latter. It was thus possible to make renewal awards to all well-recommended programs.

2. Medical Scientist Institutional Predoctoral Training Program
(23 grants, 665 trainee positions, $8,500,000)

The goal of the Medical Scientist Program is to assist medical schools and their universities in the development and operation of programs of combined scientific and medical training to highly motivated students of outstanding research and academic potential. Each institution is encouraged to utilize its unique strengths in developing its program, and each candidate's training over a six to seven year period should draw, as needed, on the full range of scientific and medical opportunities available within the institution. Trainees are awarded the combined MD-PhD degree upon successful completion of their medical-research training.

Eleven of the ongoing programs have undergone review for the renewal of their programs during the past year, ten successfully, with one awaiting a decision as to funding. One new application competed sucessfully bringing the total number of programs to 23. Although funds for this program area were increased over one million dollars during the past year, the number of trainees under support remains level: 666 in FY 79 and 665 in FY 80.

3. Basic Pathobiology Institutional Postdoctoral Training Program
(10 awards, 50 trainee positions, $1,000,000)

The goal of this program has been to provide to individuals holding a professional degree training that provides an indepth knowledge of the principles and methods required for research at the cellular and molecular level in normal and diseased states and for post Ph.D's from the basic biological, biochemical, and biophysical sciences advanced interdisciplinary training for research on fundamental problems of human disease.

This has been the most difficult of all CMBD training programs to bring into full fruition -- largely because of the difficulty of attracting post-MD's into research careers. Because of this, many programs have concentrated on post-Ph.D's and as a result, the hoped-for dialogue across the basic/clinical interface has been inadequate. A number of these programs have come up for review and some have not fared well during

review. The number of trainee positions has fallen from 117 in FY 79 to 50 in FY 80. In order to strengthen their programs, several applications submitted during the past year have sought post MD-Ph.D's who require a significant period of postdoctoral research training if they are to remain active in research. This is now being encouraged in combination with their residency training in a clinical specialty as well, where possible.

4. Cellular and Molecular Biology Individual Postdoctoral Fellowship Program (157 awards, 157 trainee positions, $2,300,000)

The Cellular and Molecular Biology Postdoctoral Fellowships under the National Research Service Award Act, are provided to enable individuals holding the Ph.D. or a professional doctoral degree to acquire special advanced research training to obtain the necessary cross-field knowledge for a research career in cellular and molecular biology. We continue to receive high quality applications that encompass the entire spectrum of the program in areas (such as membrane structure and function; cell motility; differentiation and growth; enzyme catalysis and regulation; protein structure and function and related biophysics and chemistry) that are essential for an understanding of living systems at the cellular and molecular level.

GENETICS PROGRAM

I. Narrative Report

A. Goals and Objectives of the Program: The Genetics Program continues to be directed toward gaining a better understanding of the fundamental processes and mechanisms of inheritance in health and disease. The ultimate objectives of the research and research training that we support are the prevention, therapy or cure of human genetic disease. It should be emphasized that the program considers the term "genetic disease" to encompass a broad spectrum of conditions. The term, as we use it, includes not only those conditions with single gene (Mendelian) modes of inheritance (examples are cystic fibrosis, hemophilia and Huntington's disease) or chromosomal abnormalities (for example, Down's syndrome) but also more common degenerative conditions such as atherosclerosis and diabetes. The risk of occurrence of such conditions is determined by both genetic and environmental factors.

The NIGMS Genetics Program differs in a number of ways from numerous categorical (specific, disease-oriented) research supported programs in other NIH institutes which are also concerned with such diseases:

1. A major component of research supported by the Program focuses on such fundamental topics as nucleic acid chemistry, the mechanisms of transmission and expression of genetic information, and population genetics. Model organisms such as bacteria, fruitflies, and mice are extensively employed in this type of research.

2. The program also emphasizes that genetics is not only a discipline but a mode of thinking about biology and medicine that complements the more specific, targeted approach demanded by other more categorical NIH programs. For example, the study of rare (Lesch-Nyhan disease) or more common but relatively benign (G-6-PD deficiency) conditions has often illuminated our knowledge of genetic disease far out of proportion to their intrinsic medical importance. In the future, disease models will be needed that may provide generalizable concepts applicable to common diseases as diverse as diabetes and schizophrenia.

In summary, the program considers itself as a resource for generating techniques and concepts that transcend concerns about specific genetic diseases. It seeks to accomplish its objectives by supporting research and research training in basic research in genetics. Characteristically, the preponderance of research that led to recombinant DNA technology, as well as much of the early developmental work, was supported by the Genetics Program. As the utility of these techniques to the analysis of biological and medical problems became more apparent, support for investigators utilizing these methods has become more widespread among all of the NIH awarding units.

B. Staffing:

The steady-state level of program staff continues to be seven program administrators (including a Director and Deputy Director) at the Ph.D.-equivalent level and five supporting staff (grants technical assistants and secretaries).

On August 10, 1980, Dr. Robert Gulley, who joined the program in 1979, will leave in order to resume laboratory research in neurobiology at the National Institute of Neurological and Communicative Disorders and Stroke. Dr. Gulley is a person with keen scientific insight, as well as sensitivity to the complex aspects of science administration. His contribution to the program will be missed.

In December 1979, Dr. Fred Bergmann returned to active participation in program affairs after a six-month assignment detail to the Congressional Office of Technology Assessment. He participated in the design and initial phases of an assessment of genetic technology. During his absence, Dr. Elke Jordan was Acting Director of the Program.

It is important to emphasize that the Program's staff could not possibly operate effectively without the generous support of many other groups and individuals, including NIGMS Grants Operations staff; the staff of the Office of the Director, NIGMS; consultants from the scientific community, and numerous peer review groups. In addition, our relationship with our sister NIGMS programs and other NIH awarding units have been most constructive. The scientific enterprise, including science administration, is, by-and-large, not a zero-sum game. There is mutual benefit to be gained by increased cooperation. To the many who have interacted in this spirit with our staff, our heartfelt thanks.

C. Workshops:

The Genetics Program held two scientific workshops during FY 80. Such staff-initiated workshops are generally concerned with timely scientific issues which, in the opinion of our staff and consultants, may merit increased scientific or administrative attention.

1. Workshop to Explore the Potential of TCDD as a Tool in Molecular Biology, December 11-12, 1979 (organized by Dr. Robert Gulley): TCDD (2,3, 7,8 - tetrachlorodibenzo-p-dioxin) has received public attention recently due to its presence as a contaminant in herbicides and hexachlorophene. The possible consequences of its introduction into the environment are controversial. Scientific inquiries suggest that TCDD is a potent toxin, teratogen, and carcinogen in laboratory animals. These studies also suggest that TCDD has biological properties which may be used to explore basic biological questions. Since the National Institute of General Medical Sciences (NIGMS) is committed to basic research, the Institute sponsored a workshop to explore the potential of TCDD as a tool in molecular and cell biology. The participants included applied scientists currently working with TCDD and basic scientists working in complementary areas of cell and molecular biology. It was hoped that the workshop would identify new approaches or techniques for understanding the mechanism of TCDD toxicity, and introduce basic scientists to

the potential of TCDD as a basic research tool. As a background for the discussion of these presentations, each participant presented an overview of his research area. From the discussion of these presentations, it was apparent that TCDD could be a valuable tool for basic studies of genetic regulation and expression, and the control of cellular differentiation.

2. Workshop on the Need for a Nucleic Acid Sequence Data Bank, July 14-15, 1980 (organized by Drs. Elke Jordan, Genetics Program and Marvin Cassman, CMBD Program).

A recent editorial (Nature 85, p59, May 8, 1980) has emphasized that the very successes of the techniques of molecular genetics have created new problems in intelligent handling of the large amount of nucleic acid sequence data that is now accummulating at a continuously increasing rate. The relevant improvements in technology that have made all this possible include great advances in the ability to isolate large amounts of homogenous DNA by the techniques of recombinant DNA technology, as well as the development of methodology to sequence long stretches of DNA. The NIGMS Genetics Program is pleased that its grantees have contributed substantially to progress in these areas.

The nucleotide sequences of a number of viruses - MS2, ΦX174, SV40, polyoma, and a hepatitis virus - are now known. In the near future, we can expect to know the complete sequence of some more complex viruses, and of the genomes of yeast and animal mitochondria. Thus, at present, genomes ranging in complexity from 3.6 kilobases (thousands of bases), represented by the virus MS2, to 50 kilobases (mitochondrial DNA) can be analyzed. The editorial states that "by contrast, the complete genome of E. coli is 10,000 kilobases, the Drosophila genome an order of magnitude greater, and a single human chromosome some 500,000 kilobases of DNA". Clearly, the next decade of research will generate an avalanche of important information on the nature of genes. How does one handle this enormous mass of data so that it can be used most effectively in understanding how genes make us and our fellow creatures what we are?

Appropriately, the editorial states that a "major problem arises from the quantity of sequencing that has already been amassed - of the order of 100 kilobases - let alone what the next few years will bring. The problem is how best to collate as much data as possible to bring out the salient features..." "What does not yet exist is any central data bank in which all sequences could be deposited and made freely available to the whole scientific community."

The workshop attendees discussed a number of approaches to the development of such a facility, and how it might best be structured and supported. A report developed by the attendees outlined some special recommendations. (Copies of the report are available on request. Please contact Dr. Elke Jordan, Gentics Program, NIGMS, NIH.)

D. Contracts: The Human Genetics Mutant Cell Repository, Contract NO1-GM-9-2101, the Institute for Medical Research, Camden, New Jersey.

Since April 1972, the NIGMS has supported a contract to IMR to establish and maintain a repository of viable genetic mutant cells in tissue culture. The aims of this program are to facilitate and support research programs in human and clinical genetics. The repository contains cells in low passage from individuals with hereditary diseases and from heterozygous carriers. In addition, it includes cells with useful phenotypic gene markers and cells with chromosome abnormalities, as well as normal controls.

The program staff, assisted by technical consultants, maintains a close overview of the facility and makes frequent recommendations to the contractor. Meetings of the project officer and consultants are held several times a year, frequently on site, in order to establish general policies and to recommend specific lines of action, such as addition or deletion of certain cell lines, or classes of cell lines.

The utility of the collection to the biomedical research community has increased over the years, due both to the increase in the size and diversity of the collection, and to increased knowledge about and confidence in the materials that are available from this facility. Almost 3000 cultures were sent out during 1979, of which more than half went to academic institutions, and the bulk of the remainder to other nonprofit institutions, including government laboratories. About 500 lines were shipped to foreign countries, including the USSR, Japan, Israel, and East Germany.

Some significant recent conceptual and administrative developments in the operation of the facility include:

1. The computerization of repository data.

After several years of operation of an IBM System/32, the consultants recommended conversion to a higher capacity IBM System/34. This was done in February 1980.

The system includes complete records of 4400 cell lines, including submitted information, laboratory data, shipping information and inventories; programs for preparing an annual catalog; mailing lists, etc. It is generally agreed that computerization has resulted in an increase in both the quality and efficiency of the operation.

2. New Kinds of Accessions to the Collection:

In the early years of operation, it was our stated policy that only cell lines representing conditions whose metabolic or chromosomal defect could unambiguously be demonstrated in culture would be collected. More recently, our consultants have felt that this restriction should be relaxed, since the existence of certain collections may, in fact, stimulate research on the cellular or molecular defects of important diseases. Accordingly, the collection now includes significant holdings of cell lines representing diabetes (as the type of condition which is prevalent, multifactorial and heterogenous, and where the strategy in compiling the most informative set of cell lines presents an appreciable challenge) and Huntington's Disease (as an example of a rarer "mendelian" condition whose primary defect is as yet difficult or impossible to diagnose in cultured cells).

We are continuing with a policy of acquiring selected cell lines representing defects which cannot presently be detected in culture. For example, at present serious consideration is given to establishing a collection of cell lines representing psychiatric disorders, particularly the schizoid and manic-depressive groups. Many of our consultants feel that such a collection will be very useful as the DNA probes for many human genes become available, and as research which seeks to understand psychiatric illness from a biochemical point of view continues to proliferate. It is recognized that this is a high-risk effort, and that the usefulness of such a collection to the scientific community can be increased only by excellent design and by gathering material from the most informative, well-diagnosed cases. To this end, some of the cell lines currently in the collection will probably need to be discarded.

Another new cell line collection is a response to new trends in human genetics research made possible by recombinant DNA technology. This is a collection of fibroblast cell lines representative of various hemoglobinopathies such as sickle cell anemia and the thalassemias. Although hemoglobin is not synthesized in tissue cultures (there are one or two noteworthy exceptions) the new technology permits the analysis of the genome structure of specialized genetic systems, such as those for hemoglobin synthesis. More generally, investigators can now analyze genomes without requiring phenotypic expression of a trait or synthesis of a product. The collection of lines representing hemoglobinopathies may thus be the first of a number of collections which exploit our new ability to study specialized genes in unspecialized cells such as fibroblasts.

3. High-quality Junior Staff

Other recent developments include the recruitment of high-quality junior staff for the cell bank, with expertise in cell biology, tissue culture and computer operations. The presence of such staff provides increased assurance of continuity of operation as the facility approaches its second decade of operation.

4. Increased Insistence on Accessioning of Only High-Quality, Well-Documented Samples.

Another, perhaps more gradual development, is increased insistence on accessioning of only high-quality, well-documented samples. In the earlier years of operation, the facility established many cell lines from unsolicited biopsy and post-mortem material. Much of this material was extremely valuable; however, some of the cell lines have been removed, from the collection because of incomplete documentation, questionable diagnoses, or because of redundancy.

Most of the materials that are now banked are solicited by IMR on the advice of the project officer and our consultants, and they are of very high quality and scientific importance. This change in operation has been facilitated by the development, at IMR, of methods which allow freezing and storage of clinical materials, such as blood samples or skin biopsies, and subsequent work-up as desired. Thus there is no need to establish cell lines before adequate documentation has been obtained, and before a decision is made to add this material to the catalogued collection.

II. Research Highlights

A. Transposable Genetic Elements:

1. Introduction: The FY 1979 annual report of the NIGMS Genetics Program emphasized the growing importance of research on DNA sequences (transposons) that "hop" from one region of the genome to another, via recombination at specific sequences near their ends. Much of this recent research could not have been done a few years ago; the availability of recombinant DNA probes to define the physical location of defined pieces of DNA on chromosomes, and the associated technology using restriction enzymes, high-resolution separation techniques of nucleic acid fragments etc., has enormously facilitated the research.

It is interesting to trace the development of the concepts of transposable genetic elements as a function of both time and of the experimental material used. Probably the first evidence of movable genetic elements was obtained by Barbara McClintock some 30-40 years ago. The experimental organism was corn. Some time later it was shown that antibiotic-resistance elements on bacterial plasmids are highly mobile, and can in fact, migrate from one bacterial species to another. The significance of this finding to human health, and more specifically, to policies relating to indiscriminate use of antibiotics, did not escape attention. However, it was only a few years ago that the existence of similar transposable elements was demonstrated physically (rather than on the basis of genetic evidence, as in corn) in higher organisms, such as yeast or fruit flies.

Awareness of the importance of transposable elements continues to increase. This year's report documents some evidence that the transposition of genetic elements may be a major cause of mutation; that the transposition of genetic elements may be an important mechanism for differentiation; and that "genetic switching" from one phenotype to another, in yeast, is accomplished by insertion of silent genes (cassettes) into a location on DNA that activates them.

Any important biological mechanism will, sooner or later, be found to be important to human health, and transposable elements are no exception. For example, the section on gene interconversion in yeast (see below) stresses that the conversion of one yeast mating type to another by a cassette mechanism may be a model for antigenic variation in pathogens, such as trypanosomes. Such antigenic variation confounds attempts by the immune system to combat infection, and thus it increases virulence.

The mechanism by which a large but surely finite population of genes for antibody synthesis can have the versatility to produce the almost infinite diversity of responses to almost any antigen that can be devised by man or nature has been a biological puzzle of some magnitude. It now appears that a single antibody molecule is encoded in three separate "libraries" of DNA fragments. One piece of DNA from each of the libraries is translocated to form a single DNA sequence, which is transcribed into messenger DNA. Such translocations can increase enormously the potential for diversity. (For illustration only, if each of the "libraries" contained one hundred different sequences, one million different kinds of antibody molecules [100 x 100 x 100] could be produced).

It seems possible, also, that many of the basic studies on transposable elements may have some bearing on malignancy.

A variety of host chromosomal changes are noted in the development of virus-associated cancer. It must be noted that the movement of nucleic acid, visualized microscopically as chromosomal rearrangements, is many orders of magnitude larger than the movement of nucleic acid in the transposons now being studied. However, it is considered possible that the apparent specificity of breakpoints and rearrangements in the cancer-associated chromosomal aberrations might result from movements directed by transposable genetic elements. Furthermore, it is considered likely by a number of investigators, including Barbara McClintock, that external signals, such as cancer-inducing agents, can activate transposable chromosomal elements.

Experimental attempts are now being made to determine the involvement of a transposition-like mechanism in tumorigenesis and chromosomal rearrangement. Among the scientists in this field is Ruth Sager, Sidney Farber Cancer Institute, whose NIGMS grant (GM 22874) is concerned with nuclear-cytoplasmic inheritance. NIGMS is currently funding a wide variety of basic studies of transposable genetic elements, and most of the fundamental information is likely to come from this work. These basic studies may prove to be seminal in understanding the origin of chromosomal aberrations associated with malignancy.

The development of these concepts and ideas can tell us something about the unity of Biology. Some aspects of corn genetics began to suggest the existence of transposable genetic elements. The transposition of antibiotic genes between plasmids of different bacterial species was found to be the scientific explanation of epidemics of antibiotic resistance in hospitals.* It now seems that mechanisms involving transposons may contribute to mutation, and may be a factor in the virulence of some pathogens, as well as in the immune systems which combat these pathogens. Finally, the concepts are exerting an influence on cancer research.

It is an article of faith of this Institute that all of biology, including corn genetics and the study of mating types in yeast, can contribute concepts that may ultimately prove relevant to the understanding of human disease. Research on transpons in a number of test organisms indicates that this faith is probably not misplaced.

2. Gene Interconversion in Yeast: The Cassette Model

GM 25624 - James B. Hicks, Jeffrey Strathern, Cold Spring Harbor

Ordinary baker's yeast, Saccharomyces cerevisiae, has served for many years as valuable experimental material for studies of basic cellular processes. Yeast is a simple unicellular organism, which is easily grown in cell culture, and is therefore adaptable to techniques that have contributed significantly to our understanding of molecular mechanisms in bacteria. However the yeast cell differs importantly from all bacterial cells in possessing a differentiated nucleus. For this reason, it is believed that the elucidation of cellular processes in yeast may be relevant to the understanding of similar processes in higher eukaryotic organisms.

One of the basic tenets of genetic theory assumes the extreme stability of genes, providing for the orderly transmission of traits from one generation to the next. Over the past decade, there have been reports suggesting that genes may not be as stable as has been confidently believed. Recent studies by Drs. James Hicks, Jeff Strathern, and Amar Klar of Cold Spring Harbor Laboratory, and Dr. Ira Herskowitz of the University of Oregon, concern the reproductive cycle of yeast. These studies provide striking evidence of gene modification during the life cycle, and have also led to the formulation of a molecular model to explain the observed changes.

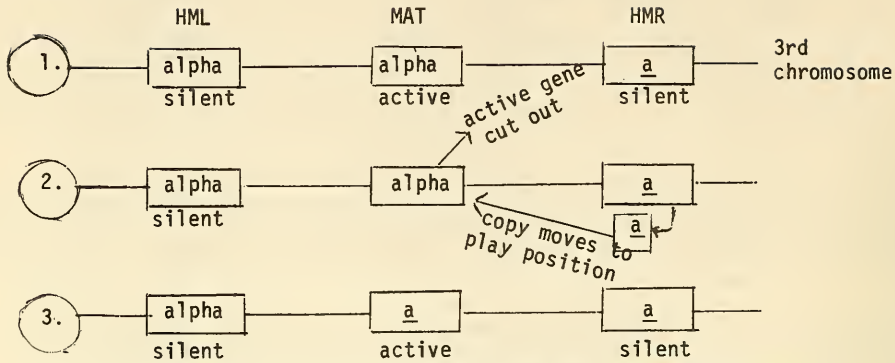
*Incidentally, these epidemics gave rise to increased research interest in plasmid biology, which was an important element in bacterial genetics, and later, was one of the building blocks of recombinant DNA technology.

