A PROGRAM IN GENETICS AND MOLECULAR BIOLOGY

Genetics Department

Stanford University School of Medicine

October 1968 - October 1973

Submitted by:

Joshua Lederberg, Principal Investigator
Department of Genetics
Stanford University School of Medicine
Palo Alto, California

This application is intended as a continuation of Grant Number GB 4430 (expiring 10/15/68) entitled "A Program in Genetics and Molecular Biology".

A PROGRAM IN GENETICS AND MOLECULAR BIOLOGY

The development and expansion of the Genetics Department in its new facilities in the Clinical Sciences Research Building of the Stanford University School of Medicine, occupied in March 1966, has been greatly aided by funding from the current "Program in Genetics and Molecular Biology" -- National Science Foundation Grant No. GB 4430. The main emphasis of this grant has been the provision of major items of equipment whose use is commonly shared by all members of the Department. The greater flexibility which has derived from this type of departmental grant compared to the individual research grants has enabled us to operate much more efficiently and to respond rapidly to equipment needs as research programs change and develop. The success of the program as an essential complement to individual funding can be judged from the variety of advances documented in the departmental bibliography. As the program has continued, and many, if not all, of the equipment needs have been met, the Department has been evolving other cooperative efforts to provide for the maximum and efficient utilization of our facilities. It has become increasingly clear from this that there is a considerable need for personnel and services which operate at a departmental rather than individual level and it is mainly for their provision that the present continuation application is made. Many areas of our individual research programs converge on common techniques and facilities, yet often no one individual is in a position to justify the entire use of a Professional Assistant or laboratory facilities for these particular needs. The operation of the departmental amino acid analyzer purchased from funds of the present

Program is an excellent example. Again the ordering and disposal of supplies and the day by day maintenance of equipment and common wash-up and sterilization facilities is most effectively accomplished by a Departmental Laboratory Coordinator.

The faculty group participating in this application consists of:

Professor J. Lederberg, Executive - Genetic chemistry of bacteria. Computer system for biochemical analysis.

Professor W. Bodmer - Genetic chemistry of transformation. Somatic cell genetics and genetics of human white cell antigens. Population genetics.

Professor E. Shooter - Molecular neurobiology. Chemical ontogeny and polymorphism of nervous system proteins. Nerve growth factor proteins.

Associate Professor L. Herzenberg - Immunogenetics and somatic cell genetics.

Assistant Professor A. Ganesan - Mechanisms of genetic recombination. In vitro synthesis of transforming DNA.

Senior Research Associate E. Levinthal - Instrumentation research.

Senior Research Associate B. Halpern - Reagents and techniques for ultramicroanalysis.

In addition, a number of research associates of senior stature are connected with these and other programs, including Dr. S. Liebes (physics of mass spectrometry). The substantial engineering developmental program in automated instrumentation under N.A.S.A. auspices continues to converge with the main research areas of the department, the development of an automated cell separator being one excellent example of the collaboration.

The Exchange Program in Genetics and Molecular Biology between Stanford University and the University of Pavia, funded in part by NSF, is also now in operation and the following Pavia faculty and students are, or will shortly be, working in the Genetics Department: Professor L. Cavalli-Sforza, Drs. M. Polsinelli, S. Barlatti and A. Cefferi and P. Pignatti.

Professors E. Glassman of the University of North Carolina and Sarane T. Bowen of San Francisco State College will be spending their sabbatical leaves in the department in 1968-69.

A detailed budget for the first year of the program is attached, as well as a general breakdown of expenditures for the subsequent second, third, fourth and fifth years.