The most common form of reproduction in yeast is by the process of asexual budding of the haploid cell. However there is also a sexual process, whereby mating (conjugation) occurs between two haploid cells to produce a single diploid cell. (Diploid cells do not mate or produce buds; they usually undergo meiosis and differentiate into dormant spores.) The haploid vegetative cells are of two mating types, a and alpha, and mating occurs only between cells of opposite mating types. The cell-cell recognition involved in the mating reaction is facilitated by chemical signals. Alpha cells produce a polypeptide alpha factor, which arrests a cells at the specific point in the cell cycle (G_1) where mating occurs. Similarly, a cells produce a factor, which arrests alpha cells in G_1. Cell type is determined by a single gene at the mating type locus, which may be either MATa or MATalpha. Each haploid cell can carry only one of the two alleles, and should transmit that gene to its descendants.

The surprising observations began with the discovery that certain strains of yeast regularly switch their mating type from one cell generation to the next. These unorthodox forms are known as homothallic strains. Their ability to change mating type depends upon the presence of a separate gene, HO, which is not linked to MAT. While the mating type locus is stable in the absence of HO, it has been shown that the HO gene alone does not induce changes in mating type. Switching depends upon the activity of two other genes, HML and HMR. The ability to interconvert mating types at high frequency indicates that these cells contain the information for both the a and alpha phenotypes, but that only one or the other is expressed.

In an effort to organize into a unified framework the complex observations concerning homothallic gene conversion, Drs. Hicks, Strathern, and Herskowitz have proposed the Cassette Model. According to this model, the mating type genes are like tape cassettes, with the HO gene operating the machine. The three other genes occupy sites on the third chromosome. The central locus is occupied by MAT, which is the "play" position; the allele expressed at MAT determines the a or alpha phenotype of the cell. The MAT locus is flanked by HML on the left arm and HMR on the right arm of chromosome III. While the active gene is the allele expressed at the MAT position, HML and HMR make "silent" copies of the MAT alpha and MATa genes. The model proposes that interconversion involves moving a silent copy (cassette) from the storage area (HML or HMR) to replace the resident allele at MAT, resulting in the expression of the alternate cassette.

Conversion from MATalpha to MAT<u>a</u>



Most of the early support for the cassette model came from genetic evidence derived from mutations affecting the three mating type genes. An example is the process of "healing." A yeast cell of mating type alpha may undergo a mutation at the mating type locus which makes it unable to mate. In homothallic strains, such sterile cells may switch in successive buddings, first to normal <u>a</u> type cells, and then to normal alpha cells, in which the defective mutation has apparently been healed. This observation is consistent with the cassette model, providing for removal of the abnormal alpha gene from the MAT locus, and replacement with a normal <u>a</u> cassette.

While the genetic predictions of the cassette model have been confirmed, the next step was to examine the molecular details of the directed transposition. Since interconversion implies the substitution of the DNA at the MAT locus, efforts were made to identify and characterize the genes. Recently it has become possible to transform yeast by using hybrid DNA, containing bacterial plasmid DNA and a chromosomal yeast gene. Thus specific yeast genes can be selected by function from total yeast DNA cloned on plasmids or bacteriophage. Plasmids containing the mating type locus have been devised and tested.

These techniques have now been used to correlate all three cassette sites (<u>HML</u>, <u>HMR</u>, and <u>MAT</u>) with specific restriction fragments. At each cassette site, the <u>size</u> of the restriction fragment is dependent upon the mating type allele present. In every case, the MATalpha fragment is approximately 200 base pairs larger than the corresponding MAT<u>a</u> fragment. Likewise, at HMR and HML, when the alpha allele is present, the restriction fragment is 200 base pairs larger than when <u>a</u> is present at that locus.

In addition to identifying the genotypic basis of the mating phenotype (<u>a</u> or alpha), these studies also confirmed a crucial tenet of the Cassette Model. The gene at the MAT locus changes at switching, but the genes at HML and HMR are unchanged. The replacement HM gene is duplicated, and the <u>copy</u> (cassette) is inserted at MAT.

Further studies have been directed at the pattern of gene conversion during development. Hicks, Strathern, and Herskowitz have devised techniques to follow individual cells through several generations. Since yeast cells divide by budding, the older or "mother" cell can be clearly distinguished from the small "bud" cell, and the two can be separated by micro-manipulation. By separating the cells at each division, keeping track of the mother and the bud, and testing each cell for sensitivity to alpha factor, a detailed picture of the frequency and distribution of interconversions has been obtained.

These experiments demonstrate that the capacity to switch mating type is not random, but follows a regular pattern during clonal growth. The homothallic yeast cells never switch at the first division; only an "experienced" cell gives rise to a switched bud. Furthermore such a cell almost always slots in a "storage cassette" of the opposite mating type. Thus a single haploid cell of either mating type can give rise to a population of yeast cells containing both alpha and a cells.

Hicks and Strathern suggest that such mechanisms of gene interconversion may have implications for many unsolved problems in the development of higher organisms. A question of major importance concerns the mechanisms which regulate the differentiation of multiple cell types from a single fertilized egg. Since every cell of a multicellular organism is believed to contain identical genetic material, the differentiation of diverse cells and tissues must depend upon differences in the particular genes expressed in each cell type. In the language of these investigators, the process of development involves the selective switching on and off of particular genes in different groups of cells, by moving genes from the storage to the "play" position.

Strathern and Herskowitz point out a possible analogy between the homothallic switching of yeast and the differentiation of the array of mammalian blood cells. All the cell types are derived from hematological "stem cells". Each stem cell must replace itself at a low rate, and also supply new red and white blood cells at a much higher rate. In comparison with a yeast cell, a larger number of genes can be assumed. If switching occurs only in experienced cells, then the first division will produce another stem cell. The second division will involve a directed switch, producing a new kind of cell with different switching potential. Subsequent cell generations will produce a series of different cell types with different developmental potential, leading in successive stages to final differentiation as erythrocytes, lymphocytes, macrophages, and the other blood cell types.

The Cassette Model may also apply to a variety of biological processes in which the appropriate response of the organism depends upon the expression of alternative allelic genes. Such a phenomenon is seen in the production of the enormous repertoire of antibodies in response to specific antigens, through alterations in the immunoglobulin molecules.

Recent research reports suggest that similar gene transformations may be involved in the baffling public health problem of trypanosomiasis. The salivary trypanosomes are parasitic protozoa transmitted by the tsetse fly. Trypanosomiasis affects both man and domestic animals, with profound

economic and health consequences, across a wide belt of Africa. The trypanosome survives in the bloodstream of the host by sequentially altering its surface antigens; accordingly the immune response developed by the host becomes abortive. The disease is therefore characterized by a relapsing infection, with each recurrence representing the development of different antigenic variants of the parasite. The number of variants which can be expressed by a single clone of trypanosomes is believed to run into hundreds. All are glycoproteins with similar molecular weights (ca 60,000), but the variants differ in amino acid composition, N-terminal amino acid sequence, or C-terminal structure.

The mechanisms by which the information encoding the surface antigens is stored and expressed is of considerable interest. Dr. J.H.J. Hoeijmakers and colleagues at the University of Amsterdam have cloned DNA complementary to the mRNAs for four immunologically distinct surface glycoproteins. Each cDNA recognizes a unique set of fragments, and this basic set is present unaltered in the nuclear DNA from the four variants. In the sequences corresponding to each of the four antigens, the basic gene is flanked by different DNA sequences, indicating genetic rearrangement. The re-arrangement is found only in the homologous variant, that is, the one expressing the antigen corresponding to the cDNA probe. Hoeijmakers interprets these results as showing that the same basic gene copy is present in all variants examined, but that the homologous variant shows an additional copy of the gene flanked by different DNA sequences. The flanking sequences are presumably related to the expression of the particular antigen. The mechanism for the expression of the copy could be another case of cassetting, analogous to the interconversion of yeast mating types.

The cassette model, developed to explain the complex observations concerning gene conversion in yeast, may prove to have wider applicability. Like the recent evidence for the existence of "jumping genes" (transposons) in bacteria, these investigations indicate that genes in higher organisms are also subject to molecular re-arrangement during the normal processes of development.

3. Transposable Elements and Spontaneous Mutation:

GM 15408 - Gerald Fink, Cornell University

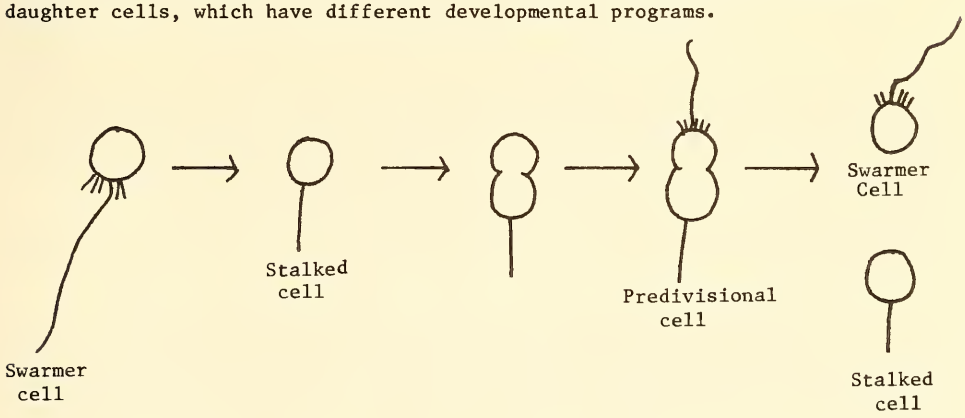
At the workshop on the use of TCDD (Dioxin) as a tool in Molecular Biology, Dr. Fink reported his recent results on the nature of insertion mutations in yeast. Dr. Fink has developed an elegant system, combining gene cloning and transformation techniques, whereby he can isolate, study the expression of, and sequence selected yeast genes. Using this method he has shown that 20% of spontaneous mutations in the His-4 gene are caused by the insertion of a 6200 base-pair piece of DNA. Further studies have revealed that there are about 35 copies of this inserted DNA in a normal yeast chromosome and that each copy of inserted DNA has a 300 base pair region repeated at each end. When the inserted DNA moves spontaneously from one chromosomal location to another, it frequently leaves behind one of the 300 base pair repeats. The yeast chromosome has about 200 copies of this 300 base pair region, probably all remnants left behind by inserted DNA that has since moved on.

The structure and behavior of these insertions are very similar to the transposon systems that have been described in prokaryotes. Although isolated cases of transposons have been reported in eukaryotic organisms, e.g., Drosophila and maize, this is the first case where complete analogy to the bacterial situation has been shown. This makes it likely that similar insertions, or transposons as they are commonly called, are widespread in higher animals and man. So far, transposons have been shown to be involved in mutations, chromosome rearrangements, control of gene action, and developmental changes. One interesting property of the mutagenic character of the transposons studied by Fink is that the rate of mutation caused by these elements is not enhanced by any known mutagen such as X-rays, UV, or chemicals. What controls the movement of transposons from one chromosomal site to another also remains to be discovered.

4. Transposable Elements and a Prokaryote Model for Cellular Differentiation:

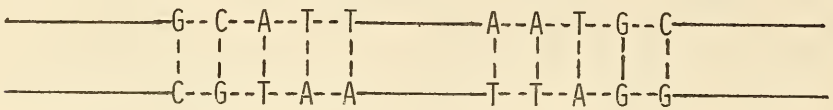
GM 11301 - Lucille Shapiro, Yeshiva University

Dr. Lucille Shapiro's group has been studying differentiation in the bacterium Caulobacter crescentus. The cell cycle of C. crescentus is characterized by precise spatial and temporal differentiation events. One form of the organism is characterized by the presence of a slender rod-shaped stalk at one pole of the cell. As the cell cycle proceeds, this stalked cell elongates and several microdifferentiation events occur on the cell surface so that the predivisional cell carries a newly formed flagellum and phage receptor sites on one cell pole, and a stalk on the other. As a consequence of the events, the pre-divisional cell expresses a polarity which, upon binary fission, will yield phenotypically dissimilar daughter cells, which have different developmental programs.

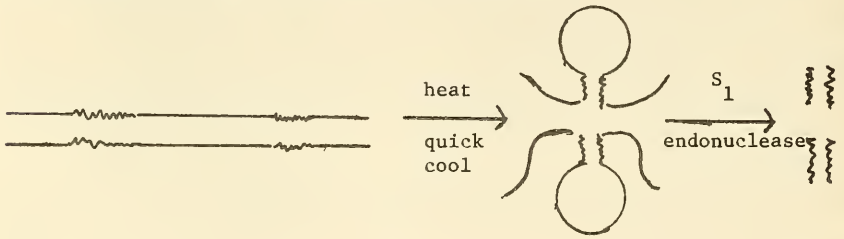


Shapiro's laboratory is trying to understand the mechanism of temporal control of gene expression in this organism by examining the organization of its DNA as a function of the cell cycle.

In collaboration with Ann Skalka of the Roche Institute, Shapiro's group has been studying invertedly repetitious (IR) sequences of C. crescentus DNA. Such sequences have the following type of structures:



IR sequences on the same strand of DNA are self-annealing; therefore, after DNA is denatured and quickly cooled, these are the only structures that tend to form a double strand. The rest of the DNA can be digested with a nuclease that attacks only single-stranded DNA. The residual double-stranded DNA is further purified on a hydroxyapatite column. What remains is about 4% of the total C. crescentus DNA, composed of two heterogeneous size classes: 100-600 base pairs long, and 1000-2000 base pairs long. The DNA is then cloned in bacteriophage λ.



Dr. Shapiro's group has recently shown that the DNA isolated from one such recombinant clone hybridizes to different restriction fragments of C. crescentus DNA depending on whether the DNA which was cleaved with restriction enzyme was isolated from swarmer or stalked cells of this organism. This result suggests that the IR sequence that was studied may be in different parts of the DNA in the two cell types The implication is that a piece of DNA may have been transposed from one region of DNA of C. crescentus to another.

Additional work will be needed to better understand this phenomenon, and its relationship to cell differentiation. One attractive possibility that will be tested is that RNA polymerase-binding sites adjacent to IR sequences may be transposed. This would result in a switch of transcription, from one population of genes to another. Such a mechanism could provide an explanation, in terms of molecular genetics, of cell differentiation. Hypotheses of this type, involving transposition of parts of the genome, are being tested in a number of laboratories interested in such phenomena as cell differentiation and development, the genetics of antibody synthesis, and the molecular evolution of enzymes.

B. Some Aspects of Genetic Regulation

1. Introduction: Regulation refers to those processes by which organisms and their component cells determine the internal economy - synthesis, maintenance, and breakdown - of many diverse cell constituents in response to the internal and external environment. Some of the regulatory mechanisms (for example, the Jacob-Monod model involving repressor substances and operator regions on DNA) turn specific genes on and off; other mechanisms also have an effect on the synthesis of proteins, perhaps by affecting the rate of protein synthesis on ribosomes, or the rate of mRNA breakdown. We term all such mechanisms genetic in order to differentiate them from another class of more "direct" kinds of regulation, as for example, the allosteric effects of certain small molecules on the activity of enzymes and other proteins, such as hemoglobin.

Some 20 to 30 years ago, two varieties of cellular regulation were discussed: one that affected enzyme synthesis (the predominant model was the one proposed by Jacob and Monod) and one that affected enzyme activity (allosteric control, feedback inhibition, etc). What is becoming more and more clear is that there is a very rich fabric of all sorts of regulatory activities operating in cells. The examples below, selected from the activities of the program's grantees, illustrate some of this diversity.

Some of the regulatory mechanisms occur only in lower organisms such as bacteria, others occur only in eukaryotes, and a number of mechanisms have been conserved over many eons of evolution and occur in both types. For example, the attenuation of transcription of mRNA by physiological events occurring on the ribosome, as described below, can occur only in bacteria and other prokaryotes, since in these organisms, one end of newly synthesized mRNA can still be attached to DNA, while the other end has already complexed with ribosomes and is beginning to synthesize polypeptides. Events occurring at the "peptide-synthesis end" of the complex (such as a slowdown due to a shortage of certain charged tRNA's) are readily transduced to the "mRNA synthesis" end. (Clearly, such a mechanism is precluded in cells of higher organisms, since the nuclear membrane spatially separates the two activities of RNA synthesis and protein synthesis.)

On the other hand, intervening DNA sequences appear to occur only in higher organisms. If these structures are involved in regulation, as some of our grantees speculate, such mechanisms must be exclusive to eukaryotes.

Less speculative are the responses of the protein synthetic apparatus of eukaryotic cells to such stimuli as starvation, hormones, viruses, and interferon. Recent work by a number of the program's grantees indicates that at least part of the physiological response to these agents is mediated by modification of components such as protein synthesis initiation factors.

Although some control mechanisms thus appear exclusively in lower organisms, and others have evolved in higher organisms, some structures which may be sites of action of control mechanisms, such as RNA polymerase binding sites, have resisted evolutionary change and are virtually identical in both bacteria (E. coli) and some eukaryotes (Neurospora).

The diversity of mechanisms used by cells to regulate their economy is indeed fascinating. It is already clear that defects in the regulation of human cells can prove to be one cause of human disease. For example, Goldstein and Brown (formerly supported by the Genetics Program and now, appropriately, by NHLBI) showed some years ago that genetic defects in cell surface lipoprotein receptors, which are involved in the control of cholesterol synthesis, can result in unregulated, high levels of cholesterol and thus, at the clinical level, in extreme cases of atherosclerosis.

2. New Models for Regulation; the Control of Amino Acid Synthesis by Attenuation of Transcription

GM 09738 - Charles Yanofsky, Stanford University
GM 10791 - Elizabeth Keller, Cornell University
GM 24956 - Wayne H. Barnes, Washington University
GM 23408 - John R. Roth, University of Utah

Background: The control of the biosynthetic enzymes for the synthesis of amino acids has been a fertile field for biochemists and geneticists for many years. Among the most investigated systems have been those for the synthesis of tryptophan (Yanofsky), isoleucine and valine (Umbarger), leucine (Calvo, in collaboration with E. Keller) and histidine (Ames and Hartman; later, Roth).

There has always been something puzzling about the control of amino acid synthesis in bacteria such as E. coli and Salmonella typhimurium. The genome of each of these intensively studied systems consists of an operon - a genetic unit of several adjacent genes that are controlled and that function in a coordinated manner. The tryptophan system is controlled, in part only, by a repressor - operator system, i.e., a system in which a protein component, the repressor, interacts with a site on DNA, the operator, that controls the binding of RNA polymerase and thus the transcription of structural genes downstream from the site of interaction. Free tryptophan in the milieu of the cell promotes the interaction, i.e., it is a physiological signal indicating that the synthesis of enzymes to make more tryptophan is not required.

However, it was clear even some 5 years ago that most of the control of amino acid biosynthesis in prokaryotes could not be explained by repressor - operator interactions. For example:

a. No repressor - operator system was found for any of the other biosynthetic systems, including those for histidine, leucine, and the branched chain amino acids isoleucine and valine.

b. Dr. Yanofsky's group showed some years ago that there is a second regulatory site within the tryptophan operon. Deletions within this site increased expression of the tryptophan genes. Apparently, it is possible to inactivate or remove a site which normally tends to reduce expression of tryptophan genes. This site, which has been termed the "leader region" maps between the operator (the site of the repressor - DNA interaction) and the structural genes of the tryptophan operator.

A transcribed mRNA sequence corresponding to about 160 nucleotide pairs of this region was detected. It appeared that this second control region has many analogies to the more representative (no repressor) control systems of the operons for enzymes of amino acid synthesis, such as those for histidine, phenylalanine, threonine and leucine.

c. The degree of transcription, and subsequent expression, of the structural genes of these systems seem to be dependent on the level of aminoacylation of the corresponding transfer RNA, rather than on the intracellular level of free amino acid. Mutations that, in any way, decrease the degree of charging of a tRNA species promote increased transcription and translation of its corresponding biosynthetic genes. It is as though the cell "senses" the level of free amino acid indirectly by way of its cognate tRNA.

In summary, these findings in the area of bacterial physiology and formal bacterial genetics imply the existence of a control mechanism in which a high rate of protein synthesis acts as a brake on transcription of some selected sets of enzymes of amino acid biosynthetic pathways. The effect is specific - a lack of histidine and thus, more directly, a lack of histidyl tRNA, acts to increase only the enzymes of the histidine pathway. Furthermore, the now classic Jacob-Monod repressor-operator system is not involved.

The Solution: A molecular mechanism implicit in an m-RNA sequence. The explanation of these phenomena had to await the development of some very modern methodologies, including the cloning of the whole operons of several amino acid biosynthetic systems in bacterial plasmids, isolation and ordering of their restriction enzyme fragments, and DNA sequencing techniques, such as were developed by Maxam and Gilbert. The leader sequences, (i.e., the sequence just proximal to the structural genes) of a number of systems have now been analyzed by a number of groups: Calvo (Leucine operon of Salmonella), Yanofsky (tryptophan operon of E. coli), Roth and Barnes (histidine, Salmonella) and Gardner (threonine, E. coli). There are many features of all the sequences that are remarkably similar, and these features support a model first proposed by Lee and Yanofsky around 1977, which is called the transcriptional attenuation mechanism. This model, also termed the arrested ribosome model, predicts a number of structural features of the mRNA of the leader sequence, all of which have been found in the systems which have been examined.

The leader sequences (defined as sequences proximal to the structural genes, and where mutations have an effect on the rate of expression of the system) are all about 120-180 nucleotides long. They are transcribed into mRNA, and at least a portion of this mRNA near the 5' (left-hand) end is translated into a peptide. In these translated regions, each of the sequences contains a sequence of triplet codons corresponding to the cognate amino acid. For example, mRNA transcribed from the leader sequence of the tryptophan operon contains two adjacent tryptophan codons UGG UGG; that for leucine contains four leucine codons CUA CUA CUA CUC. Most strikingly, the histidine sequence contains seven histidine codons in a row: CAC CAC CAU CAU CAC CAU CAU.

An examination of the sequence data shows that a number of alternate potential base pairing conformations are possible for each of the leader mRNA sequences which have been studied. The diagram below (from Oxender, Zurawski and Yanofsky, PNAS 76. 5524-5528, 1979) illustrates this feature.

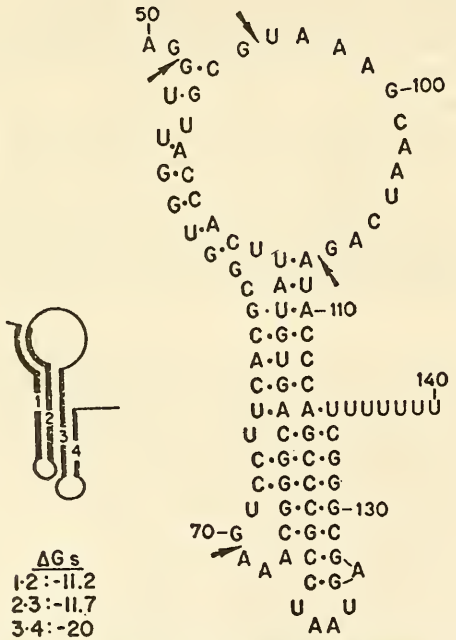


Diagram 1. Proposed secondary structures in *E. coli* terminated *trp* leader RNA. Four regions can base pair to form three stem and loop structures.

An understanding of how the information encoded in such a sequence can lead to control of the synthesis of a group of enzymes can provide one with a heightened appreciation of the explanatory and predictive power of modern molecular genetics.

One must imagine this sequence as a short piece of RNA dangling from the bacterial DNA and being elongated by RNA polymerase transcription somewhere downstream near the 3' end, around position 140. At this point, all of the structural information for the structural enzymes is contained only in DNA, and has not yet been transcribed. The sequence shown above should be considered a "sensor" that "decides" from physiological data such as the intracellular concentration of phenylalanyl-tRNA, whether to continue transcription and to commit the cell to the production of enzymes leading to more tryptophan, or to abort this process, already briefly underway.

As so frequently happens in bacterial systems, the transcription of DNA into mRNA, and the translation of mRNA into protein are coupled. Thus, one must imagine not only an RNA polymerase molecule "working" around position 140, but also a ribosome attaching around position 20 and moving downstream along stem 1 and, in fact, translating the sequence into a short peptide, starting at position 25. Note the two tryptophan codons UGG UGG around position 54-59 which code for a part of this peptide.

Diagram 1 indicates that the mRNA sequence discussed here can have two alternate base-paired structures. We can have stem-loop structure 1-2 and 3-4, or stem loop structure 2-3 (i.e. a nucleotide in region 2 can bond to one in either region 1 or region 3, not to both). Which of these two base-paired alternatives is favored depends on the position of the ribosome as it is moving along the sequence (see diagram 2).

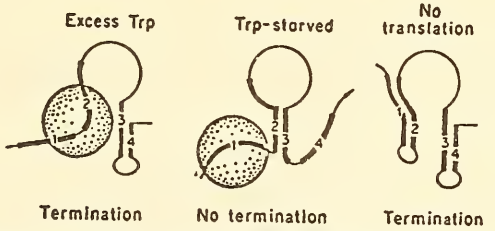


Diagram 2. Model for attenuation in the *E. coli trp* operon. Under conditions of excess tryptophan the ribosome (the shaded circle) translating the newly transcribed leader RNA will synthesize the complete leader peptide. During this synthesis the ribosome will mask regions 1 and 2 of the RNA and prevent the formation of stem and loop 1-2 or 2-3. Stem and loop 3-4 will be free to form and signal the RNA polymerase molecule (not shown) transcribing the leader region to terminate transcription. Under conditions of tryptophan starvation, charged $tRNA^{Trp}$ will be limiting and the ribosome will stall at the adjacent Trp codons in the leader peptide coding region. Because only region 1 is masked, stem and loop 2-3 will be free to form as regions 2 and 3 are synthesized. Formation of stem and loop 2-3 will exclude the formation of stem and loop 3-4, which is required as the signal for transcription termination. Therefore, RNA polymerase will continue transcription into the structural genes. Under conditions in which the leader peptide is not translated (i.e., *trpL29 in vivo*, transcription *in vitro*, or starvation for amino acids occurring early in the leader peptide), stem and loop 1-2 will be free to form as regions 1 and 2 are synthesized. Formation of stem and loop 1-2 will prevent the formation of stem and loop 2-3 and thereby permit the formation of stem and loop 3-4. This will signal transcription termination.

If the system is starved for tryptophan, the ribosome is arrested while traversing region 1, which contains the tryptophan codons, and the formation of stem-loop 2-3 is favored. Excess tryptophan or absence of protein synthesis favors the formation of stem-loop 3-4. It turns out that stem-loop 3-4 is a structure that leads to termination of RNA synthesis. Thus, a low concentration of tryptophan tRNA is compatible with continued synthesis of mRNA and expression of the genes of the tryptophan pathway; a high level of tryptophan tRNA (or, alternatively, absence of protein synthesis) will lead to termination of gene transcription.

Such a mechanism can explain how the lack of a specific amino acid can derepress a specific pathway via a system as general and global as protein synthesis. The model predicts that mutants can be found in which one of the relevant pathways could be derepressed by a deficiency of amino acid(s) that are not cognate to the pathway! A search for such mutants is underway.

Significance: An efficient control mechanism has been described involving a short stretch of transcribed RNA whose secondary structure is sensitive, via a complex series of events, to a specific physiological state of the cell, such as starvation for an amino acid. No control protein, such as a repressor, need be synthesized, since the control mechanism is inherent in the structure of an mRNA and common cell components, such as ribosomes and transfer RNA.

The concept that mRNA sequences can have alternate secondary structures, and that the choice between such structures may be determined by physiological elements external to the genetic apparatus - metabolites, hormones, macromolecules etc. - is an attractive one in considering possible modes of regulation for higher organisms.

The story is a happy one: it illustrates how the new techniques - cloning, sequencing, restriction enzyme mapping - can solve old puzzles and lead to new insights. It is too early to know whether this work on prokaryotes has led to new concepts that may be applicable to biological problems in higher organisms.

3. The Control of Transcription in Higher Organisms

GM 20158 - David Hogness, Stanford University
GM 22395 - Donald Brown, Carnegie Institute
GM 27973 - Daniel Vapnek, University of Georgia
GM 28777 - Norman Giles, University of Georgia

As described in the previous section, a control region in prokaryotes, located adjacent to the 5' end of the structural gene, consists of a recognition and binding site for RNA polymerase, the promoter region, and a nearby site for transcription initiation. Recently, Dr. David Hogness has identified a similar control region for several eukaryotic genes. By sequencing four tandem repeat histone genes in Drosophila, he identified sequence homologies adjacent to the 5' end of each gene. Immediately upstream from the initial codon of each structural gene, a homologous sequence of 50 noncoding base pairs constitutes the leader sequence. Thus, he believes that the sequence is the transcription initiation site for these genes. Twenty to thirty base pairs upstream from this sequence, another homologous sequence (5'-TATAATA-3') is present which is identical to that of E. coli promoters. These homologies have been found in 15 different structural genes, including histone genes in sea urchin, mammalian globin genes and the fibroin gene in the silkworm. Thus for these genes, there may be similarities between prokaryotic and eukaryotic transcriptional control mechanisms.

Such work on the genes of higher organisms has been facilitated by recently developed technology which extends the scope and power of recombinant DNA methodology.

Recombinant DNA technology involves the use of (1) restriction endonucleases, enzymes that cut DNA at very specific nucleotide sequences, (2) viruses or plasmids that act as carriers for the excised DNA segments, and (3) assorted other enzymes that promote the trimming and rejoining of the isolated DNA segments with the plasmids or viruses. Recently it has become possible to introduce bacterial plasmids into eukaryotic organisms. This means that one can not only study bacterial or eukaryotic genes in bacteria, but also introduce bacterial and especially eukaryotic genes into a eukaryotic organism and study their expression in this environment.

A natural infection process occurs when bacterial plasmids are mixed with the normal host bacterium. For higher organisms, however, certain tricks are usually necessary to achieve entry of the plasmids. Dr. Norman Giles and his associate, Dr. Mary Case, have learned how to introduce the E. coli plasmid, pVK88, into Neurospora crassa, a bread mold often used for genetic studies. Using the snail gut enzyme, glusulase, Drs. Giles and Case digested the cell wall of Neurospora cells, making naked cells or spheroplasts of them. Next the spheroplasts are mixed with the pVK88 plasmid, which is actually the standard E. coli plasmid pBR322 containing the Neurospora gene qa-2 encoding dehydroquinase. This enzyme catalyzes the first step of the breakdown of quinic acid and is easily assayed. Using spheroplasts of a mutant missing the qa-2 gene, transformation of Neurospora, if it occurs,

would be demonstrated by the appearance of new strains containing the missing enzyme. Dr. Giles' experiments show that the dehydroquinase sequences, with or without the pBR322 sequences, are in fact integrated into the Neurospora DNA at various sites and are expressed. The cells which were enzyme deficient are now complemented by the transforming DNA and produce original levels of enzyme.

This ability to insert isolated eukaryotic genes back into the organism of their origin now allows one to study the controlling elements of these genes and the consequences of their modification. Questions may now be asked such as (1) do promoters exist in eukaryotes and does their structure modulate the level of the associated gene(s)? (2) what is the nucleotide sequence on the cloned Neurospora DNA that is recognized by E. coli or Neurospora RNA polymerase, etc.? To answer these questions, Dr. Giles is using the DNA cloned from the qa-2 gene as a probe to detect the presence or absence of qa-2 specific messenger RNA(mRNA). The results show that the mRNA for the qa-2 gene is present in cultures induced for quinic acid metabolism but not in uninduced cultures, suggesting that expression of this gene is regulated at the transcriptional level. The details are now being examined.

The technique of re-introducing Neurospora genes into their original host after passage in E. coli has been utilized by Dr. Daniel Vapnek, a colleague of Dr. Giles. He has demonstrated that the promoters of Neurospora structural genes are also very similar to those of E. coli. This finding extends the data of Hogness and others showing that binding sites for the RNA polymerase on DNA have been highly conserved throughout evolution.

Clearly, research on RNA polymerase binding sites will continue to be of great basic interest in understanding the mechanisms of genetic expression. However, such research has not escaped the attention of others more concerned with the industrial aspects of genetic engineering. Learning how to modify promoters in order to increase their efficiency in coaxing RNA polymerase molecules to transcribe stretches of DNA may be crucial to the economical use of "genetic engineering" methods to produce products such as interferon, growth hormone, and insulin.

4. Some Unusual Aspects of the Control of Transcription of DNA by Eukaryotes

a. A Possible Role of Intervening Sequences in Regulating Phenotypic Expression

GM 26378 - Peter Rae, Yale University

Unlike the prokaryotic genome, the amount of DNA in the eukaryote's genome exceeds by a very large factor that which is necessary to code its proteins. This excess of DNA is explained only in part by the number of genes present as multiple copies in the genome. Since 1977, it has been recognized that eukaryotic structural genes occur in pieces separated by intervening DNA sequences. These sequences are transcribed along with the coding regions into precursor RNA, but later are spliced out to produce

mature RNA. Initially, these sequences were believed to be associated only with genes producing specialized cell products such as insulin, ovalbumin and immunoglobin. Recently, however, intervening sequences have been identified as a ubiquitous feature of the eukaryotic genome, including genes coding for rRNA, tRNA and dihydrofolate reductase.

There is no consensus concerning the function of the intervening sequences. Dr. Walter Gilbert (GM 09541) has evidence that intervening sequences divide genes into units, called minigenes, which code for different functional parts of the molecule. For example, the coding sequence of the hemoglobin gene is divided into several segments by intervening sequences. Each segment codes for a different functional region of the hemoglobin molecule, e.g., the heme binding region. Gilbert, and others, feel that minigenes facilitate evolution by increasing the probability of recombination, and also the probability that such recombination occurs between, rather than within, functional genetic units. However, the concept that intervening sequences divide genes into minigenes coding for different functional units appears not to be applicable to all molecules. For example, rat serum albumin, an apparently functionally homogeneous protein, is coded by a gene which is divided by thirteen intervening sequences (Dr. James Bonner, GM 13762).

Dr. Peter Rae has suggested that intervening sequences may be involved in regulating phenotypic expression. He has shown that three quarters of the gene copies coding for 28S ribosomal RNA in Drosophila embryos have intervening sequences; yet, in the cells which produce an abundance of this RNA during development, the 28S RNA genes with the intervening sequences are not copied. From this intriguing observation, it seems possible that the presence or absence of intervening sequences in a gene may determine the time or conditions under which that gene is expressed.

b. A Control Region for Transcription of DNA Located Within a Structural Gene

GM 22395 - Dr. Donald Brown, Carnegie Institute

Ribosomes are composed of some 50 proteins and three RNA molecules. The smallest of the three RNA species is 5S RNA. (The S refers to Svedberg units, which are a measure of mobility in the ultracentrifuge). Dr. Donald Brown has found that at least some of the signals needed to begin transcription of 5S RNA from the DNA of the frog Xenopus seem to lie within the DNA region coding for the actual sequence of the 5S RNA. This is very different from any gene that has ever been studied in prokaryotes; there, the regions that control transcription of DNA into RNA - operators, promoters, etc. - all lie outside of the structural gene.

Dr. Donald Brown used genetic engineering, sequencing techniques and an in vitro transcription assay. Restriction enzyme fragments were isolated containing the entire 120 base pairs of the 5S RNA gene and part of the plasmid used to clone the gene. Exonuclease III, followed by S_1 nuclease, was used to digest sequentially the 5' end of the fragment. These fragments, enzymatically deleted in their 5' flanking regions, were returned to the plasmid and were tested for their ability to support transcription in the assay system. Fragments lacking the entire 5' flanking

region synthesized the 5S RNA or slightly smaller RNAs. Fragments deleted as far as 50 nucleotides into the gene synthesized discrete RNAs that were initiated in the plasmid vector and transcribed throughout the remainder of the gene. Fragments deleted 55 or more nucleotides into the gene synthesized little or no RNA. When fragments were digested from the 3' end, discrete RNAs were obtained unless the fragment was digested beyond nucleotide number 81 in the gene. Thus a control region appears to be located within the gene between nucleotides 55 and 81. The relationship of this control region to the initiation site was determined by inserting additional bases between nucleotide numbers 41 and 42 in the gene. Transcription of the expanded gene was initiated at a different nucleotide in the flanking sequence or in the gene. The distance of the new initiation site from the normal initiation site was identical to the number of nucleotides inserted. Hence, for this gene, the initiation site appears to be located at a fixed distance from the control region, rather than being determined by a particular type of nucleotide or sequence of nucleotides. In other words, it can be inferred from Dr. Brown's data that there is a sequence within the gene that is sensed as a reference point for a "molecular ruler" that measures a fixed distance back along the gene to define the second point where RNA synthesis will be initiated. The significance of this new finding, including the occurrence of similar control regions within other eukaryote genes, remains to be assessed.

5. Another System for Cell Regulation: The Control of Protein Synthesis in Eukaryotic Cells by Initiation Factors

GM 15399 - Umadas Maitra, Albert Einstein College of Medicine
GM 22135 - John W. Hershey and Rob Benne, University of California
GM 26796 - William C. Merrick, Case Western Reserve University
GM 21424 - Jolinda A. Traugh, University of California, Riverside

There is increasing evidence that a major site for control of protein synthesis in eukaryotic cells is the macromolecules involved in initiation of protein translation. The translational control mechanism affects the overall pattern of protein synthesis in the cells, resulting in broad and pleiotropic effects. The translational pattern described in eukaryotic cells is very different from the transcriptional control (Jacob-Monod) and attenuation of transcription models in prokaryotic cells (see Section 2,b.) which provide for a fine-tuning type of control. In eukaryotic cells, translational control during protein synthesis initiation has now been clearly linked to such broad effects as (1) physiologic response to the energy state of the cell, (2) the induction of protein synthesis by hormones, (3) takeover of protein synthesis apparatus in cells by viruses, and (4) response to interferon. At this time a solid base of information describing the molecular events related to this control is emerging. Such information is significant because the control of differentiation, development, and response to cellular physiologic states in eukaryotes has previously been known only at a phenomenological level.

At the initiation phase of protein translation, several components must operate together to produce one complex, highly cooperative molecule which represents the first association of tRNA and ribosomes. A protein factor, eIF-2, is essential to achieving this cooperative structure. In the systems that are best studied, eIF-2 occurs in very small amounts which are recycled in a catalytic fashion. eIF-2 participates in binding the chain initiating transfer RNA species, methionyl tRNA, to the smaller subunits of ribosomes.