NATIONAL SCIENCE FOUNDATION

Washington, D. C. 20550

RESEARCH GRANT BUDGET SUMMARY

Institution:	Principal Investigator:	Program Name:	Duration:	Grant Number:
Stanford University School of Medicine	Joshua Lederberg Genetics Department	A Program in Genetics and Molecular Biology	10/15/68 to 10/15/69	

	NSF Funded Man Months (Cal)	Grantee Man Months (Cal)	NSF Grant	Grantee Share
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Investigator				
Joshua Lederberg	1			3,369
b. Faculty Associates				
W. Bodmer, A. Ganesan, L. Herzenberg, E. Levinthal, E. Shooter				
2. Other Personnel: Technical				
a. Administrative Officer				
D. Stuedeman	4		4,000	
b. Laboratory Coordinator				
M. Thomas	12		7,600	
c. Professional Asst. to operate Dept. Amino Acid Analyzer				
V. MacPhee	12		8,400	
d. Professional Asst. to operate Dept. Tissue Culture Lab	12		8,400	
e. Lab. Technician for Dept. Animal House				
F. Rodriquez	12		6,600	
f. Machinist	2		2,000	
Total			37,000	3,369
B. FRINGE BENEFITS (11.6%)			4,292	391
Total			41,292	3,760
C. PERMANENT EQUIPMENT				
1. Large liquid N_2 tank			5,000	
2. Spinner culture apparatus			5,000	
Total			10,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT				
Materials for use in departmental facilities, e.g. wash up room and sterilization, Media Room, Animal Room; chemical and glassware for departmental stores; reference materials and library			7,000	

RESEARCH GRANT BUDGET SUMMARY (continued)

	NSF Grant	Grantee Share
E. OTHER DIRECT COSTS		
Maintenance on equipment (purchased on previous grant)	2,000	
Minor laboratory rearrangements	2,000	
Rent of two complete terminals for use with ACME	4,500	
Computer time for batch processing on campus computer facility at $400/hr.	2,000	
Total	10,500	
F. TOTAL DIRECT COSTS	$ 68,792	3,760
G. INDIRECT COSTS (57% S&W)	21,090	1,920
H. TOTAL COSTS	89,882	5,680
REQUESTED FROM NSF	89,882	

Stanford University
School of Medicine

A Program in Genetics
and Molecular Biology

BUDGET FOR 2ND AND SUBSEQUENT YEARS -

	2nd	3rd	4th	5th
PERSONNEL	43,300	45,465	47,738	50,125
EQUIPMENT	10,000	10,000	10,500	10,500
EXPENDABLE SUPPLIES	7,000	7,300	7,600	7,900
OTHER	10,500	11,000	11,500	12,000
DIRECT COSTS	70,800	73,765	77,338	80,525

The figures for Personnel have been increased by 5% each year.

Justification for Budget

A. Personnel

As the research programs of the various faculty members have developed and expanded it has become clear that they overlap in many areas, e.g. in protein sequence work and tissue culture, and we have therefore set up or are setting up such facilities on a departmental rather than individual basis. This provides for the most efficient utilization of facilities and equipment and also, in instances where a person's needs are relatively small (but vital) and do not justify individual requests for funding, for the greatest flexibility in meeting specific research needs. The department has operated an amino acid analyzer on this basis with great success for the past nine months and the budget therefore requests continuing stipend for a Professional Assistant to run this instrument.

Three faculty members at the present use tissue culture and the operations again are on a scale that coordination of equipment and media would be of great benefit. It is envisaged that a Professional Assistant would be in charge of such a coordinated effort and that this person would have also sufficient capability to assist each of the faculty groups as required. The use of the Genetics Department Animal Facility has increased far beyond the load for one person and a second Animal Room technician was recently hired. In that much of his work is for general members of the Department rather than one Faculty member, his stipend is now included in the departmental application.

Two positions are listed for a part salary for the Senior Laboratory Coordinator and full time salary for his assistant. Between them they take

care of the routine management of the departmental facilities and its technical staff, the organization of the wash and sterilization facility, much of the ordering of supplies and equipment and any research that this may involve, and generally the day to day interactions with the service engineers of the building.

A continuing part time salary for a machinist is also included in this application. His services in repairing and modifying equipment and installing minor laboratory fittings continue to be invaluable.

C. Equipment

This category is now much reduced in level compared to the current grant and will be used as indicated to buy, again, equipment for departmental use, for example, for cell storage and culture.

D. Expendable Equipment and Supplies

These items include materials for use in the common wash up and sterilization facility, the animal house and for departmental stocks of glassware, scintillation vials and chemicals and solvents used in relatively large amounts. Also it includes funds for the purchase of necessary reference materials (e.g. Automatic Subject Citation Alert, ASCA) and other library supplies.