The binding events that initiate protein synthesis proceed in a series of orderly, sequential reactions. Guanosine triphosphate first binds to eIF-2 to form the binary complex eIF-2•GTP complex. Methionyl transfer RNA (met-tRNA), the initiator tRNA for all peptides, then binds to form the ternary complex eIF-2•GTP•met-tRNA. This set of reactions is extremely responsive to the physiological state of the cell. A loss of high-energy ATP immediately results in a corresponding loss of GTP and an increase in GDP, since the degree of phosphorylation of adenine and guanine nucleotides is linked via the efficient nucleoside diphosphate kinase system. GDP is a very potent inhibitor of the reaction forming the eIF-2•GTP complex. Thus a slight decrease in cellular GTP concentration and concomitant increase in GDP results not only in a decrease in concentration of a reactant, but in an increase in a potent inhibitor. Thus, if the cell is under stress or otherwise in a low energy state as reflected by a lowered ATP level, the cell will shut down the energy-expensive process of protein synthesis at a very early stage, prior to commitment of appreciable energy resources. The shutdown is a generalized control which affects translation of all proteins and thus has broad or "pleiotropic" (multiple product) effect. When the energy state of the cell is sufficient for translation to continue, the met-tRNA ternary complex is formed, and mRNA (in capped form) is bound to an eIF-2 subunit, permitting protein synthesis to proceed. The details of this sensitive control mechanism, at the molecular level, will remain a topic of interest to all biologists for quite some time.

A most important concept to emerge in studying the regulation of protein synthesis is that rapid changes in the rate and type of protein synthesis occur by covalent modification of such protein initiation factors. In some systems, modification of a small recyclable pool of initiation factors mediates a major response. The establishment of the antiviral state by interferon and the coordination of heme and globin biosynthesis during reticulocyte maturation involve partial phosphorylation of the initiation factor eIF-2. During the sequential expression of genetic information during cell growth and differentiation and during the viral takeover of the host's translational machinery, other protein synthesis initiation factors undergo phosphorylation.

The significance of these phosphorylation reactions in regulating the overall economy of the cell is still unclear. The data of some investigators (for example, Dr. Daniel Levin, working with a system that synthesizes hemoglobin) indicate that phosphorylation directly inactivates essential initiation factors such as eIF-2. Others feel that phosphorylation does not directly inactivate this protein, but alters its subsequent utilization in the ribosome cycle. Others, such as Dr. N. Gupta, feel that phosphorylation has no direct role in initiation events.

It is clear that there is still controversy and some lack of consensus in this very active research area. As the issues become resolved over the next few years, we should have a far clearer picture of how environmental factors - stress, hormones, interferon, etc. - impact on the synthesis of cellular macromolecules, such as proteins.

6. Regulation of Cytochrome C and Mitochondrial Genes in Yeast. GM 26061 - Richard Zitomer, State University of New York

Much genetic research is currently performed with yeast, because this single-celled eukaryotic organism has many of the features of mammalian cells. For example, yeast cells contain mitochondria, and thus interactions between the nuclear genome and extranuclear genetic elements, such as mitochondrial DNA, are readily studied. At the same time, yeast can be manipulated easily, and is in this way, similar to the prokaryotes, such as bacteria. Yeast cultures can yield large numbers of organisms (rare mutants are readily detected) and large amounts of tissue (trace substances are readily isolated). Furthermore, as summarized below, it was recently shown that yeast cells take up and express purified DNA.

Dr. Richard Zitomer's research (GM 26061) concerns the cytochrome c genes of yeast, the most extensively studied yeast genes. There are two cytochrome c genes, the major one coding for 95% of the cytochrome c protein. Cytochrome c is a respiratory protein which functions in conjunction with a large number of other respiratory proteins in the mitochondria to generate cellular energy. The amount of cytochrome c in a yeast cell is regulated by the cellular energy status: when the sugar glucose is available, cells do not accumulate respiratory proteins, but rather ferment the sugar to obtain energy. When glucose or an equally energy-rich sugar is not available, cells respond by producing high amounts of respiratory proteins, including cytochrome c. In addition, some respiratory proteins are encoded in the mitochondrion rather than in the nucleus where most genes, such as cytochrome c, are encoded. The expression of the mitochondrial genes is regulated coordinately with the nuclear respiratory genes, suggesting a communication system across the nuclear and mitochondrial membranes analogous perhaps to the cell-cell communication in a multicellular organism. Dr. Zitomer's goal has been to determine how the cytochrome c genes are regulated in response to the availability of energy sources and how the coordinate regulation between nuclear and mitochondrial respiratory genes is achieved.

To achieve this goal, Dr. Zitomer has used purified cytochrome c genes, cloned by recombinant DNA techniques, and a large number of mutants with altered cytochrome c activity. With these tools, cycl, the major cytochrome c gene of yeast, has been purified. Since a gene is expressed through a series of steps including 1) transcription of a messenger (mRNA) from the DNA, 2) modification and transport of the mRNA to the cytoplasm, 3) translation of the mRNA into protein, and 4) processing of the protein, regulation may occur at one, several, or all of these steps.

Using the purified cycl gene to identify and quantitate the cytochrome c message (by hybridizing the complementary mRNA to the DNA), Dr. Zitomer has shown that it is the level of mRNA itself which is regulated in response to energy source. Furthermore, it is the availability of a rich energy source rather than the energy derived from it to which transcription of the cycl gene responds: the rate of transcription of the cycl gene is dramatically decreased within two minutes after addition of glucose to cells, even when utilization of the glucose is inhibited. In the absence of glucose, alteration of mitochondrial function, including changes in the rate of mitochondrial protein synthesis, affects cycl gene expression. However, the expression of the cycl gene in the presence of glucose is unaffected by inhibition of mitochondrial function.

Dr. Zitomer is also studying the sequences along the DNA that are important to the expression and regulation of the cycl gene. These sequences are, besides the region which codes for the protein, the initiation and termination sites for transcription and the region at which regulatory elements interact with the DNA either to promote or to prevent transcription. By a series of experiments in which fragments of DNA comprising the cycl gene and its surrounding sequences were matched to the mRNA, the positions where the mRNA starts and ends on the gene have been mapped. Based on extrapolation from bacterial models, the regulatory region appears to be located near the initiation site.

Finally, Dr. Zitomer has begun to exploit a new technique which gives additional advantage to studies of gene regulation in yeast compared to most other eukaryotes. Yeast cells are capable of taking up purified DNA and incorporating this DNA into the genome, passing the genetic information on this DNA to future generations and expressing the genes encoded in it. (Note, however, that this can also be done in Neurospora, work of Dr. Vapnek, Section B,3.) By adding the cytochrome c genes, both in their normal form and in altered forms, to the cells, it will be possible to examine how specific changes in a gene affect its expression and regulation. Such changes can be induced in precise locations in the gene and can help to determine the exact function of the various gene regions.

Studies such as those by Dr. Zitomer are very exciting and powerful and the information gained with lower eukaryotes such as yeast will be critical to the understanding of gene regulation in higher eukaryotes, including man.

C. Polynucleotides which Exist in a Left-Handed Helix.

GM 12121 - Richard E. Dickerson, California Institute of Technology
GM 17371 - Arnott Struthers, Purdue University

Some attention has been given in the popular media and scientific press to the recent discovery of small polynucleotides which have a left-handed helical configuration (Z-DNA). This configuration was not predicted by the conventional Watson-Crick right-handed model of DNA B-DNA. The polynucleotides used in this work are crystals of a double-stranded deoxyhexamer $(CG)_3$ with alternating cytosine and guanosine bases on each strand. The B-DNA conformation can change to the Z-forms under some circumstances, all of which are not specified. The three dimensional arrangement of the Z-DNA form a "jagged" helix compared to the "smooth" helix of the B or right-handed DNA. This work was originally reported in Nature (282:680, Dec. 13) by Rich et al. from MIT. They speculated that the "jagged" edges of the helix might make the DNA more susceptible to either regulatory or mutagenic agents, and this is therefore, a potentially exciting discovery. While these speculations appear plausible and grounded in logic, they are as yet not fully substantiated.

The original observation was supported by an NCI grant to Alexander Rich; however other related work has been supported by NIGMS. Dickerson has reported the discovery of a left-handed helical conformation for the tetramer d(CGCG) in high salt concentration He has extended the observations made in Rich's laboratory by showing (1) that the left-handed polynucleotide helix can occur in helices containing several hundred base pairs and (2) that the left-handed helical conformation also exists when alternating bases of adenine and thymine respectively are substituted for cytosine and guanine.

Richard Roberts, a leading figure in the analysis of nucleic acid sequences, has pointed out that "depicting DNA as a sterile one-dimensional string" may be quite misleading. The work discussed above, demonstrating a radically different conformation or "texture" of stretches of DNA, may be only an extreme example of the fact that the DNA double helix is not perfectly smooth and regular. According to Roberts, local distortions in conformation may be the "very elements by which other macromolecules such as repressors or RNA polymerases, interact with DNA."

We are yet some way from defining the rules which relate primary DNA sequences to three-dimensional structures. Clearly, the model of DNA proposed by Watson and Crick defines the structure of DNA only in a general, global way, and quantitatively minor deviations from this structure may be disproportionately important to DNA function. Much more experimental and theoretical work is needed in this area. Dr. Roberts feels that "if we are ever to understand the nature of the interactions which control the behavior of macromolecules, it will be essential to expand both our experiments and our theories into this third dimension." The computer, and particularly computer graphics, is likely to prove an important tool in these endeavors.

D. The Control of Nucleic Acid Packaging in Viruses

GM 24365 - Phillip Serwer, The University of Texas Health Science Center

Viruses are subcellular particles that invade cells and introduce viral information via nucleic acid, either RNA or DNA, into the cell. Such viral information will either direct the construction of progeny viruses, usually resulting in destruction of the host cell, or alternatively, become incorporated into a host chromosome. The former is the course taken by several disease-causing viruses including Poliovirus, some Herpes viruses, and Poxviruses. It has been hypothesized that some human cancers are caused by viruses taking the latter course.

It is desirable to understand the life cycle of viruses for the following reasons: a) Such information should assist in the detection, isolation and inhibition of disease-causing viruses; and b) viruses, although subcellular, exhibit the apparently purposeful activity of living organisms; the study of viruses therefore may quickly reveal some mechanisms basic to all living organisms.

The object of Dr. Serwer's research is to characterize in detail the life cycle of bacterial virus T7, a parasite of the intestinal bacterium Escherichia coli. Dr. Serwer has chosen T7 rather than an animal virus because the techniques for genetic manipulation are more advanced for bacteriophages and also because bacteriophages grow more rapidly. T7 infection shuts off the DNA and protein synthesis of its host, properties that are desirable for clean experiments.

During the assembly of bacteriophage T7 and several animal viruses, a proteinaceous container (capsid) is assembled which subsequently draws into its interior ("packages") a linear molecule of DNA. The capsid protects its "packaged" DNA from DNA-digesting enzymes prior to injection of the DNA into a host bacterium during the next infectious cycle. In some smaller RNA plant and bacterial viruses with 180-subunit capsids, spontaneous interactions between the capsid subunits and the viral nucleic acid are sufficient to direct "in vitro" assembly of the capsids. However, the larger bacteriophages (with 420 capsid subunits), including P22, T4, and T7, require non-capsid proteins to direct assembly of the capsid. Evidence indicates that these latter bacteriophages assemble a DNA-free capsid which subsequently packages DNA. Thus, interactions with DNA probably do not assist capsid assembly in the larger phages.

The long-range goals of Dr. Serwer's research are to determine both the mechanism by which the capsid is assembled and the mechanism by which the DNA is packaged. Dr. Serwer's approach to achieving these goals is to utilize virus-infected cells in order to isolate and characterize precursors of the mature virus. He hopes to describe the molecular events that occur during viral assembly and to determine, in particular, the forces that control packaging and assembly.

The characteristics of the T7 precursors which have been isolated to date suggest that: (1) the force which induces packaging of T7 DNA is a difference in pressure between the inside and the outside of the capsid. DNA packaging may, therefore, be sensitive to osmotic properties of the environment of virus-infected cells, and that (2) electrostatic repulsion and attraction control the binding of the capsid to DNA during packaging. Furthermore, Dr. Serwer hypothesizes that binding of the capsid (negatively charged) to DNA (negatively charged) is controlled by a positively charged "extendable projection" which is attached to the capsid.

A combination of biochemical and ultrastructural experiments has provided evidence for the "extendable projections." Preliminary experiments show that treatment with trypsin (which does not digest the capsids) resulted in the cleavage of a 3500 dalton fragment from a capsid protein ("P10") and in the loss of "P19", previously known as a minor, but not integral, capsid protein. Correlated with these protein losses was the loss of a visible external projection from the base of the core of the capsid. No structural changes were noted in the capsid itself. Trypsin treatment of the capsid also increased the negative charge, which indicated that a positively charged peptide had been removed.

To achieve these results, Dr. Serwer has developed sophisticated new techniques to isolate and characterize viruses and viral precursors. He is particularly adept at stabilizing fragile intermediates. Bacteriophage mutations are used as a tool to find and to serially order intermediate structures involved in DNA packaging. Dr. Serwer is beginning to utilize kinetic labelling and intends to utilize *in vitro* re-entrance of nucleic acid into the viruses to study the packaging pathway.

These studies may have an important spin-off. The work has required the development of improved methodology for isolating and visualizing viruses and their components. Such methodology may substantially increase our ability to monitor viruses and virus-related particles in the environment (water supplies, for example) and to identify viruses or virus-like particles related to disease. It is also possible that extendable charged projections may be a general mechanism controlling interactions between macromolecules and interactions between cells. Dr. Serwer's progress is particularly important because, as first suggested by Demarr in 1952, viral DNA packaging is a potent target for antiviral chemotherapy.

E. Novel Genetic Processes in the Spread of Drug Resistance

GM 21887 - Walter Guild, Duke University

Resistance to commonly used antibiotics began to appear among several species of pathogenic bacteria in the late 1960's. By 1977, strains of *Streptococcus pneumoniae* resistant to as many as 12 antibiotics were found in South African hospitals, and resistant strains accounted for a majority of the *Pneumococcus* isolates in Paris in 1978. These results have stimulated work to find the genetic basis of the resistance. The first assumption made was that "plasmid resistance factors," small, circular DNA molecules carrying genes which can confer drug resistance on many other

species of bacteria had finally become established in the pneumococci. However, work done by Dr. Walter Guild implies a different mechanism for pneumococcal resistance. He has found that transfer of chromosomal genes from one cell to another and between species may occur by the process of conjugation in the "gram positive" bacteria, a major group that includes, among others, the pneumococci, streptococci, staphylococci, and many common soil bacteria. This discovery is a major step in understanding the nature and origin of resistance genes in the clinically significant pneumococci.

Conjugation, which was discovered in E. coli by Joshua Lederberg in the 1940's, has proved to be a major factor in bringing that organism to its central role in genetic investigation. Conjugation consists of a protected transfer of DNA in E. coli and other gram negative bacteria through hair-like bridges between donor cells and recipient cells. The transfer of the bacterial chromosome through this appendage can be measured by the appearance of genes in the recipient cell in a time-dependent fashion. Certain plasmids have been discovered because of their ability to transfer more rapidly than the chromosome in this conjugation process. It is now known that certain plasmids can also insert themselves in the chromosome and lead it to transfer to another cell on the correct signal. In E. coli, a set of 20 "tranfer genes" is required to encode all the information necessary for conjugation to occur.

By contrast to the gram negative bacteria, transfer of chromosomal genes by conjugation had never been confirmed in gram positive bacteria, which have very different wall and membrane structures. While studying the transfer of genes in pneumococcus by the process of transformation, the uptake of free DNA by cells, Dr. Guild undertook studies concerning proliferation of antibiotic resistance in this organism. He examined pneumococcus for plasmids that might be transferred through transformation. Although he was able to show that plasmids could be detected in pneumococcus following their deliberate introduction from another species, Dr. Guild could not find plasmids in the clinically isolated strains. He could show, however, that the genes of interest were located on the bacterial chromosome and did not reside on free plasmids. These antibiotic resistance genes were present as large insertions, suggesting that part or all of one or more plasmids had at some time been inserted into the bacterial chromosome.

Dr. Guild has found from one to four such inserted genes in chromosomes of the various strains examined. The multiple drug resistance may be accounted for by the fact that two of these genes each confers resistance to large groups of antibiotics. In addition, he has shown that three of the genes are essentially identical to genes carried on plasmids in other bacteria, thus giving a strong clue to their origin. Equally interesting is Dr. Guild's observation that some of the resistant strains also carry mutations in certain housekeeping genes, such as those for ribosomal proteins, thus conferring resistance to still other antibiotics affecting ribosome function.

A striking finding is the observation that some of the blocks of inserted genes in pneumococcus transfer by conjugation from the chromosome of the donor cell to the chromosome of the recipient. These conclusions are based on four lines of evidence: 1) the transfer is DNAase resistant, contrary to transfer by transformation; 2) the gene transfer occurs only at high cell density, as when cells are pressed together on a filter; 3) recombination of markers on the two bacterial chromosomes is not observed in this conjugation process, whereas recombination occurs under transforming conditions; and 4) the transfer is endonuclease-independent: whereas a specific endonuclease is required for the transformation process, endonuclease-deficient mutants are completely capable of transferring genes by conjugation.

Dr. Guild has also been able to detect transfer of chromosomal resistance genes to pneumococcus by conjugation with several species of streptococcus. Since similar interspecies transfers have been reported by other laboratories, it now appears that streptococci undergo a previously unknown form of chromosomal conjugation. The recognition of interspecies spread of resistance genes by conjugation is an important facet of understanding the epidemiology of resistance to antibiotics.

Dr. Guild is now determining whether these chromosomal resistance genes are integrated after transfer at a few specific sites or at many different sites, and whether there are specific insertion sequences that facilitate the integration, such as have been described for other transposable elements.

We have mentioned previously (see Section II-A-1; Transposable Genetic Elements) the importance of studies of drug resistance factors in gram-negative bacteria such as E. coli in furthering the development of plasmid biology and of transposable genetic elements. It is gratifying that a related mechanism has now been found in a completely different group of microorganisms, the gram-positive bacteria. If experience with the gram-negative bacteria is a guide, this finding will develop into a tool for the study of the fundamental genetics of organisms such as pneumococcus and streptococcus. Concurrently, Dr. Guild's work provides new concepts and approaches for more applied research in microbiology, on topics such as the origin of drug-resistant strains and new strategies for prevention or therapy.

F. Some Aspects of Ribosome Biology

1. Regulation of Bacterial Cell Growth Through Translation Control of Ribosome Biosynthesis

GM 20427 - Masayasu Nomura, University of Wisconsin

Dr. Nomura has achieved a significant breakthrough in our understanding of the regulation of ribosome biosynthesis. The ribosome, the organelle responsible for protein synthesis, plays an essential role in

cell growth and its regulation. In fact, in bacteria the number of ribosomes in a cell is directly proportional to the growth rate of the cell. For this reason, a major goal of current biological research is to understand how cells regulate the production of ribosomes.

Bacterial ribosomes contain three different RNA molecules and fifty different proteins. Therefore, comprehension of ribosome biosynthesis requires understanding a set of reactions beginning with the transcription of over sixty genes and ending with the assembly of nucleic acid and protein into a complex organelle. Understanding the regulatory process also requires a knowledge of how synthesis of the RNA and the protein components are coordinated. Until the present time, attention has been directed toward regulatory mechanisms operating at the transcriptional level.

Dr. Nomura has demonstrated that regulation of ribosome synthesis occurs during translation. Such regulation occurs by a feedback mechanism which regulates the availability of the mRNA needed to synthesize "r-protein" (ribosomal protein). He has shown that the rate of r-protein synthesis is not coordinated with the rate of r-protein gene transcription. Dr. Nomura has, furthermore, specifically identified certain r-proteins which act as repressors for r-protein synthesis.

In order to examine the regulation system, Dr. Nomura has studied the effect of inserting into E. coli a transducing phage, spc, containing extra copies of r-protein genes. Thus, this E. coli strain is partially diploid (merodiploid) with respect to the r-protein genes. In comparing the rate of r-protein transcription of the merodiploid with that of a control "haploid" strain, he found that the rate of synthesis of r-protein mRNA in the merodiploid strain is about two-fold higher than that in the control strain. However, this r-protein mRNA was found to be more rapidly degraded in the merodiploid strain than in the control strain, and thus the steady-state amount was only slightly higher in the merodiploid strain than in the control strain. Therefore, because of this increased degradation, no gene dosage effect was observed on the appearance of the fourteen r-proteins encoded by the spc or E. coli genomes.