E. Other Direct Costs

With the availability of ACME (Advanced Computer Facilities for Medical Education) this Department makes large scale and effective use of these major facilities. The cost of the rent of two of our ACME terminals is included in this category as is also computer time for the overnight batch processing of some of our larger requirements.

Existing Facilities

The department covers some 11,000 square feet in the new Clinical Sciences

Building of the Stanford Medical Center complex providing integrated areas for microbial, cell and human genetics, molecular neurobiology and computer work. Common facilities for wash up and sterilization are available as are hot and cold room facilities. The Instrumentation Research Laboratories are housed in 6,000 sq. feet of appropriately designed areas on the Ground Floor beneath the Genetics Department.

The department is equipped with all the usual apparatus for work in the fields outlined above. Major items of equipment include:

Tricarb scintillation spectrometer

Spinco analytical ultracentrifuge

Spinco and IEC preparative centrifuges

Spinco amino acid analyzer

Zeiss spectrophotometer

Bendix and Quadripole mass spectrometer with interfaces to the IBM 360/50 computer

Existing Funding. No other funding matches the purpose of this application, however, component elements of our investigations are supported as follows:

From National Institutes of Health

Grant No.	Grant Title	Dates	Direct Costs
AI-5160	Genetics of Bacteria	9/1/64-8/31/68	$ 253,000
GM-14108	DNA Synthesis & Genetic Recombination	6/1/66-5/31/69	104,300
GM-14650	Genetics of Human Tissue Antigens	12/1/66-11/30/69	124,000
NB-04270	Molecular Neurobiology	12/1/67-11/30/70	202,000
GM-12075	Genetics of Immunoglobulins	6/1/64-5/31/69	149,500
CA-04681	Genetic Studies with Mammalian Cells	9/1/67-8/31/72	272,000
GM-295-10	Final year of current period of Training Program	7/1/68-6/30/69	115,000
Application Pending	Renewal of Training Program in Genetics	7/1/69-6/30/74	756,650
GM-35002	Career Award: Dr. Walter F. Bodmer	1/1/67-12/31/71	120,000

From National Science Foundation

GB 5862	Genetic Chemistry of DNA Mediated Bacterial Transformation	12/1/66-11/30/68	76,950
GB 6878	Subunit Structure of Nerve Growth Factor	9/1/67-8/31/69	44,500

From National Aeronautics and Space Administration

NASA NsG 81-60	Cytochemical Studies of Planetary Microorganisms (Instrumentation Research Lab.)	9/1/67-8/31/68	410,000

Joshua Lederberg - Genetics of Bacteria

The DNA transfer system ("transformation") in Bacillus subtilis offers a particularly favorable opportunity to study the physical and chemical properties of DNA molecules in relation to their biological activity. Over the past several years my principal co-investigators have been Professor Walter F. Bodmer and Professor A. T. Ganesan. Their work now has become well differentiated and receives independent support, but the studies summarized in the attached publications were initiated during the current term of the grant.

The main questions we have examined have concerned:

The linkage system, as manifested in DNA transfer;

The fractionation of biologically active DNA, assigning different genetic activities to different average base compositions;

The effects of fragmentation of DNA molecules by physical shearing, and by nuclease attack;

The mechanism of integration of DNA in the course of the transformation process;

The association of the DNA replication process with a cell-membrane bound polymerase.

In collaboration with Professor Arthur Kornberg and other members of the Department of Biochemistry we had also attempted to demonstrate the replication of biologically active DNA by E. coli DNA polymerase. Those experiments were, however, unsuccessful -- in considerable part, as we now know, because of our ignorance of polynucleotide ligase.

The main impact of these studies has been to verify the correspondence of the properties of genetic activity of DNA with distinctive sequences of nucleotides, and to provide some further technical facilities for the ultimate description of genetic information in chemical terms. During the period covered by this report many workers have entered similar fields and the literature now contains many examples of the building of new edifices on previously established foundations.

I propose now to concentrate on the natural or experimentally induced occurrence of insertions and inversions in B. subtilis. In enteric bacteria, mapping studies have shown a remarkable congruence of gene sequences among bacterial species which have already diverged significantly in DNA homology as tested by hybridization-reanealing experiments. Conservation of gene order is also evident in the organization and persistence of operons, showing coordinate repression; these are frequent in bacteria but rare in higher forms. Most or all of the examples of inversions found experimentally in bacteria can be explained either as methodological artifacts (1) or as consequences by crossing over of episome-bound segments with redundantly homologous segments of the whole DNA (2).

These observations were vague forerunners of the now accepted understanding that bacteria differ from eukaryons in 1) The simple organization of the chromosome as a single polynucleotide sequence, and 2) The absence of any common mechanism for "holo-ligation repair" of the chromosome. The recent clarification of the dark repair of single-strand breaks in DNA (reviewed in 3) has pointed up mechanisms of template-directed repair (hemiligation) in bacteria. These are fundamental to recovery from UV-damage, to recombination mechanisms, and probably to the post-editing of newly replicated DNA. There is, on the other hand, no good evidence for any normal mechanisms for joining or rejoining broken DNA strands except for hemi-ligation, i.e., when the broken strand is entwined with a complementary template strand that spans the broken ends.

It may be previous to define a process that may not exist, but we are then looking for holo-ligation, i.e., some way to join broken strands, or double helices, without the help of a homologous template. The potential extent and limitations of holo-ligation relate to many current problems in chemical genetics and cell biology. These include, for example:

1) Genetic variation in recovery from X-ray damage and the mechanism of recovery from double-strand scissions.

2) A sharper confrontation with chromosome structure of eukaryons in which broken chromosomes can indeed be rejoined (but is this a covalent nucleotide assembly?)

3) Transcription problems: framing, the reading of inverted sequences, and strand selection.

4) The barrier to promiscuous recombination of DNA from different sources.

Point 4 may be the most far-reaching for every level of genetic manipulation, both investigative and applied. The rejection of foreign DNA by competent Bacillus subtilis cells may illustrate one evolved mechanism whereby a cell has been able to protect itself against unlimited intrusion by foreign genes, arising by chemical scrambling or by virus infection. The research utility of freely moving genes from another species into bacteria needs no elaboration, e.g. for the study of transcription-control, and to facilitate analyzing the genetic competence of DNA from differentiated tissues of higher organisms.

Important practical utilities would follow from the incorporation of human genes into suitable cryptic virus DNA for kind of transductional therapy for human genetic disease (4).

References:

1. Glansdorff, N., 1967. Pseudoinversions in the chromosome of Escherichia coli K-12. Genetics 55:49-61.

2. Berg, Claire M. and Roy Curtiss III, 1967. Transposition derivatives of an Hfr strain of Escherichia coli K-12. Genetics 56:503-525.

3. Hanawalt, Philip C., 1968. Cellular recovery from photochemical damage. Ch. 11, Photophysiology, Vol. III (A. C. Giese, ed.) Academic Press, New York.

4. Rogers, Stanfield, 1966. Shope papilloma virus: A passenger in man and its significance to the potential control of the host genome. Nature 212:1220-1222.

Personnel

Postdoctoral:

S. Barlati	Polysome aggregation in B. subtilis
M. Polsinelli	Genetic translocation in B. subtilis

Predoctoral:

H. Eisenstark	Conditional lethals in B. subtilis; toxicity of DMSO
I. Majerfeld	Adding homopolymer terminations to B. subtilis DNA
W. Spiegelman	Regulation of lambda bacteriophage
L. Okun	High molecular weight transforming DNA

JOSHUA LEDERBERG

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Stanford University School of Medicine
Palo Alto, California
94304

Phone:
(415) 321-1200 Ext. 5801

Education:

1938-41	Stuyvesant High School (New York City)
1941-44	B.A., Columbia College
1944-46	Enrolled as medical student, Columbia University College of Physicians and Surgeons
1946-47	Ph.D., Yale University. Sc.D. (h.c.) 1960; also Wisconsin (1967); Columbia (1967).