From these results, Dr. Nomura concluded that E. coli cells have the ability to regulate the rate of r-protein synthesis, regardless of the rate of transcription of r-protein genes. Dr. Nomura has proposed a model which involves selective inhibition of r-protein mRNA translation by a feedback mechanism. The model assumes that r-protein can recognize and inactivate its own mRNA. As long as the assembly of ribosomes removes free r-proteins, the corresponding mRNA escapes the inactivation and continues to direct synthesis of r-protein.

Dr. Nomura further postulates that a specific nuclease(s) is involved which recognizes and digests r-protein mRNA when it is complexed with r-proteins, but does not utilize free mRNA as a substrate. A precedent for such a nuclease exists in colicin E3; that enzyme cleaves various rRNA's in 70S ribosomes but fails to cleave them in the free state. It is not yet known what determines the normal rate of decay of r-protein mRNA, and the possibility is not excluded that transcriptional regulation of r-protein mRNA synthesis also occurs.

The model has been tested using an in vitro protein synthesizing system with various template DNA molecules carrying r-protein genes. Dr. Nomura found that r-protein L1 inhibited the synthesis of both proteins of the L11 operon, L11 and L1, but not the synthesis of other proteins. Similarly, S4 inhibited selectively the synthesis of the three proteins S13, S11, and S4. S8 also showed preferential inhibitory effects on the synthesis of some proteins encoded in the spc operon, L24 and L5. Inhibition was shown to take place at the level of translation rather than transcription. Thus, at least some r-proteins (L1, S4, and S8) have the ability to cause selective translational inhibition of the synthesis of certain groups of r-proteins whose genes are in the same operon as their own. These results support the hypothesis that certain "free" r-proteins not assembled into ribosomes can act as autogenous feedback inhibitors to regulate the synthesis of r-proteins.

Dr. Nomura's results and hypothesis contribute substantially to our understanding of how an organism regulates its growth. Selective mRNA inactivation may account for regulation of other macromolecular aggregates as well, and may provide an attractive alternative to the current theories of transcriptional regulation.

2. Mammalian Mitochondrial Ribosomes are Significantly Different from Cytoplasmic Ribosomes

GM 15438, GM 23322 - Thomas W. O'Brien, Univ. of Florida,

Dr. Thomas O'Brien has undertaken studies of the structure, function, and biosynthesis of mitochondrial ribosomes, or "mitosomes," in a manner analogous to the current characterization of cytoplasmic ribosomes of prokaryotic and eukaryotic cells being carried out by several laboratories. In order to study mitosomes, Dr. O'Brien first developed a method for large-scale preparation of the scarce mitosomes. Successful purification of mammalian mitosomes has been achieved utilizing 30 kg of beef liver to obtain 40-65 mg of purified mitosomes or mitosomal subunits.

Using sedimentation and gel electrophoresis among other techniques, Dr. O'Brien has made considerable progress in characterizing mitosomes. His efforts have led to some startling findings: First, mitosomes contain twice as much protein as RNA. This is in sharp contrast to cytoplasmic ribosomes, which contain a 1:1 ratio of protein to nucleic acid. Second, there is little overlap between the protein composition of mitochondrial and cytoplasmic ribosomes. Third, when compared by two-dimensional electrophoresis, the ribosomal proteins of mitochondria from beef liver were found to be markedly different from their counterparts in human, rat, rabbit, or chicken tissues.

These observations on mitochondrial ribosomes cast a new perspective on the previously held concepts of ribosomal structure. Cytoplasmic ribosomes from many sources exhibit a highly-conserved protein composition. In fact, cytoplasmic ribosomal structure is so invariant that cytoplasmic ribosomes taken from almost any source will function in a variety of in vitro protein assembly systems. The highly conserved protein composition of ribosomes has been considered to be essential to the highly cooperative molecular interactions in which ribosomes participate to fulfill their role in protein synthesis.

Dr. O'Brien has determined that all 52 of the mitosomal proteins vary from species to species; that is, at the resolution obtained, it has not been possible to pick up any invariant proteins or protein regions. This is, again, in contrast to the cytoplasmic ribosomal proteins which are highly conserved between species. For example, although the cytoplasmic ribosomes from cows and rats have conserved proteins, the corresponding mitosomal proteins do not. Dr. O'Brien believes that, because of the high protein content, mitosomal architecture is different from cytoplasmic ribosomal architecture. He feels that mitosomes may rely more on protein contact domains than on protein-nucleic acid interactions, and that such protein-protein domains may be more tolerant of variation than are the protein-nucleic acid interactions.

In addition to the benefits of primary information about mitochondrial ribosomes and the evolutionary significance of these findings, the differences observed between cytoplasmic and mitochondrial ribosomes may be exploited in some useful ways. For example, it has been known for some time that mitochondrial ribosomes respond differently to antibiotics than do cytoplasmic ribosomes. This feature has been used in a variety of experimental situations, such as experiments requiring manipulation of the energy supply of the cell. Dr. O'Brien's observations suggest some reasons for altered antibiotic sensitivity and, furthermore, may permit exploration of some detailed subunit structure-function questions. Thus, chloramphenicol is bound to bacterial ribosomes at the L16 protein. Eukaryotic cytoplasmic ribosomes do not bind chloramphenicol and, therefore, they cannot be used to compare the structure and function of subunit regions. The bovine mitochondrial ribosomes do bind chloramphenicol on a large protein, L-2 (three times the size of the bacterial protein L16). Structural homology may now be sought between these two chloramphenicol-binding proteins.

Another possibly significant aspect of this work lies in the current interest in differential sensitivity to antibiotics among individuals. Dr. O'Brien has speculated that aplastic anemia, a condition which can be induced by chloramphenicol, may be related to individual variation in mitochondrial ribosomes. He is exploring this possibility in a research project funded by the NIGMS Pharmacological Sciences Program (GM 25888).

G. Gene Transfer in an Intact Animal

GM 18586-09 - Winston Salser, Univ. of California, Los Angeles

Transformation is a process of inserting exogenous DNA into the genome. Transformation of mammalian cells has been accomplished *in vitro* using a number of approaches; however it has previously not been possible to establish transformed cells in an intact animal. This step is, of course, essential if it is to be possible to treat inherited disorders by replacing the defective genes. In the April 3, 1980 issue of *Nature* (284: 422-425), Drs. Cline, Stang, Mercola, Morse, Ruprecht, Browne and Salser reported the successful transfer of genes bearing methotrexate resistance to hematopoetic cells in a mouse. The paradigm for this experiment, which was supported in part by NIGMS, is summarized as follows: Bone marrow stem cells were isolated from two strains of mice. The cells from one strain, identified by a chromosomal marker, were transformed by exposure to DNA containing the gene for methotrexate resistance. The cells from the other strain were exposed to wild type DNA. A 1:1 mixture of the cells was injected into mice, which previously were irradiated to destroy the bone marrow. These mice were treated with methotrexate over various intervals; subsequently, karyotypes of blood and hematopoetic cells were examined to determine from which of the two injected strains of cells these cells were derived. It was hypothesized that the methotrexate treatment would provide a selective advantage to the transformed methotrexate resistant stem cells over the methotrexate sensitive non-transformed line.

These experiments clearly demonstrated a selective advantage for the cells transformed with DNA from methotrexate resistant cells, indicating a successful gene transfer to the host animal. If transformed cells can be established in human bone marrow, the clinical implications of this work will be significant. Gene transfer could potentially be used in chemotherapy to permit the use of higher doses of methotrexate without compromising the hematopoetic system. Moreover, other genes could also be transferred by attaching the genes to the DNA carrying the methotrexate resistant marker DNA. Subsequent methotrexate treatment would establish the transformed cells carrying the desired gene. With this approach, defective genes could, in principle, be replaced in patients with sickle cell anemia or β-thalassemia.

NIGMS support for Winston Salser (GM 18586) played an important role in this important breakthrough and will be instrumental in the future in developing the possible implications of the work. The project proposes experiments to transfer globin genes to Friend cells to study their expression *in vitro*. It briefly discusses the preliminary data for the methotrexate work and suggests the future use of the methotrexate system to facilitate the transfer of the globin gene to intact animals. The appropriate globin gene has already been isolated, cloned and sequenced. Experiments to transfer this globin gene to bone marrow are now under way.

The cited work illustrates an important benefit of basic science to applied efforts. Dr. Cline, a hematologist supported by NIAMDD and NCI, spent the last year on sabbatical leave in Dr. Salser's lab and is a coinvestigator on the GM project. Dr. Cline has pioneered work on the hematopoetic system and developed *in vitro* bone marrow systems. Dr. Salser has developed gene sequencing and cloning techniques for studying gene expression. The merging of these basic and applied approaches put into use during Dr. Cline's sabbatical with Dr. Salser, has provided hope for significant breakthroughs in the treatment of diverse clinical problems.

PHARMACOLOGICAL SCIENCES PROGRAM

Fiscal year 1980 brought a "new look" to the Program. After fifteen years as the Pharmacology-Toxicology Program, on November 19, 1979 we officially became the Pharmacological Sciences Program. The new name does not represent an abrupt change in the direction or content of the program; rather, the new name reflects the gradual evolution that the scientific substance of the program has undergone since its beginning in 1964.

Since that time the areas represented in the program have diversified and developed into fields unimagined a decade ago. The program was broadened with the inclusion of the biorelated chemistry area in 1972 and now represents one of the largest sources of support for innovative organic chemistry applied to biological problems at the National Institutes of Health. Environmental toxicology has expanded into an important discipline in itself and is now the responsibility of the National Institute of Environmental Health Sciences. Other areas of biology are being brought to bear upon pharmacological problems. These include molecular biology, immunology, and especially genetics. The staff of the Pharmacological Sciences Program has taken "crash" courses to learn to deal with such new areas (to pharmacologists and chemists) as recombinant DNA and the "MUA's" (memoranda of understanding and agreement) which the administration of this technology requires.

Activities related to clinical pharmacology, both intramural and extramural, were some of the highlights of FY 1980. William Z. Potter, M.D., Ph.D., became a formal member of the Pharmacological Sciences program staff as Coordinator for Clinical Pharmacology with responsibilities for the new clinical pharmacology area in the Pharmacology Research Associate Program. In addition to his work with the fellows and the NIGMS clinical pharmacology lecture/grand rounds series, Dr. Potter maintains a joint appointment in the Laboratory of Clinical Psychobiology, National Institute of Mental Health. The first formal clinical pharmacology fellow, Dr. Richard Burns, completed his training in the PRAT program and has joined the staff of NIMH.

January was a sad time in the National Institute of General Medical Sciences as Dr. Folke Sjöqvist completed his year as an Expert Consultant to the Director, NIGMS, and returned to the Department of Clinical Pharmacology, Karolinska Institute, Stockholm. In addition to his research collaboration with intramural NIH scientists, Dr. Sjöqvist consulted with the Pharmacology Research Associates and preceptors, assumed a major role in the Clinical Pharmacology Trainee Workshop (see below) and served as a resource to the Director, NIGMS and to the Pharmacological Sciences program in research areas in both clinical and basic pharmacology. His stimulating presence is sorely missed.

The Pharmacological Sciences Program sponsored a four-day workshop in January on the NIH campus for trainees in clinical pharmacology. Two trainees from each extramural clinical pharmacology training program and from those anesthesiology training programs with a pharmacological orientation were invited to attend as were the intramural Pharmacology Research Associates. The meeting opened with a plenary session at which Dr. Sjöqvist presented some past, present and future perspectives of clinical pharmacology; Dr. Alexander Levitzki, a grantee from Hebrew University in Jerusalem, and Dr. Fusao Hirata of NIH presented lectures on aspects of receptor function. The afternoon was devoted to poster sessions at

which each trainee discussed research data or protocols. During the next two days NIH intramural clinical scientists presented clinical pharmacology grand rounds in the areas of cancer chemotherapy (intraperitoneal dialysis), pediatric psychopharmacology, and endocrine receptor pharmacology. A series of four three-hour mini-courses occupied the mornings with two courses presented concurrently; the two trainees from each university arranged their schedules so that one attended each course. These courses covered pharmacokinetics and drug metabolism, design of controlled clinical investigations, stable isotopes and mass spectrometric techniques in clinical pharmacology, and receptor studies in clinical pharmacology. In addition to intramural faculty, visiting faculty included Dr. Arthur Atkinson, Northwestern University, Dr. Paul Insel, University of California, San Diego, and Dr. Raymond Woosley, Vanderbilt University.

The workshop culminated with a lively panel discussion by the trainees related to the future of clinical pharmacology. The trainees were very complimentary to the Pharmacological Sciences program staff, especially Drs. Carrico, Potter, and Sjöqvist who organized the meeting. They noted that they had never before realized the breadth of research opportunities in clinical pharmacology and they enthusiastically recommended that future workshops be held, noting that they had particularly enjoyed their interaction with outstanding role models, as well as the opportunity to meet with each other.

Research Training

Under the authority of the National Research Service Act, the Pharmacological Sciences Program supports research training in the pharmacological sciences and in clinical pharmacology. Support for multidisciplinary training in the pharmacological sciences is provided through institutional fellowships. In FY 80, 38 institutions in 25 states received such awards to support a total of 365 trainees. Because of the multidisciplinary nature of the training programs, these trainees received their doctoral degrees in many different areas of biological sciences including not only pharmacology but physiology, medicinal chemistry, immunology, neurobiology and biochemistry. However, it is intended that all trainees supported in the area of pharmacological sciences receive a thorough exposure to pharmacological principles and be able to apply such principles to all aspects of health related research.

Support by the Pharmacological Sciences Program for postdoctoral training is accomplished by institutional fellowships, individual fellowships and the Pharmacology Research Associate Program. Institutional fellowships for training M.D.'s in clinical pharmacology support 43 fellows at 13 institutions in 11 states to pursue integrated training in pharmacological laboratory principles and clinical research.

Individual postdoctoral fellowships are awarded in the areas of pharmacological sciences and clinical pharmacology. In FY 80, 43 young scientists received support from this program. Of these, one was in the area of clinical pharmacology.

The Pharmacology Research Associate Program of the National Institute of General Medical Sciences provides opportunities for 22 outstanding postdoctoral fellows to spend two years doing research in the intramural laboratories of the NIH. While any laboratory at the NIH is open to fellows with a pharmacological background,

there are currently 55 active formal preceptors directing research in such areas as regulation of monoaminergic systems, mechanisms of action of chemotherapeutic agents, elucidation of the roles of endogenous mediators of physiological responses, and interactions between xenobiotics and biological materials resulting in toxic responses. During FY 80, one fellow was involved in training in clinical pharmacology under this program.

The clinical pharmacology area of the Pharmacology Research Associate program was further strengthened by the appointment of Dr. William Z. Potter (see above) as coordinator for the clinical training portion of the program and by a two semester seminar series in clinical pharmacology approved for continuing medical education credit.

Applications for Research Career Development Awards are accepted by this program only from those investigators who have expressed a commitment to research careers in clinical pharmacology. It is the intent of the Pharmacological Sciences Program and the National Institute of General Medical Sciences that such individuals, given this opportunity, will form a cadre of superb investigators capable of applying basic pharmacological principles to problems in clinical medicine. No new awards were made in FY 80; however, support has continued for two individuals in the area of clinical pharmacology. Prior to July 1, 1977, Research Career Development Awards were made in diverse areas of the pharmacological sciences and support has continued to two individuals in these areas.

Organization and Management

The Pharmacological Sciences Program is divided into two major sections, pharmacological sciences and biorelated chemistry. Dr. Carl Kuether has responsibility for the biorelated chemistry area, and, during FY 80, Dr. Christine Carrico has had primary responsibility for the pharmacological sciences portion. In addition, each administrator handles institutional and individual fellowship training activities.

As described above, Dr. William Z. Potter joined the program on October 1, 1979, and Dr. Folke Sjöqvist returned to Sweden in January, 1980. Dr. Anthony Zavadil left NIGMS in October to accept a position with the Food and Drug Administration. Mrs. Janice Milligan, secretary to the program director, retired on August 22, 1980.

Research Highlights

"Selective Destruction of Cytochrome P-450 by Drugs"
GM 25515 (Ortiz de Montellano) University of California, San Francisco

Catalytic processing of certain drugs by hepatic cytochrome P-450 results in irreversible (covalent) attachment of the drugs to the heme prosthetic group of the enzyme. The previously unsuspected generality of this process has been revealed by the observation that gaseous ethylene, the simplest unsaturated hydrocarbon, efficiently destroys the enzyme. In addition to defining the minimal requirement for cytochrome P-450 destruction, the activity of ethylene is of singular interest because it has been reported as a physiological metabolite in man and is also an important plant hormone. Catalytic inactivation of cytochrome P-450 by ethylene may thus play a normal physiological role.

Dr. Ortiz de Montellano is postulating structures for the prosthetic heme-drug adducts based on extensive spectroscopic studies, the synthesis of a model compound (N-methyl protoporphyrin IX), as well as the study of the particularly simple adduct with ethylene. Definitive evidence has been obtained that drugs, after catalytically accepting an oxygen atom, are bound to a nitrogen in the porphyrin framework of heme. In the case of ethylene, the attached group is a 2-hydroxyethyl function, the resulting adduct being N-(2-hydroxyethyl) protoporphyrin IX (isolated as the dimethyl ester). Although this adduct formally is the result of alkylation of the porphyrin by ethylene oxide, it has now been demonstrated that epoxides are not intermediates in the destructive process. A number of drugs commonly ingested in overdose quantities (ethchlorvynol, novonal, secobarbital, for example) have been shown to destroy cytochrome P-450 and thus to interfere with their own metabolism and excretion. It has been shown, in collaboration with Dr. R. Schmid's group, that administration of heme to patients leads to replacement of the impacted heme, reconstruction of the enzyme, and renewed drug metabolism. This may be a possible therapeutic modality for treatment of certain serious drug overdoses.

"Prostaglandin Hydroxylation"
GM 22688 (Kupfer) Worcester Foundation for Experimental Biology

Dr. Kupfer and his associates are attempting to establish whether prostaglandins (PGs) are endogenous substrates of the liver and kidney cytochrome P-450 monoxygenase systems and whether there are prostaglandin-specific enzymes which catalyze prostaglandin hydroxylation.

They have obtained evidence that the hydroxylation of PGs at the terminal carbon (ω-) and at the penultimate position (ω-1) are catalyzed by different liver monoxygenases. However, it also appears that they are non-specific monoxygenases which catalyze hydroxylation of PGs at both positions. This evidence is based primarily on kinetic considerations established in the rabbit, that the affinity of PGs for the ω-hydroxylating enzyme is much higher than for the (ω-1) -hydroxylating enzyme. Additionally, the administration of phenobarbital (a typical inducer of monoxygenases) altered the nature of the enzymatic activity, most probably by stimulating the synthesis of a novel PG-hydroxylating enzyme(s) which is less specific than the constitutive enzymes in that the new enzyme catalyzes the hydroxylation of PGs at both positions.

The secondary metabolism of PGs after the initial hydroxylation at the ω-position appears to occur via further oxidation of the ω-hydroxyl to ω-carboxyl, as determined from the urinary PG products. An enzyme which can oxidize the ω-hydroxyl [but not the (ω-1) -hydroxyl] to the ω-carboxyl has been demonstrated in liver cytosol. The question as to whether this involves a two step mechanism catalyzed by two enzymes has not yet been answered.

In collaboration with Drs. B. S. S. Masters and R. Okita (University of Texas Health Sciences Center at Dallas), the investigators have demonstrated with a reconstituted system, composed of purified kidney cytochrome P-450 and NADPH-cytochrome P-450 reductase, the hydroxylation of PGA_1 at the ω-position. This activity is inhibited by antibodies to kidney P-450, but not by antibodies to isolated liver P-450. This is the first demonstration of prostaglandin hydroxylation by a reconstituted purified enzyme system.