Experience:

1945-46	Research assistant in zoology (with Professor F. J. Ryan), Columbia University
1946-47	Research fellow of the Jane Coffin Childs Fund for Medical Research at Yale University (with Professor E. L. Tatum)
1947-59	Professor of Genetics, University of Wisconsin
1950	Visiting Professor of Bacteriology, University of California, Berkeley
1957	Fulbright Visiting Professor of Bacteriology, Melbourne University, Australia
1957-59	Chairman, Department of Medical Genetics, University of Wisconsin
1959-	Professor, Genetics and Biology, and Executive Head, Department of Genetics, Stanford University
1961-	Director, Kennedy Laboratories for Molecular Medicine, Stanford University

Special field: Genetics, chemistry and evolution of unicellular organisms and of man.

Distinctions:

1957	National Academy of Sciences
1958	Nobel Prize in medicine (for studies on organization of the genetic material in bacteria)

Public responsibilities:

1961-62	President (Kennedy)'s Panel on Mental Retardation
1950-..	President's Science Advisory Committee panels. National Institutes of Health, National Science Foundation study sections (genetics)
1958-..	National Academy of Sciences: committees on space biology
1960-..	NASA committees; Lunar and Planetary Missions Board
1967-	NIMH: National Mental Health Advisory Council

Personal Data:

b. May 23, 1925; Montclair, New Jersey

Walter F. Bodmer - Genetic Chemistry of DNA Mediated Bacterial Transformation; Somatic Cell Genetics and the Genetics of Human White Cell Antigens; Population Genetics.

1. Genetic Chemistry of DNA Mediated Bacterial Transformation

The main aim of this research is to further the understanding of the processes of integration and recombination during DNA mediated transformation in Bacillus subtilis. Earlier work from our laboratory was instrumental in showing that donor DNA was incorporated into small single-stranded regions of the recipient genome (Bodmer and Ganesan 1964) and in establishing the extent to which DNA synthesis may be involved during transformation (Bodmer 1965). This work clearly showed that there is no major amount of DNA synthesis during uptake and integration of donor DNA, though the amount of repair synthesis that may be involved and the specific enzymatic steps leading to final integration remain incompletely understood. Understanding these problems remains a major part of our research effort. Specific research projects currently underway or being planned are

a) The properties of a mutant which is a genetic rearrangement will be investigated during transformation, with special reference to testing for heterozygosity in the products of transformation.

b) Attempts are being made to assess the true extent of repair synthesis during transformation. High specific activity P^{32} labelling during transformation and 5-Bromouracil labelling during transformation accompanied by physical separation of competent cells, are the two techniques currently envisaged for these studies.

c) A double marker transformation assay has been shown to be a likely indicator of "cross-correction repair" during transformation. The

effect of UV sensitive mutants on this system are being investigated.

d) work with multiply marked recipient strains is continuing with a view to determining further the relationship between DNA integration during transformation and regions of initiation of DNA synthesis. Experiments are also planned to test whether protein synthesis is required for reinitiation of DNA synthesis following transformation.

e) Further experiments are planned on the early events following uptake of donor DNA during transformation, in particular to determine the nature of the early native material found following addition of donor DNA. In addition, efforts will be made to characterize the transition from non-covalent to covalent association between donor and recipient DNAs.

2. Human Somatic Cell Genetics and the Genetics of Human White Blood Cell Antigens.

a) Our laboratory,in collaboration with Dr. Rose Payne of the Department of Medicine, has been instrumental in identifying some of the major antigens of the HL-A human white blood cell antigen polymorphism and in interpreting the relationships between the genetic determinants for these antigens (Payne et al 1964, Bodmer and Payne 1965, and Bodmer et al. 1966). During the last 18 months we have developed a convenient microcytotoxicity assay combining Terasaki's micro droplet assay with the technique of fluorochromasia (Bodmer et al 1968). We have used this assay extensively for population genetic and other studies directed towards further development of knowledge of the genetics of the human white cell antigen systems. Current areas of investigation are

i. We are attempting to devise more sensitive assays for the antigens using anti-human gamma-globulin serum both for cytotoxic and agglutination assays.

ii. New antigenic specificities are being characterized by absorption analysis of a number of sera, combined with population and family studies.

iii. We have undertaken a major population genetic study, in collaboration with Professor L. L. Cavalli-Sforza, of the pygmies of Central Africa and of other African populations. We plan to extend these population studies to other racial groups through collaboration with other investigators.