"The Role of Prostaglandins D_2 and I_2 in Man"
GM 15431 (Oates) Vanderbilt University

Systemic mastocytosis is a disease of unknown etiology characterized by abnormal proliferation of tissue mast cells in various organs. A frequent symptom is attacks of flushing which can be accompanied by life-threatening hypotension and tachycardia. In the past, the signs and symptoms of the disease have been attributed to histamine release from mast cells. Recently two patients with this disease have been investigated. The first patient had marked elevated urinary histamine excretion, five to eight times normal, but was essentially refractory to intravenous treatment with both the H_1- and H_2-receptor antagonists, chlorpheniramine and cimetidine, respectively. Daily attacks continued until the hypotension and tachycardia progressed to irreversible shock and death. The second patient had only marginally elevated urinary histamine excretion but he too was unresponsive to histamine antagonist therapy. The lack of effect of such treatment raised the question of involvement of mediators other than histamine. Examination of both patients' urines by mass spectrometry revealed a large quantity of an unknown compound with prostaglandin-like properties which had not been previously observed over several years of research with specimens from patients without mastocytosis. Isolation and structure determination indicated that this unknown was 9-hydroxy-11,5-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid; a compound previously shown to be a metabolite of PGD_2 in the monkey. Previous studies had demonstrated the presence of PGD_2 in rat serosal mast cells and more recently Dr. Oates' group has shown that human lung mast cells produce PGD_2 as essentially the only cyclo-oxygenase product. This is the first evidence that PGD_2 is produced in man, and although the spectrum of biological activity of this compound is not completely known it is a potent systemic vasodepressor, pulmonary arterial vasoconstrictor and bronchioconstrictor. Significantly, treatment of the surviving patient with both antihistamines and the prostaglandin synthesis inhibitor, aspirin (4.2g/day) has almost completely relieved him of his symptoms for over 10 months. Previously, he was suffering attacks requiring hospitalization about every two weeks. It, therefore, appears that PGD_2 may be a previously unrecognized important mediator of the signs and symptoms of systematic mastocytosis. As the mediators released from mast cells are thought to participate in allergic responses such as asthma, the discovery in these patients with mastocytosis that human mast cells release PGD_2 may have broader implications in the understanding and treatment of allergic diseases.

Prostacyclin (PGI_2) is another prostaglandin which is unstable and is synthesized by the endothelial cells lining the inner walls of blood vessels. Since PGI_2 is an extremely potent inhibitor of platelet aggregation, it could serve as a natural protection against intravascular thrombosis. However, it is not known whether a sufficient quantity of PGI_2 is synthesized by the body to function in this capacity. The development of an assay for the stable metabolite of PGI_2, dinor-6-keto-PGF_1, which is excreted in the urine has permitted the investigation of this question. Analysis of 24 hour urines from 30 healthy subjects indicated that only 0.6 g/day of metabolite was excreted. In order to estimate PGI_2 from this figure the fraction of PGI_2 converted to its metabolite in subjects receiving 0.1 to 2 mg/kg/min PGI_2 by infusion over 6 hours was determined. Only about 7% of the administered prostaglandin appeared as the dinor-6-keto PGF_1 metabolite in urine, and there was a linear relationship between the dose of PGI_2 infused and the metabolite excreted in excess of baseline. Interestingly, even the smallest dose of PGI_2 significantly elevated the metabolite levels above normal levels. Based on these data the mean production rate of endogenous PGI_2 was estimated to be about 0.21 ng/kg/min. This is about 5 times lower than the infusion rate of PGI_2 which affects platelet aggregation in vitro. Thus it appears that prostacyclin is not a systemically active hormone in man. It is more likely that PGI_2 is important in highly localized interactions of platelets and blood vessel walls.

"Studies on the Pathogenesis of Drug-Induced Lipidosis"
GM 24979 (Hostetler) University of California, San Diego

Cationic amphiphilic drugs such as chloroquine and diethylaminoethoxyhexestrol (DH, Coralgil^R) cause phospholipid accumulation in the tissues of man and animals. In the early 1970's DH was implicated with substantial human morbidity in Japan. Previous studies by Dr. Hostetler's group suggested that interference with lysosomal phospholipid degradation might be a major mechanism of the phospholipidosis-causing drugs.

This has led to the study of the degradation of phosphoglycerides by lysosomal phospholipases. Dr. Hostetler has noted that bis(monoacylglycero) phosphate (BMP) was degraded to monoglyceride by cleavage of its phosphodiester bond while some BMP was degraded to lysophosphatidylglycerol by deacylation. The former observation suggested the possibility that phospholipase C might be present in lysosomes; previously this enzyme had been reported only in bacteria. Using [1-^{14}C]dioleoylphosphatidylcholine (^{14}C-PC), they found that a rat liver lysosomal protein fraction converted the ^{14}C-PC to ^{14}C-LysoPC as well as to ^{14}C-mono- and diglyceride. The latter reaction suggested the presence of phospholipase C; the reaction had a pH optimum of 4.4, was not dependent on divalent cations, and was not inhibited by sulfhydryl reagents or non-ionic detergents. In addition to phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and phosphatidylglycerol could all serve as substrates in this reaction demonstrating for the first time the presence of a nonphosphatidylinositol-specific phospholipase C in mammalian tissues.

It was also found that both phospholipase A and C of rat liver lysosomes were strongly inhibited by DH and chloroquine at pH 4.4 and 5.4. Since no alternative routes of lysosomal phospholipid degradation are known, an important mechanism for phospholipid storage may be the concentration of these two drugs in lysosomes with inhibition of these two pathways for catabolism of phospholipids.

"Pharmacokinetics and Immunosuppressive Activity"
GM 26691 & 26551 (Benet) University of California, San Francisco

A major research goal has been to develop a correlation of drug pharmacokinetics with a measure of immunosuppressive activity that may be useful in defining the appropriate drug dosage regimen to be used in kidney transplant patients. Dr. Benet's group was able to develop sensitive and specific analytical measures for the two most frequently used immunosuppressive agents, azathioprine and prednisolone, and to define the pharmacokinetics of these drugs and their active metabolites, 6-mercaptopurine and prednisone, in patient populations routinely receiving the drug. This knowledge was applied to the development of an intermediate index of immunosuppressive activity which could be correlated with drug pharmacokinetics. Previously developed methodology for measuring immunosuppressive activity was extremely laborious and poorly correlated with drug kinetics; however a modification of the mixed lymphocyte reaction does appear to correlate with prednisolone plasma levels. The mixed lymphocyte reaction was chosen as a suitable intermediate index of immunosuppressive activity since it has previously been shown that a measure of mixed lymphocyte reactivity did allow one to predict in which patients a kidney transplant would remain viable and in which patients the transplant would be rejected. In eleven patients, the inhibition of the mixed lymphocyte reaction could be correlated with plasma levels of total prednisolone as well as with free (not bound to protein) concentrations of prednisolone. In a further preliminary analysis of four patients receiving both an oral and i.v. dose of prednisolone, a significantly better linear correlation between the mixed lymphocyte reaction and unbound prednisolone concentrations as opposed to that found for total levels was noted. Although these results are preliminary, the utility of this measure of immunosuppressive activity is promising since the investigators believe that the unbound (free) concentrations of prednisolone should relate to the pharmacodynamic response better than total levels.

One other interesting finding has emerged from preliminary studies related to the correlation of plasma levels with the inhibition of the mixed lymphocyte reaction. Greater concentrations of prednisolone are required following oral dosing, as opposed to intravenous dosing, to cause the same extent of inhibition of the mixed lymphocyte reaction. These data might imply that the metabolism of drug on its first pass through the gut and liver following oral dosing yields greater concentrations of metabolites which could partially counteract the effects of the unchanged drug.

These findings could serve as basis for adjustment of the dosage regimen in kidney transplant patients so as to optimize the immunosuppressive activity while minimizing toxic side effects or the lack of efficacy due to underdosing of the drugs.

"Non-Invasive Methods for Study of Drug Metabolism in Children"
GM 24796 (Saenger) Montefiore Hospital and Medical Center

Numerous methods for the determination of the activity of drug metabolizing enzyme systems have been developed for use in animal models or for in vitro studies. Only a few of these methods are applicable to clinical studies in man and even fewer can safely be employed in children. Drugs metabolized by the liver appear to be cleared from the plasma at a faster rate by children than by adults. These quantitative differences between the rates of drug metabolism in children and

adults suggest that data about the effect of drugs on drug metabolism in adults cannot automatically be applied to children.

6β-Hydroxycortisol is a highly polar metabolite of cortisol formed in the endoplasmic reticulum of hepatocytes by mixed function oxygenases, and changes in the urinary excreton of 6β-hydroxycortisol are thought to be a useful index of enzyme induction. In a first application of this non-invasive approach for the study of drug metabolism in children, Dr. Saenger and his co-workers measured urinary excretion in normal children and in children and adolescents receiving anticonvulsant therapy with phenobarbital or diphenylhydantoin. 6β-Hydroxycortisol excretion increased four to seven fold during anticonvulsant therapy. The marked increase in the ratios of 6β-hydroxycortisol/17-hydroxycorticosteroid and 6β-hydroxycortisol/free cortisol suggests that the measured increase in urinary 6β-hydroxycortisol is a true indication of a hepatic microsomal enzyme. This preliminary evidence suggests that urinary 6β-hydroxycortisol is a convenient and important probe for the non-invasive detection and characterization of enzyme induction and possibly also inhibition in childhood and adolescence.

"Molybdenum and Sulfur Complexes - Models for Metalloenzymes"
GM 22566 (Zubieta) State University of New York, Albany

The major objective of Dr. Zubieta's research is to develop molybdenum sulfur chemistry in order to elucidate the chemical structure and structural properties of the active sites of the redox active molybdoenzymes. The research can be loosely divided into three sections involving different aspects of molybdenum chemistry.

This group is developing the chemistry of molybdenum in the higher oxidation states (IV, V, VI) with thiolate (RS^-) and thioether (RSR) ligands. These ligands model the types of sulfur available in the biological systems better that the dithioacid ligands used previously. An example is the reaction between molybdenum(VI) and a saturated linear chain ligand with an SNNS arrangement of donor atoms according to the reaction:

$$[MoO_2(acac)_2] \quad + \quad SNNS \longrightarrow [MoO_2(SNNS)]$$

The chemistry of these compounds appears to be as rich and varied as for the dithiocarbamates. In addition, the complex $[MoO_2(SNNS)]$ is unusual in that it exhibits quasi-reversible electrochemical behavior which may be relevant to understanding the redox associated structural changes in the molybdoenzymes.

Hydrazine is thought to be an intermediate in the reduction of dinitrogen at the molybdenum active site of nitrogenase. In order to elucidate the interaction between molybdenum and nitrogenous substrates these investigators have been studying the interaction between substituted hydrazines and molybdenum(VI). Hydrazines react with dioxo Mo(VI) complexes by replacing one or both of the oxo ligands. The hydrazido groups are end-on bonded in a nearly linear fashion with substantial multiple bond character to the Mo-N bond. In addition, the hydrazido complexes can be protonated at the hydrazido ligand. In the reaction

$$[Mo(NNR_2)_2(dtc)_2] \xrightarrow{H^+} [Mo(NNR_2)(NHNR_2)(dtcO_2]^+$$

the protonated hydrazido ligand has been shown by crystallographic studies to assume a side-on bonded configuration. This novel compound is the first to contain both end-on and side-on bonded hydrazido ligands. Further protonation of this compound yields hydrazine but no trace of ammonia.

The active site of the nitrogenase enzyme is apparently composed of a molybdenum-iron cluster with sulfur ligands in an Mo:Fe:S ratio of around 1:6:4. Oxo coordination is absent at the nitrogenase active site. Dr. Zubieta has recently initiated an effort to utilize a new molybdenum starting material which affords a route to molybdenum-iron clusters which are distinct from the $MoFe_3S_4$ clusters previously reported. The $MoFe_3S_4$ clusters are now thought to be precluded as models for the active site because of the Mo:Fe:S ratio and EXAFS (extended X-ray absorption fine structure) data. Presently, molybdenum-iron cluster compounds appear to present a breakthrough in preparing the first compounds which might be considered as true models for the active site of the redox active molybdoenzymes.

"Sustained Drug Delivery Systems"
GM 26698 (Langer) Massachusetts Institute of Technology

One of the most common methods of preparing sustained-release preparations is to mix a drug uniformly with a carrier in a matrix system. When the carrier is a polymer and the rate-limiting step is diffusion, release rates are never constant because the drug at the surface of the matrix is released first and has only a short distance to travel. Thus, the release rates are rapid. At later times the drug near the center of the matrix is released; however, it has farther to travel so that the release rate is slower. For many drugs, it would be desirable from a pharmacological standpoint if the drug were released at a constant rate. Dr. Langer and his colleagues have now developed a design to accomplish this. The central concept was to develop a matrix geometry in which the amount of available drug increased in such a way as to precisely compensate for the distance effect. The matrix designed can be envisioned as follows: Consider a canteloupe melon in which the orange pulp is the drug-polymer matrix. Then cut the melon in half and coat all of the melon-half except where the seeds were with an impermeable barrier. With such a shape, the drug is forced to be released focally from the central concavity in the face of the resultant hemisphere. Now although the drug distances will increase, an increased amount of drug will be available as the distance increases. A theoretical analysis which proves that such a concept should achieve constant drug release rates has been developed. Preliminary experiments indicate that the theoretical analysis is valid and fabrication procedures for designing these systems have been developed.

Previously, all polymeric drug delivery systems were capable only of releasing drugs at decreasing or, at best, constant rates. There has never been a way to release drugs at increased rates and no method has ever been developed that could trigger increased drug levels on demand. Such a system has now been developed by modifying previously developed techniques for fabricating macromolecular delivery systems in such a way as to incorporate magnetic beads into the matrix. By applying an appropriate magnetic field, the rate of release increases by as much as two-fold. This system has been turned on and off repeatedly in vitro over a 5-day period with reproducible results. The concept, though still at an early stage of development, could potentially be used to obtain a pattern of modulated sustained release. Potential applications would include the delivery

of insulin in diabetes and the development of birth control systems that could release drugs to correspond to the menstrual cycle.

"The Relationship Between Agonist Occupation, Activation and Desensitization of the Cholinergic Receptor"
GM 24337 (Taylor) University of California, San Diego

By employing a cloned line of mammalian muscle cells in culture, it has been possible to measure simultaneously the binding of agonists to nicotinic cholinergic receptors and the resultant response. Since cholinergic receptor activation results in a change in cation permeability, a unidirectional flux of $^{22}Na^{+}$ from extracellular to intracellular space can be readily monitored. Analysis of activation and occupation parameters reveals that both the permeability response and ligand binding exhibit positive cooperativity indicating that more than a single agonist molecule per receptor is required for activation. Dr. Taylor's group has examined, in parallel, parameters associated with agonist occupation, activation and desensitization following progressive degrees of irreversible association of α-toxin with the available binding sites. Since α-toxin binding appears equivalent at each site on the receptor, the parabolic relationship between prior toxin occupation and the resulting loss in functional capacity is also accommodated by a relationship where activation of the permeability response requires agonist occupation of two sites per functional receptor. Furthermore, the binding of α-toxin and agonist is mutually exclusive, but α-toxin association with either of the two sites on the receptor oligomer is sufficient to block function. Consistent with this model, when a major fraction of sites is occupied by α-toxin, the concentration dependence for either activation or desensitization is unchanged and retains positive cooperativity. In contrast, progressive occupation of receptor by α-toxin leads to a decrease in apparent affinity and a corresponding loss of homotropic cooperativity for agonist occupation at the remaining sites. At high fractional occupancy by α-toxin, where the dominant receptor species binding agonist would contain a single bound toxin, and can be considered as a hybrid, the Hill coefficient falls below 1.0. For reversible antagonists, the binding isotherms exhibit Hill coefficients less than 1.0 and are not altered following fractional irreversible occupation by α-toxin. Thus, the two binding sites, which can bind either agonist or antagonist, on the receptor oligomer are not intrinsically equivalent and the state transition which gives rise to positive cooperativity exists only for agonists. Functional antagonisms may also be described from the binding isotherms where the association of antagonist with either one of the subunits on the receptor is sufficient to block the permeability response. Thus, "hybrid" receptor molecules containing an agonist bound to the subunit and α-toxin or reversible agonist associated with the other show a low or no probability for activation.

Overall, the nicotinic receptor can be described functionally as a dimer with non-equivalent binding sites. Agonists elicit state transitions for the rapid activation and the slow desensitization steps. Both of the transitions are characterized by homotropic cooperativity and fit a Monod-Wyman-Changeux model. This description of the receptor is entirely consistent with the receptor's subunit structure of α_2, β, γ and δ where α contains the respective binding sites. Nonequivalent intersubunit contacts for the two α-subunits would also be predicted and should account for the nonequivalence in binding.

PHYSIOLOGY AND BIOMEDICAL ENGINEERING PROGRAM

SCOPE and OBJECTIVES

The Physiology and Biomedical Engineering (PBME) Program encompasses research on the application of the engineering, physical, chemical, biochemical, and physiological sciences to the solution of significant biomedical problems, as well as the development of new instruments and methods to facilitate such research. It ranges from laboratory research on cells and tissues to clinical research, and encompasses certain aspects of behavioral science and epidemiology.

The research objectives are twofold. New knowledge and understanding of biologic function at the organ and organismic levels is sought, in order to better enable the physician to prevent, diagnose, and treat a variety of clinical conditions, including those following trauma or burn injury. The second major objective is to develop instruments and methodology for research activities in all the Institute's programs.

The PBME Program also supports research training relevant to its research objectives.

The professional staff administering the research and associated research training is divided into three sections: (a) biomedical engineering--Drs. Americo Rivera and Bert Shapiro; (b) instrumentation--Drs. Sue Badman, Matti Al-Aish, Marvin Cassman, and Americo Rivera; and (c) physiological sciences--Drs. Emilie Black, Elizabeth O'Hern, William Taylor and Lee Van Lenten. Dr. Robert Melville, of the instrumentation section, left the Institute in February for a position in FDA, and Dr. Lee Van Lenten joined the physiological sciences section in April, from the Office of Review Activities.

Overall, the program budget for FY 1980 was $45.7 million, an increase of about $5 million over the FY 1979 budget. These figures do not include the P41 awards that resulted from the instrumentation initiative of 1979, which although administratively placed in PBME, was designed to advance research in all the programs of the Institute. It had been announced as a once-only competition, but because of the continuing need for such expensive, sophisticated instruments, and the laudatory comments from both Congress and the scientific community on the administration of the program, the Institute staff has sought to set up a continuing program for such special instrumentation awards, recognizing that the availability of sophisticated instruments is one of the justifications of research center grants.

The Program continues to emphasize studies of general biologic relevance, with the goal of uncovering physiologic or pathologic mechanisms. Acknowledging the initial value of astute clinical observation and careful description, the objective is to understand the underlying biologic processes so that more purposeful treatment or even prevention of disease becomes possible. This is particularly true of the Program's trauma and burn research effort. Severe injury results in a bewildering series of systemic changes in the body, many of them excessive and harmful; and causes and effects must be unraveled for truly effective treatment to be designed.

The biologic emphasis is present in the research and development of instrumentation and methodology, as well, but, in addition, the Program lays stress on projects to develop new concepts rather than on refinements or validation of existing technology.

RESEARCH

Outlined below, for each of the three sections, are brief descriptions of areas of research support and some selected activities in FY 1980.

1. BIOMEDICAL ENGINEERING SECTION

This section is concerned with the application of engineering knowledge to the solution of significant physiological and other biomedical problems, and also to the development of new instruments and devices for physiological research. Areas of current research support include physiological systems analysis, biomathematics, biomaterials, biomechanics, prosthetics, physiological instrumentation, and biomedical information processing.

At present this section is supporting three research centers, one program project, and 45 regular research projects.