iv. We are investigating the potential uses of cattle *isoantisera* for typing human white cell antigens and the general question of species cross-reactivity with respect to these antigenic systems.

b) During the last year we have initiated a program of research in human somatic cell genetics. This is based on using the technique of cell fusion mediated by inactivated Sendai virus. Hybrid lines are being made by fusing human peripheral blood lymphocytes with base analogue resistant mouse cell lines derived from known inbred mouse strains. Hybrids are initially recognized by their chromosome constitution. A major source of genetic markers are the white cell antigens being studied in our laboratory. A micro-mixed agglutination technique for the identification of these antigens on cell cultures is being developed and is being applied to study the distribution of these antigens on our human-mouse hybrids. Other genetic markers will, of course, be

studied, in particular electrophoretically distinguishable variant enzymes. The karyotypic and genetic constitution of hybrid lines is being studied and attempts are being made to correlate the presence of combinations of human genetic markers with the chromosomal constitutions of the hybrids. These studies will be coupled with attempts to obtain control changes in the hybrid karyotype. Other lines of human cells will be used for studies related to the problem of gene expression as a function of differentiated cell type.

3. Population Genetics

The emphasis of work in this area is on the theoretical analysis of population genetic models and their interpretation, particularly with relation to the problems of human genetics. For some years I have been involved in an active program of research in theoretical population genetics with Professor Karlin of the Mathematics Department. This unique relationship has provided an opportunity for combining a sophisticated mathematical approach with direct contact with experimental genetics. Recently, work has been done to extend understanding of models for the Rhesus blood group incompatibility system. In collaboration with Professor L. L. Cavalli-Sforza, new models have been developed to explain observed differences in gene frequency in terms of observed migration patterns, using a migration matrix approach. Further work is planned on the problem of the interaction of linkage and selection, particularly with respect to the conditions under which there is selection pressure for tighter linkage and the problems of dealing with more than two loci. In addition, some work has been done on using data on amino acid substitutions to match observed evolutionary rates with those expected from population

Walter F. Bodmer - Genetic Chemistry of DNA Mediated Bacterial Transformation; Somatic Cell Genetics and the Genetics of Human White Cell Antigens; Population Genetics.

genetic theory. A major survey of theoretical population genetics, undertaken in collaboration with Professor Karlin and Mark Feldman, a graduate student, is nearing completion.

Personnel

Postdoctoral

A. J. Darlington	Genetic chemistry of DNA mediated bacterial transformation
V. Miggiano	Human somatic cell genetics
T. Iha	Human white cell antigen genetics
F. Scudo	Theoretical population genetics

Predoctoral

M. Nabholz	Human somatic cell genetics

Research Assistants

L. Wang	Genetic chemistry of DNA mediated bacterial transformation
J. Bodmer	Genetics of human white cell antigens
G. Gerbrandt	" " " " " "
M. Tripp	" " " " " "

WALTER F. BODMER

CURRICULUM VITAE

Personal Data:

Born: Frankfurt am Main, Germany, January 10, 1936
British citizen, U.S. Immigrant
Wife: Julia Gwynaeth Bodmer
3 children
Soc.Sec. No. [redacted]

Education:

1953-56	B.A., Clare College, Cambridge University
1956-59	Ph.D., Clare College, Cambridge University

Experience:

1958-60	Research Fellow, Clare College, Cambridge
1960-61	Demonstrator, Department of Genetics, Cambridge University
1961	Official Fellow, Clare College, Cambridge
1961-62	Fellow, Visiting Assistant Professor, Department of Genetics Stanford University School of Medicine.
1962-66	Assistant Professor, Department of Genetics, Stanford University School of Medicine.
1966-68	Associate Professor, Department of Genetics, Stanford University School of Medicine.
1968-	Professor, Department of Genetics, Stanford University School of Medicine.

Special Field: Chemical Genetics, Human and Population Genetics.