Workshop

In line with its interest in encouraging the transfer of knowledge and technology between the engineering and biological communities, this section recently sponsored a workshop on "New Opportunities in Biomedical Research Through Totally Implantable Telemetry." The meeting, held at Stanford University on August 20-22, 1980, was organized and chaired by Dr. James Meindl, Director of the NIGMS-supported Biomedical Engineering Center for Integrated Electronics and Medicine at Stanford.

Major objectives of the workshop included the identification of areas of physiological research in which significant new information can be obtained with totally implantable telemetry systems, and the formulation of new implant systems for future biomedical research. Participants, approximately 50 in number, included biomedical investigators with a variety of relevant interests and experiences, and engineers actively engaged in research and development aspects of implantable telemetry devices and systems. An important feature of the program, in addition to scheduled reports and discussion periods, was the provision of opportunities for participants to visit the nearby Stanford integrated circuit laboratories, and also selected biomedical research laboratories in which advanced implant telemetry systems were in actual operation. It is anticipated that the proceedings of the workshop will be published in an appropriate biomedical journal in the near future.

2. INSTRUMENTATION SECTION

This section is concerned with research and development related to innovative instruments and supportive methodologies in the areas of cellular and molecular biology, biochemistry, biophysics, and radiologic imaging of internal structures. Among the topics of interest are the development of new types of chemical or physical separation techniques, and other general laboratory techniques for use in basic biomedical research; the development of spectroscopic techniques and the extension of these to the study of

ultrafast molecular phenomena; and the development of instruments for use in basic research on problems requiring the identification, separation, enumeration and manipulation of cells or subcellular organelles. Microscopy involving all types of radiation, development of specimen preparation techniques and methods of image analysis, development of the electron microprobe, and extension of these areas not only to conventionally prepared tissue but to live specimens or to resolutions at the molecular level are encouraged. In the area of imaging by ultrasound, x-ray and other forms of electromagnetic radiation, research on instrumentation as well as on the effects of these radiations on biological tissues is supported.

At present this section is supporting one research center, four program projects, and 198 regular research grants.

Workshop on Scanning Transmission Electron Microscopy

The first high resolution scanning transmission electron microscope (STEM) was developed by Dr. Albert Crewe at the University of Chicago and completed in 1970. Since that time NIH and other federal agencies have supported its further development, and attempted to encourage its application to biomedical research problems. It is the perception of the NIH staff that there is a growing impatience and concern within the biological community at the slow pace of applications to research and significant contributions of STEM technology to biological problems. Because of this concern, a meeting to discuss problems and opportunities in the area of scanning transmission electron microscopy was held on December 10, 1979, under the joint sponsorship of the PBME Program and the Biotechnology Resources Program of DRR. The participants included Drs. Michael Beer (Johns Hopkins University), Robert Josephs (University of Chicago), Karl Piez (NIDR), Lionel Rebhun (University of Virginia), Thomas Reese (NINCDS), Jerry Shay (University of Texas), Joseph Wall (Brookhaven National Laboratory), representatives from DRS/NIH, DRG/NIH, the NSF, and several visitors. The meeting was chaired by Dr. Donald Engelman of Yale University.

The participants reviewed present STEM capabilities, which fall broadly into three areas: electron energy loss spectroscopy (EELS), heavy atom imaging, and mass determination. Improved detection systems are likely to improve EELS capabilities, and cold stages (liquid helium) should overcome mass loss and movement problems in heavy atom imaging. They then discussed their current or projected needs for STEM capabilities. These ranged from electronic contrast adjustment for locating small organelles in unstained tissue to improved specimen preparation techniques.

In general discussion, two problems were identified which had been responsible for the slow growth in use of STEM by the biological community. The first was the lack of widespread knowledge about the different types and capabilities of STEM. These instruments fall into at least three general catagories, i.e.:

1) High resolution STEM instruments with field emission guns;
2) Conventional microscopes with STEM attachments and x-ray analysis capablities; and
3) Microprobe instruments for detailed wavelength or energy dispersive quantitative analysis of ions.

The committee decided that a review article on these various aspects of STEM technology would help potential users. Dr. Beer, who coordinated a session on STEM applications at the Electron Microscope Society of America meetings in August, 1980 has agreed to publish the results of that session in the form of such a review.

The second problem identified by the group was the need for improvement of various aspects of sample preparation. The participants recommended that NIGMS consider publicizing its interest in encouraging systematic exploration of new methods for the preparation of specimens for examination in the STEM. Research areas of particular interest include studies of heavy atom labelling procedures, the use of frozen sections, development of thin support films, studies of sectioned material, and the evolution of new fixation techniques. These methods would have application in other kinds of microscopy as well. Staff of the PBME program is in the process of developing a program announcement which will highlight the Institute's interest in these areas.

Shared Instrumentation Program

One of the most important aspects of the FY 1979 instrumentation initiative was the attempt to make equipment in an intermediate price range available to grantees on a shared basis. The Institute has therefore begun a follow-up evaluation, by asking all awardees to report on the number of users, core and otherwise, and on any problems which have developed in making the instrument available as a shared facility. While the responses will be only a preliminary indication of the program's successes and problems, this and subsequent follow-up information will provide important feed-back to enable the Institute to determine the appropriate structure of any future program of a similar nature.

3. PHYSIOLOGICAL SCIENCES SECTION

This section of the program fosters research in physiology, biomedical engineering, biochemistry and immunology that have relevance to significant problems in the areas of trauma/burn and anesthesiology. It is also concerned with research in behavioral sciences and adaptation and with investigations into the basic mechanisms of pain.

At present this section supports 11 research centers, including nine in trauma/burn and two in anesthesiology. There are five program projects of which three are in trauma/burn and one each in epidemiology and behavioral sciences. The 116 regular research grants include 58 in trauma/burn, 29 in anesthesiology, 24 in behavioral sciences and adaptation and five in epidemiology.

Much of the research in this section is clinical in nature, and is hampered by a shortage of appropriately trained investigators. The research also tends to be multidisciplinary, requiring expertise in a broad range of scientific and professional disciplines. In order to encourage the development and the bringing together of such expertise, the Program has offered support for New Investigator Research Awards (R23), and research center grants, respectively.

Research Centers

The trauma/burn research centers provide a mechanism for a multidisciplinary approach to the body's total response to severe injury. During the first 24 hours, systemic responses often result in respiratory failure, cardiac collapse, life-threatening infections, and abnormal metabolic changes. Through the collaborative efforts of the basic scientists in such fields as biochemistry, immunology, physiology, and the medical specialists working in the area of trauma/burn, attempts are made to reduce death and disability, and to mitigate pain. In these research centers laboratory findings can be immediately applied to the treatment of the severely injured patients.

This year the Institute initiated the support of two additional trauma/burn research centers making a total of nine. One of these new centers is headed by Dr. Thomas Hunt, Professor of Surgery at the University of California, San Francisco. Dr. Hunt has devoted several decades to the study of wound healing, a high priority item on the list of needed research in the trauma/burn field.

The body's metabolic response to wounds, including those factors which inhibit or induce biological repair, are not completely understood. The role of collagen in wound repair, the nutritional needs of repair, the importance of the injured and reconstructed vascular system, the signals that cause inflammatory cells to go into a wound, are all important questions which Dr. Hunt expects to pursue in his investigations on wound healing.

An ideal formula for fluid resuscitation has not yet been discovered. In the search for an answer, basic underlying mechanisms of fluid transport and distribution are being studied. At the University of Texas in Dallas, Dr. Charles Baxter, Director of the other newly activated NIGMS research center, is devoting his time to the circulatory and fluid volume changes following severe burn injury. In 22 burn patients, Dr. Baxter was able to validate animal studies which demonstrated the rapid translocation of sodium and water into the intracellular fluid compartment in the early phase of post burn shock and the rapid recovery of the active sodium transport 24-48 hours post burn.

The other seven trauma/burn research centers are located in Boston, New York City, Albany, Salt Lake City, San Francisco, and Seattle. The research center in Boston like the one in Dallas is devoted entirely to the study of severe burn injuries. Here important studies are being performed on the metabolic response to burn injury, artificial skin, and smoke inhalation. A collaborative study between the investigators at the University of Washington on the perfection of an Argon laser for debridement of dead tissue following burn injury has resulted in a technique which should greatly reduce blood loss and infection at the critical early post burn period.

Considerable collaboration also exists among the centers in research training of postdoctoral fellows, an area of considerable interest to the Institute due to the manpower shortage in this field. Five of the research center directors are also directors of the NRSA postdoctoral institutional fellowships and exchange fellows in an attempt to broaden their scope of knowledge.

In addition to these centers, the Institute also supports three program projects devoted to research on the sequence of organ failures, the metabolism of body fluids, and the biochemical indices related to nutritional status of the traumatized patient.

The FY 1980 anesthesiology research center grant activity shows a further retrenchment, as the center grant mechanism no longer appears to satisfy the needs in this field. This trend is partially offset by the growing program of individual (R01) grants in anesthesiology, including awards for new investigators (R23). Most academic centers still do not have a critical mass of investigators working on research problems relevant to anesthesiology, and particular attention is therefore given to the support of research training and on getting young scientists started on research careers.

New Investigator Research Awards (NIRA)

New Investigator Research Awards (formerly known as Special Grants for New Investigators) in Anesthesiology were initiated by the National Institute of General Medical Sciences in FY 1977. A year later similar awards were established in Trauma and Burn research. At the present time the Institute is supporting 16 grants in the former area and eight in the latter. Although it is still too early to predict the effectiveness of these awards in facilitating the transition of individuals from training status to that of established investigator, encouraging results are beginning to emerge, particularly from the Anesthesiology segment of the program. It is gratifying to note, for instance, that of three young Anesthesiologists who completed their three-year awards during FY 1980, two competed successfully for regular research project (R01) grants. The program will be monitored with interest and care as it grows and matures over the next few years.

RESEARCH TRAINING

Predoctoral

The training program in Systems and Integrative Biology (SIB) plays an important role in the NIGMS training picture. As part of the PBME program, it has provided predoctoral training in both physiology and biomedical engineering. Programs are multidisciplinary and awarding standards emphasize the need for training in quantitative biological research. There has been a strong insistence that biomedical engineering programs provide a strong biological orientation to trainees so that they can truly enter the world of biomedical research. There is a similar emphasis on appropriately quantitative training for the more physiologically oriented trainees. There were 29 active grants as of September, 1980. Two new awards were made while seven grants were terminated. The failure to renew these awards was due to several factors. In some cases the programs have failed to integrate different disciplines or courses of training, i.e. to provide a strong program. However, a major factor has been financial stringency. The two new programs, at the University of Chicago and the University of Texas at Galveston, were rated as truly excellent, new programs with much promise. Their projected budgets are initially small, and even at steady-state levels will remain modest.

The SIB training also has a place in the overall institute training approach. The SIB programs concentrate on biomedical problems above the cellular level, in many respects taking up where the more cellular Cell and Molecular Biology training grants leave off. There is often a meshing of the neurobiological interests of many SIB preceptors with the interests of preceptors in the Pharmacological Sciences training program. Since several of the SIB programs have a neurobiological emphasis, appropriate for a program stressing integration, regulation and interactions, the trainees may continue in research areas not supported by NIGMS. This is consistent with the NIGMS intent to prepare basic researchers for all areas, including those relevant to the categorical institutes.

Reviewers, and program directors (both present and potential) have expressed some interest in a clearer definition of this program. Now that NRSA awards have been available for five years, and that some grants have been rereviewed, a clearer picture is emerging of the needs and realities of training in this area. We have moved away from the departmental approach that characterized the old T01 grants. SIB is unique in NIGMS as the only predoctoral training grant not reviewed by a standing committee. This has allowed greater flexibility in obtaining appropriate expertise on the committee but has let to some loss of continuity.

Postdoctoral

Institutional National Research Service Awards are offered by the NIGMS for postdoctoral training in Anesthesiology and in Trauma/Burn Research. These programs are limited to postdoctoral scientists (Ph.D., M.D. or equivalent) for training in trauma/burn research and to individuals with an M.D. degree for training in anesthesiology. Multidisciplinary in nature, the programs provide two years in basic research training with an optional third year. The training is intended to enhance the capabilities of the participants to advance the state-of-the-art in these disciplines.

The anesthesiology training program was active in four training sites at the start of FY 80: University of Virginia School of Medicine in Charlottesville, with two trainees; Massachusetts General Hospital in Boston with four trainees; University of Washington in Seattle with three trainees; and the University of Pennsylvania in Philadelphia with four trainees An award to Columbia University made in 1978 was never activated and was canceled in FY 80. A new training site at the University of Utah was added during the year, starting with one trainee in the first year. At year end there were five training sites offering anesthesiology training for 14 individuals. The corresponding figures for trauma/burn research were ten and 28.

RESEARCH HIGHLIGHTS

1. "Metabolism of Glucose"
   GM 21700-06 (Burke) Harvard University

Studies by the investigators in the burn research center at Harvard Medical Center have focused on the development and validation of a new technique to quantitate glucose metabolism in vivo without the radiation hazard associated with the administration of radioactive tracers. They have utilized the stable, non-radioactive ^{13}C-labeled glucose molecule and mass spectrometry and have

validated the new technique against the ^{14}C-glucose (radioactive) technique which previously had been validated both theoretically and experimentally. They found that less than half of infused glucose is directly used for energy, and that the percentage falls as the infusion rate is increased to levels approximating those frequently used clinically. The predominant fate of the glucose that is not used for energy is its conversion to fat. This process of fat synthesis is felt to have several detrimental effects. Fat has been shown to accumulate in the liver, thereby probably impairing liver function. From their calculations it became evident that the extra energy expenditure associated with fat synthesis could raise the metabolic rate as much as 35%, thus reducing the desired effect of the calories supplied by the infused glucose. Perhaps of more concern, CO_2 production is increased to a much greater extent than the metabolic rate as a consequence of fat synthesis from infused glucose. The group has found that conventionally used glucose infusion rates may cause a doubling in the rate of CO_2 production. This places an extra ventilatory load on the patient, and in the burn patient with respiratory injury this can be a significant problem. Also they found that pharmacologic doses of glucose cause a disruption of the normal profile of plasma substrates - most notably, free fatty acids. The low free fatty acids in the plasma were found to impair the synthesis of pulmonary surfactant. The experiments have provided theoretical and experimental evidence in favor of providing calories in the form of a mixture of carbohydrate and fat - something which intuitively seems reasonable but has frequently not been practiced clinically.

2. "The Design and Evaluation of an Artificial Skin"
GM 23946-04 (Yannas) Massachusetts Institute of Technology

Over the past ten years Dr. Yannas and associates at MIT have collaborated with Dr. John Burke at Shriners Burn Institute in an attempt to design an artificial skin. They have recently achieved permanent closure of full-thickness wounds without use of an autograft. The results of a recent, extensive series of animal experiments show, Dr. Yannas feels, that they have succeeded in designing a semi-synthetic membrane which is applied only once, closing up the full-thickness wound sufficiently well to prevent fluid loss and infection. This membrane is neither rejected nor is it surgically replaced but is instead metabolically replaced in 3-5 weeks by epithelialized neodermal tissue.

The design objective of Dr. Yannas' study called for a suturable bilayer membrane which displaces air pockets from a carefully prepared wound bed, free of weak boundary layers, and which maintains the moisture flux through the wound at a level of 0.1-1mg/cm^2/hr. In addition to moisture permeability, optimization of the surface energy, modulus of elasticity and energy to fracture were among the essential attributes of the design. The bilayer membrane consists of a 0.1-mm thick top layer of conventional medical grade transparent silicone elastomer bonded onto a 0.4-mm thick membrane fabricated from a novel collagen-glycosaminoglycan (GAG) graft copolymer.

In this design, Dr. Yannas indicates, the silicone layer acts simply as a transparent physical barrier which controls moisture flux and confers suturability to the graft. The collagen-GAG layer is a non-antigenic, highly porous membrane which performs as a biodegradable template for synthesis of connective tissue under conditions which can be partly controlled by the investigator.

Dr. Yannas further reports that full-thickness skin grafting experiments with guinea pigs show that these membranes control infection and fluid loss of wounds satisfactorily without requiring change or other traumatic manipulation over several weeks. Formation of a thin, fully epithelialized scar is virtually complete by Day 50 following grafting. No evidence of rejection has been detected. Wounds grafted with this membrane do not begin contracting until about Day 10, compared with open wound controls which are almost fully contracted by that time. Grafted wounds become fully contracted by Day 35 to 45, at which time the silicone layer is spontaneously jettisoned by advanced epithelial cell sheets which move at the interface between the silicone and collagen-GAG layers.

Work is now in process by Dr. Yannas to advance Stage 2 of the design. This stage calls for a membrane which, in addition to controlling infection and fluid loss, minimizes the occurrence of contracture and scarring during wound healing. In addition, use of their membrane for treatment of massively burned patients began during the early part of October 1979 at Shriners Burn Institute, Boston Unit, under supervision of Dr. J. F. Burke.

3. "The Role of Anesthetics in Organ Protection"
GM 24531-02 (Tinker) Mayo Foundation

As evidence has accumulated showing that barbiturates can exert specific protective effects against brain ischemia, the use of barbiturates for this purpose has become more widespread, notably in treatment of patients with increased intracranial pressure as a result of, e.g. trauma. Barbiturates appear to decrease intracranial pressure by increasing cerebral vascular resistance and thus decreasing intracranial blood volume.

Several studies have encouraged the hope that barbiturates may be useful in treatment of cerebrovascular accidents in humans, as well as after cardiac arrest.

Dr. Tinker has presented disturbing evidence of adverse effects resulting from such use of barbiturates. In a recent study aimed at ameliorating the effects of acute coronary occlusion he has demonstrated a deleterious effect of barbiturates on dog hearts. Using four anesthetic regimens on a dog model of myocardial ischemia (ligated left anterior coronary artery) he tested 1) light anesthesia and a high heart load, i.e., low concentration of halothane coupled with nitrous oxide, 2) anesthesia with pentobarbital, 3) higher concentration halothane or 4) enflurane in reasonable clinical concentration on four groups of dogs. Comparisons were made between the ischemic area of the heart and a normal area of the same dog heart. He found that enflurane and halothane (both concentrations) resulted in far less myocardial damage than pentobarbital. Extensive myocardial damage was found in all animals treated with pentobarbital.

Evidence of myocardial damage has been present in the experiments which focused on brain ischemia, e.g., cardiopulmonary problems developed in primates given large doses of barbiturates and dogs subjected to global ischemia treated with barbiturates required more cardiovascular support than non-barbiturate treated controls.

Tinker's research has emphasized the need for caution in use of barbiturates to protect the brain from ischemia.

4. "Interfacial Behavior of Biomaterials"
GM 24858-02 (Hench) University of Florida

Approximately 110,000 patients are restored annually to a life of nearly painless mobility by the implantation of artificial joints; 73% are hip joint replacements and 27% are knee joint replacements. There have been, however, several problems associated with the metal and polymer parts used in the past for these repairs. The significant amounts of wear which occurred in the mating surfaces of the artificial joint have been reduced by the synthesis and selection of improved materials. Furthermore the inflammation of adjacent tissue has been reduced by the selection of substances which are relatively inert to the biological tissue. This latter alteration has greatly reduced the rejection reactions for these devices.

The plastic luting agents (cements) used to anchor the artificial joint to the bone, however, have left something to be desired. With time, these plastic cements become loose, allowing the artificial joint to "wobble" causing pain and rapid deterioration of the remaining bone. The ten percent failure rate experienced in the last two-year period has been of this type. Surgical removal of the artificial joint is required, often with undesirable complications.

Dr. Larry L. Hench has developed an alternative biomaterials system which eliminates the need for luting agents. Dr. Hench and his colleagues have synthesized a bioglass coating for use on aluminum oxide ceramic joints and stainless steel hip joints. Implanted bioglass coated joints in sheep and monkeys have shown that the bioglass-bone surfaces have interacted to form a stronger bond than normally obtained with plastic luting agents. No toxic effects were noted in these animals.