Public Responsibilities:

1964-67	National Science Foundation, Genetics Panel
1964-67	National Institute of Allergy and Infectious Diseases, Committee for Collaborative Research in Transplantation and Immunology
1968-	Associate Editor, American Journal Human Genetics

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination

The *Bacillus subtilis* genome replicates sequentially from a fixed origin, as judged by isotopic transfer and gene frequency distributions in transformation experiments. An *in vitro* system using *E. coli* polymerase for the replication of DNA from *B. subtilis* yielded products which were biologically inactive. Thermal denaturation of these molecules was spontaneously reversible. These abnormalities may reflect interruptions and lack of control in coherent replication in the *in vitro* system.

Earlier experiments in our laboratory suggested that *in vivo* the nascent DNA might be bound to a particulate fraction which also contained DNA polymerase of high specific activity. Using the nascent DNA already present, the polymerase in the particulate fraction was able to synthesize DNA (Judged by the incorporation of the labeled deoxytriphosphates into cold acid precipitable material) when supplied with all four deoxytriphosphates and Mg^{++}. Our approach to the problem of synthesizing biologically active DNA *in vitro* has been to study the pattern of replication by this particulate fraction, starting from relatively crude preparations and following it through different steps of purification of the polymerase.

Careful isolation of the active protein complex free of cell membrane and other components has resulted in a purification of 100 to 150 fold compared to the initial lysate. This preparation has been found to differ in some respects from the highly purified preparations of DNA polymerase from *E. coli* and *B. subtilis*. Unlike the latter, our preparation preferred native bihelical DNA to denatured DNA as a primer. Double stranded DNA was 6 to 8 times more efficient as primer than denatured DNA. In our system dAT-copolymer

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination. (continued)

was only 2 to 4 times more active as a primer than the native DNA, while with the purified polymerases dAT-copolymer has been reported to be 20 times more active.

The enzyme complex sediments with a unimodal distribution in sucrose gradients. Of the ions tested Mg^{++} was the most effective in the reaction. Traces of Na^{+} and K^{+} stimulated the reaction in the presence of Mg^{++}. In a reaction, 20% of the amount of primer added was synthesized in 30 minutes. There was no significant amount of exonuclease activity found in the polymerase. The endonuclease activity associated with the preparation can be partially inhibited by RNA.

When transforming DNA labelled with N^{15}, deuterium and H^{3} was isolated from genetically marked *B. subtilis* and used as a primer in a reaction of our partially purified preparation with light (N^{14}, H) C^{14} labelled deoxytriphosphates, it was possible to demonstrate synthesis of DNA molecules of lighter density as observed by CsCl density gradient centrifugation. 80% of the molecules are denaturable and 90% are rendered into acid soluble mononucleotides by *E. coli* Exonuclease-1. The products composed of hybrid molecules containing one strand of heavy DNA and one strand of light DNA. The hybrid molecules are biologically active. In addition to the hybrid, 4% of the biological activity was associated with completely light and hybrid. Of these light molecules, at least 10% carry 3 known genetically linked genes, while the majority are only active for single gene transformation. The enzyme complex is presently studied by different physical techniques, to detect various activities, that form the active complex.

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination

Personnel

Postdoctoral

F. Gillin	Genetic control of DNA Synthesis in *B. Subtilis*

Predoctoral

P. Laipis	*In vitro* synthesis of transforming DNA

Research Assistant

N. Buckman	Mechanism of genetic recombination *In vitro* synthesis of transforming DNA

Curriculum Vitae

A. T. GANESAN

Personal Data: Born Mannargudy, Madras State, India, May 15, 1932.
American citizen.
Wife: Ann K. Cook Ganesan (Ph.D. Stanford Univ., 1961)
No children.
Soc. Sec. No. [redacted]

Education and Experience:

1947-51 — B. Sc., Annamalai University, Madras State, India
Physics, Chemistry and English. Major: Botany

1951-53 — M.A., Annamalai University (degree conferred Sept. 1954)
Plant Physiology and Genetics

1953-55 — Research Fellow, Department of Biochemistry, Indian Institute of Science, Bangalore, India
Awarded Institute of Science Fellowship

1955-57 — Research Associate, Botany Department, Indian Agricultural Research Institute, New Delhi, India
In charge of plant tissue culture
Instructor in Genetics - Lectures and Laboratory

1957-59 — Awarded Rask-Orsted Foundation of Denmark fellowship for study at Carlsberg Laboratory, Copenhagen, Denmark.
Fermentation genetics and some aspects of yeast cytogenetics.
Also worked for a few weeks during this period at the Genetics Department, University of Copenhagen on Neurospora genetics.