Recently Dr. Hench has shown that bioglass is also non-toxic to tissue cultures of rat embryo bone cells, skin fibroblast cells and lymphocyte cells. In addition the implantation of bioglass into the soft tissues of rats and rabbits produced adhesion to connective tissue with minimal fibrous tissue reaction. Injection of powdered bioglass into the peritoneal cavity of these animals had no effects on their visceral organs. In addition the injection of bioglass powder into the blood stream was cleared without affecting the animals' lymph nodes. Furthermore embryonic bone cells adhered very strongly to bioglass and deposited matrixes of hydroxlapatite and collagen depending on the silicon dioxide content of the bioglass surface. These data support the findings of the non-toxic nature of bioglass and its bone adhering qualities. The data indicates that bioglass surfaces should be ideal for use in prosthetic devices which must be anchored into the bone structure. Work is continuing to further establish the biological properties of bioglass before it is used in human beings. The data thus far is very encouraging.

5. "Laser Assisted Scalpel for Excision of Severe Burns
GM 24990-02 (Carrico) University of Washington, Seattle

The development of an effective laser assisted quartz scalpel promises to aid significantly the early excision of severely burned skin. An Argon laser is coupled through a flexible glass fiber to a transparent, sharpened, quartz scalpel blade. The energy from the Argon laser is internally reflected until it reaches the sharpened edge of the blade where it focuses on the freshly cut

tissue. Thus, the tissue is cut with the knife and all bleeding is stopped by the intense laser beam. Experiments have now been completed in animals which demonstrate that the laser/scalpel performs as rapidly as conventional methods but does so with about 1/4 the blood loss. Since excising the burned skin of a single leg alone may require the replacement of 5 or 6 pints of blood, use of the laser scalpel will not only conserve a precious resource but also reduce the hazards of multiple transfusions in already desperately ill, burned patients.

During the past year experiments were designed to determine which type of laser would provide the best hemostasis with the least destruction of normal tissue. Comparisons were made between a Neodynium/Yttrium (Nd:YAG) laser and the Argon laser. In collaboration with Dr. John Dixon at Salt Lake City, Dr. David Auth, the principal investigator on this project, performed detailed experiments which showed that the Argon laser produced an equal reduction in blood loss but considerably less tissue destruction than the YAG laser. Clinical protocols to further validate the use of the Argon laser for excision of burned tissue in patients will begin shortly.

6. "Acoustic Microscopy for Biomedical Investigations"
GM 25826-02 (Quate) Stanford University

Much of our current understanding of cellular structure and function has been gained with the aid of microscopic techniques. With light microscopy, advances in the methods of fixation and staining, (i.e., immunofluorescence microscopy) and in optical systems, (i.e., phase contrast, Nomarski, and polarized light microscopy) have permitted major increases in knowledge about both living and fixed biological material. The electron microscope, in both the transmission and scanning modes, has greatly extended our understanding of the fine structure of nonliving preparations.

Dr. Calvin Quate of Stanford University has been supported by GM 25826 to develop a new technology, acoustic microscopy, for use in biological research. Because acoustic microscopy can, in principle, detect differences in mechanical properties such as viscosity, elasticity, and thickness, its most exciting potential may be in imaging living cells. For instance, cell motility is a prime target for study: the viscosity of localized areas of the cell can change during cell motion. Stress fibers form as the cell moves, and changes in the elasticity of a given fiber can reflect tension or relaxation. If investigators could visualize which fibers were under tension in a moving cell, they could greatly increase their understanding of the mechanisms of force generation during movement. Before acoustic microscopy can be applied in such studies, however, formidable problems remain to be solved: resolution, possible deleterious effects of the high frequency sound waves, and the mechanisms and meaning of the contrast achieved.

Resolution depends on the wavelength of the acoustic radiation in the fluid surrounding the cells. It can be increased by heating the fluid, but this is incompatible with living material beyond the range of 35-45^{o}C. Resolution can also be increased by using a lens with a shorter focal length. This shortens the working distance and limits the thickness of the cells to be examined. Using a fluid which has a lower acoustic attenuation than water increases the resolution. Most of these fluids kill cells, although some, such as liquid helium, may be useful in studies of frozen cells.

By making comparisons among sequential acoustic, electron and light micrographs of single cells Dr. Quate and co-workders have been able to identify nuclei, nucleoli, mitochondria, actin cables, cell attachment sites, filopodia, ruffles and other cell surface projections. The smallest biological objects which they are presently able to detect acoustically, such as filopodia, have diameters of 0.1 - 0.2 μm. The resolution is thus close to that of a light microscope with an oil immersion lens.

7. "Biomedical Use of Plasma Desorption Mass Spectrometry
GM 26096-02 (MacFarlane) Texas A & M

Non-volatile materials have been the bane of mass spectroscopists since the inception of the technique. In particular, polar molecules which do not have a high equilibrium vapor pressure are especially difficult to deal with. Since many such compounds are of biological importance, it is clearly of interest to develop an approach to overcome this limitation. One method that has been used is to heat the material to high temperature. However, many molecules of interest (e.g. peptides, nucleotides, and antibiotics), decompose on heating. A more common approach is by derivitization with e.g. dimethylsilyl groups at polar sites, to increase the vapor pressure. MacFarlane and his colleagues have developed a new technique called californium-252 plasma desorption mass spectrometry (PDMS) which shows great promise.

The PDMS technique involves the formation of very high energy ions due to the spontaneous nuclear fission of californium-252. These impact on thin films of the substance of interest, and through fast chemical reactions form molecular ions. These ions are then ejected from the surface as a result of a shock wave produced along the fission track, and can then be analyzed in the mass spectrometer. Analysis can be carried out on systems of molecular weights greater than 2000, with molecular ions up to 7000 being reported. This is substantially higher than other mass spectrometric techniques currently in use.

Two recent examples of application of this technique are particularly impressive. One involves the molecular weight determination of polytoxin, the most powerful toxin obtained from marine animals. Because of its high mass (approx. 3300), low volatility and thermal instability, this molecule represents one of the most difficult problems encountered for a mass spectrometric measurement of a natural product. Thin deposits (approx. 10 mg/cm^2) of polytoxin were prepared and irradiated by a ^{252}Cf source. A significant peak occured at M=2704 mass units. The N-acetyl derivative showed a peak at M=2746, which, corrected for a molecular weight of 42 for the acetyl group, is in excellent agreement with the underivatized results. The above two compounds must be the sodium salts of the toxin, as confirmed by analysis of the cesium salts, which gave results that reflect the (Cs-Na) mass difference. The molecular weight of the free toxin is then 2681.

A second study provided both sequence and molecular weight information of olideoxyribonucleotides up to decanucleotides, protected by isobutyryl and benzyl groups. The positive ion spectra were dominated by the molecular ion, and thus unambiguously provided the molecular weight as well as giving an indication of purity. The negative-ion spectra showed a complex fragmentation pattern which represented a nested set of oligonucleotides. The mass assignments allowed a determination of the sequence.

Thus, this approach allows a verification of the molecular weight and base sequence of synthetic oligonucleotides, as well as an indication of purity. Prior deblocking is not required, and the method is essentially non-destructive, because only 10^3-10^6 molecules are ionized and desorbed.

CONTRACTS

The support of contracts and interagency agreements constitutes a relatively minor activity of the PBME Program.

1. "Reconstruction and Testing of a Superconducting Electron Microscope"
(Contract NO1 GM 2116)
Duke University ($249,067, FY 1980 Funds)

A contract was awarded to Duke University on June 30, 1980 with Dr. J. David Robertson as Principal Investigator and Robert Worsham, the original builder of the microscope, as Project Engineer.

Phase I involves reassembly and restoration of the microscope. When the microscope is reassembled the investigators will demonstrate to a site visit team its operation at the contract specifications -- its previous level of performance. (nine months; $249,067 total cost).

Upon such a satisfactory demonstration the contract will proceed to Phase II, evaluation of the microscope. Phase II requires the investigators to demonstrate in a systematic way 1) the improvement in electron exposure that can be tolerated by glucose embedded catalase, or a similar specimen, at liquid helium temperature compared to that obtained at liquid nitrogen and room temperature; 2) that high resolution electron diffraction patterns can be obtained from crystals of biological interest using the superconducting microscope; and 3) whether the instrumental contrast transfer function extends to better than 3 Å. With these results a complete evaluation of the practical potential of the superconducting microscope for contributing new information on high resolution biological structure will be obtained, as well as an evaluation of its performance as a potential "user-oriented" instrument. (one year; $336,327 total cost).

Since, if Phases I and II are wholly successful, the future use of the microscope is expected to be supported through a research or research resource grant, Phase III provides support for one year, keeping the microscope intact and under vacuum to allow an orderly transition to such support. (one year, $34,630 total cost).

2. "Refurbishment and Renovation of the Infrared Heterodyne Ratiometer"
(Contract NO1 GM 52124)
Andros Incorporated (Proposed $35,000 FY 1980 Funds)

The generally accepted method for assaying the stable isotope of carbon (atomic weight of 13 instead of 12) is by high resolution mass spectroscopy, requiring an instrument costing in excess of $250,000. NIGMS funds were used to construct a $^{13}CO_2/^{12}CO_2$ Ratio Analyzer using innovative ideas from NASA - Ames and the Andros Corporation in the hopes of developing an alternative analytical instrument and procedure as sensitive and accurate as the mass

spectrometer but less costly to manufacture, maintain and operate. This ratiometer is being prepared for use in one of the proposals received in response to a request for applications. The successful applicant will use this ratiometer to gather important human data of $^{13}CO_2$ produced by the ingestion of ^{13}C labeled compounds and will validate the new analytical procedure.

MINORITY ACCESS TO RESEARCH CAREERS

SCOPE

The Minority Access to Research Careers (MARC) Program is institutionally based and is designed to assist minority colleges and universities in the training of greater numbers of scientists and teachers for careers in the biomedical sciences. It was designated as a formal program of the National Institute of General Medical Sciences in December 1975. Applications assigned to this program are reviewed by the Minority Access to Research Careers Review Committee, which was chartered specifically for the review of such applications.

OBJECTIVES

The primary objectives of the program are to assist minority institutions in the training of greater numbers of scientists in the biomedical disciplines and to help strengthen the research and teaching capabilities of these institutions. The support mechanisms used to accomplish this mission are: The MARC Faculty Fellowship, the MARC Visiting Scientist Award and the MARC Honors Undergraduate Research Training Program.

RESEARCH TRAINING

The MARC Faculty Fellowship program provides opportunities for advanced research training for selected faculty members of four-year colleges, universities and health professional schools in which student enrollments are drawn substantially from ethnic minority groups. These institutions may nominate faculty members to apply for MARC Fellowships for support for a period of up to three years of advanced study and research training in graduate departments and laboratories, either as candidates for the Ph.D. degree or as investigators obtaining postdoctoral research training in the biomedical sciences. Faculty Fellows are expected to return to the nominating institutions at the completion of such training.

MARC Faculty Fellows are selected on a competitive basis. Peer review of applications is provided by the MARC Review Committee, which is composed of consultants, including minority representatives from appropriate scientific fields, chosen primarily from the academic community. Evaluation is based upon the applicants' qualifications and potential for research and teaching as evidenced by academic records, reference reports and publications as well as by a research training proposal, the proposed training situation (i.e., the sponsor's and the institution's training facilities and staff), and other relevant information.

The second of the three support mechanisms, the MARC Visiting Scientist Award, has as its purpose the provision of financial support which allows outstanding scientist-teachers to serve in the capacity of visiting scientists at four-year colleges, universities, and health professional schools where student enrollments are drawn substantially from minority groups. The primary intent is to strengthen research and teaching programs in the biomedical sciences for the benefit of students and faculty in these institutions by drawing upon the special talents of scientists from

other institutions. Reciprocal benefits should accrue to the MARC awardee through the added experience gained by involvement in innovative science education and research development programs.

An applicant's proposal must include arrangements for the visiting scientist to reside in the campus community and to participate fully in programs of teaching, development of research, and/or counseling as outlined by the institution. Evidence of negotiations and of some tentative agreement between the applicant institution and the scientist teacher nominated to serve as the MARC Visiting Scientist should be provided in the application. Individuals nominated as Visiting Scientists in this program should be recognized biomedical scientist scholars. Proposals may request support for the MARC Visiting Scientist Awards for periods ranging from an academic quarter to a maximum of one year. Stipends are determined on an individual basis according to the nominee's current salary or other possible source of stipend support for the proposed period in residence.

The third mechanism of support (and the newest component of the Program) is the MARC Honors Undergraduate Research Training Grant Program. This activity was initiated at the suggestion of the Congress, Institute consultants and staff, as a means of emphasizing the value and importance of providing biomedical training at the undergraduate level in minority institutions. The objective of the program is to increase the number of well-prepared students who can compete successfully for entry into graduate programs leading to the Ph.D. degree in the biomedical sciences, to help develop a strong science curriculum, and to stimulate an interest in undergraduate research as preparation for graduate study in the biomedical sciences.

HIGHLIGHTS

The past year has afforded the Institute perhaps the best opportunity to date to review the actual status and achievements of the MARC Program elelements. In this regard, a progress report evaluating the MARC Honors Undergraduate Research Training was provided to the National Advisory General Medical Services Council in March 1980.

Historically, it had been noted that more than 70 percent of minority students who enter college go to minority institutions, and that more than 50 percent of current minority doctoral degree holders in the biomedical sciences obtained their baccalaureate degrees at minority institutions. Thus it was decided that one way to increase the number of minority biomedical scientists would be through a special program designed to strengthen the science environment at minority institutions. The MARC Program initially offered awards in two areas; faculty fellowships, which provide advanced training at another institution for faculty at minority institutions; and visiting scientist awards, which allow well-known scientists from majority institutions to serve as visiting professors at minority institutions. After much study, a third component was added and the MARC Honors Undergraduate Research Training Program was announced in 1977. This program is not aimed at the general population of minority science students, but rather at those very talented individuals who have demonstrated a desire to attain eventually the Ph.D. degree in preparation for a career in the biomedical sciences.

To date, 155 applications for the Honors Undergraduate Research Training Program have been received, of which 74 were approved, and 35 have been funded.

Twelve awards were made in FY 1977; five additional awards were made in FY 1978, and in FY 1979 five more were added. At that time, since this has been regarded as a pilot effort, it was felt that an interim evaluation of the program should be made; thus the MARC Program gathered data from the initial group of 12 institutions which received awards (now in their fourth year of support). Of 24 graduates in 1978, 50 percent entered graduate schools. Following approval by the Advisory Council to fully establish the program, an additional 13 awards were made in FY 1980 bringing up the present total to 35 awards. This year, there were 73 graduates, 66 percent of whom have applied to graduate school and 25 percent to professional school.

A progress report was also presented to the Council in June 1980 regarding the MARC Faculty Fellowship Program which had begun in 1972. It was initiated after staff of NIGMS, with the advice of consultants, had tried to learn as much as possible about the activities of minority institutions. It was clear that many individuals who taught at minority institutions had the potential to do biomedical research, and the institutions contacted verified that they identify a number of such persons. The faculty fellowship was established as a faculty development instrument with two facets: training for those individuals who had not yet obtained the Ph.D. degree in order that they might complete degree requirements; and support for those who had completed the degree, but who could further benefit from postdoctoral experience. It was the intent of the program that individuals who received support should return to their home institutions to do research and teaching.

Statistical data gathered to date show that 150 fellows have been supported, of whom 80 were predoctoral and 70 were postdoctoral. Of the 96 who have completed the program, 73 have returned to their home institutions; 14 obtained positions at other minority schools, four became M.D.s; and of the remaining five who did not return to a minority institution, four are currently in a research environment. Forty-nine predoctoral fellows have completed their MARC training; 34 of these earned the Ph.D. degree, and all have returned to minority schools to continue research and teaching. The average length of time spent on the fellowship was 2.9 years for predoctoral fellows, and 1.3 years for postdoctoral fellows. About 50 applications are received each year. It was noted that some schools have nominated several individuals for the program, having identified the program as a means of faculty development. In addition, schools which have vigorous training programs are making nominations. Previously, it had been found that professors in minority schools were so involved in instruction that they could not do research; and that many young instructors wanted to continue their education and obtain a doctoral degree, but had no financial aid. Since the initiation of the fellowships program, many of the fellowship recipients have returned to their institutions, and have become involved in the Honors Undergraduate Training Program and the Minority Biomedical Support Program; and many have obtained research grant support. In addition, the program has inspired the institutions to give the returned fellows release time, and to give them seed money for getting involved in research. In every sense, this program has had a very positive impact on the individuals and the institutions involved.

Program staff, in their continuing effort to improve the program, have undertaken a major evaluation of these components of MARC in order to assess improvements and accomplishments. This activity is being conducted in a cooperative manner, utilizing the minority scientific community itself as part of the evaluation process. Clearly, the more significant accomplishments

of the Program are the following:

- The "honors" concept of the MARC Program has encouraged recipients of awards to institute a different and more rigorous program for students in biology and chemistry; many have incorporated an honors thesis requirement for all graduating MARC students.

- The provision of funds for seminars and lectures, release time for faculty, up-to-date equipment and sufficient supplies, the addition of fulltime and parttime faculty in disciplines not formerly represented have all contributed to academic enrichment; in addition, specialized courses have been set up to prepare students to take administrative tests, an area where minority students have traditionally been weak.

- The scientific activities of grantee departments have been strengthened, and as a result, it has become more common for MARC faculty and trainees to be invited to present papers at local, regional, and national scientific meetings.

- The "public relations" aspects of the MARC Program, with their emphasis on research, have produced a new awareness of the possibilities available in biomedical research among students historically motivated towards careers in medicine, dentistry, and education.

- The fact that a number of MARC students are performing at an excellent level in graduate schools have provided "role models" not normally available in minority schools; this in turn has resulted in a 50 percent increase in the number of science majors at those minority institutions.

Following the report of the MARC Honors Undergraduate Training report at the last Advisory Council Meeting, it was noted that a number of students graduating from the Honors Program are reluctant to continue their education outside of a minority college setting, and are not aggressive at seeking out existing training opportunities. In an effort to resolve this problem, the MARC Program in consultation with Institute staff and advisors, had drafted an announcement which would invite applications for Individual National Research Service MARC Predoctoral Fellowships. The draft document, distributed at the meeting, described this newest initiative of the MARC Program as follows:

> "The MARC Prodoctoral Fellowship Program provides support for research training leading to the Ph.D. degree in the biomedical sciences for selected students who are graduates of the MARC Honors Undergraduate Research Training Program. It is expected that such training will be conducted in graduate degree programs of the highest quality.
>
> Students eligible for the awards will be limited to MARC undergraduate trainees who have successfully completed the course of study prescribed by an institution having a MARC Honors Undergraduate Training Grant. In addition, applicants are expected to graduate with distinction.
>
> Support is not available for individuals enrolled in medical or other professional schools, unless they are enrolled in a combined degree (M.D.-Ph.D.) program.

Awards are conditional upon acceptance into a certified doctoral (Ph.D.) program. in biomedical research.

MARC Predoctoral Fellows are selected on a competitive basis. Applications will be evaluated by the Minority Access to Research Careers Review Committee of the NIGMS. A maximum of up to five years of support may be recommended, based on the merit of the application and evidence of satisfactory progress in the doctoral program in which a successful applicant is enrolled. The stipend will be the same as is paid to other NIH predoctoral fellows and trainees (currently $5,040 per annum). Funds will be provided for tuition plus an allowance of $3,500 for fees, supplies and travel.

Following some discussion of the rationale and logistics for this program element, it was moved that Council endorse the new MARC predoctoral individual fellowship program and, funds permitting, implement it immediately; the motion passed unanimously, though it was suggested that the program be initiated on a limited basis.

The program is being announced presently, and MARC Program staff is currently developing plans for implementation of this support mechanism. Significant response to this newest effort will provide the basis for decisions regarding the future directions of the MARC Program.

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