1959-1963 — Ph.D., Stanford University, Palo Alto, California
National Institutes of Health trainee under Professor Joshua Lederberg, Department of Genetics, Stanford School of Medicine

Thesis: Physical and biological studies on transforming DNA from B. subtilis.

March 1963 - Aug. 1965 — Research Associate, Department of Genetics, Stanford University School of Medicine

Sept. 1965 - — Asst. Professor, Department of Genetics, Stanford University

Current interests:

1. In vitro replication of biologically active DNA.

2. The mechanism of genetic recombination.

L. A. Herzenberg - Immunogenetics and somatic cell genetics.

Immunoglobulin loci. Four H-chain gene loci, Ig-1, Ig-2, Ig-3 and Ig-4, have been found in the mouse, corresponding to the H-chains in the classes γG_{2a}, γA, γG_{2b} and γG_1 respectively. The alleles at Ig-1 through Ig-3 were first defined serologically with alloantisera.

Detection of the reaction of alloantisera with immunoglobulin was by immunodiffusion in agar or, where more sensitive detection methods were necessary, by inhibition of precipitation of radioactive labeled antigens. The latter method was used for most of our analyses of allotypic differences.

Among some 70 inbred mouse strains, there are 8 alleles at the Ig-1 locus. These alleles display 11 distinct antigenic specificities on γG_{2a} immunoglobulins. Each allotype is cross reacting and is determined by various combinations of relatively few specificities.

Three other immunoglobulin loci closely linked to Ig-1 have been uncovered. Ig-2, Ig-3 and Ig-4 determine γA, γG_{2b} and γG_1 H-chains respectively. Ig-2 and Ig-3 were demonstrated by the same methods as Ig-1; i.e., cross reacting specificities found with alloantisera.

A fourth linked H-chain locus, Ig-4, has been defined, using allelic differences in electrophoretic mobility of γG_1 immunoglobulins and Fc fragments. Thus, 4 of the 5 H-chains in mice are controlled by closely linked genes (forming the H-chain chromosome region).

Allelic electrophoretic mobility differences have been found for the previously identified H-chain loci, Ig-1 through Ig-3 as well. One or more of the serologically defined allotypes at each locus is strictly

L. A. Herzenberg - Immunogenetics and somatic cell genetics. (continued)

associated with each electrophoretic mobility allotype. The use of electrophoretic mobility for allotype detection is new and of potentially great use for genetic studies of immunoglobulins.

Three heavy-chain allotypic specificities have been found on two of the mouse immunoglobulin classes, γG_{2a} and γG_{2b}. The other described immunoglobulin allotypic specificities in the mouse are restricted to one or another H-chain class. This work helps understand the possibly evolutionary origins of shared or common specificities and is of importance to theories of generation of diversity in immunoglobulins.

Detection of Allotypic Antigens with Heterologous Antisera. Rabbits were immunized with either normal mouse immunoglobulins or isolated mouse myeloma proteins from BALB/c or (BALB/c X NZB)F_1 plasma cell tumors. The antisera were tested for antibodies directed to allotypic antigenic specificities by the method of inhibition of precipitation of I^{125} labeled antigens.

Three γG_{2a} and one γG_{2b} allotypic specificities were detected with one or another of these rabbit antisera. These specificities each corresponded in mouse strain distribution with one of the allotypic specificities previously defined through the use of mouse isoantisera.

Plasma Cell Tumors. A new series of 15 myeloma proteins from plasmacytomas induced in the Walter & Eliza Hall Institute, Melbourne, Australia, has been characterized here as to immunoglobulin type, physicochemical properties and allotypic specificities. Several of these tumors are now

L. A. Herzenberg - Immunogenetics and somatic cell genetics. (continued)

being maintained in this laboratory and are under further study. These are the first plasmacytomas available from (BALB/c X NZB)F_1 hybrid mice heterozygous for allotype. Since they each produce large quantities of relatively homogeneous immunoglobulin, they greatly facilitate analysis of the genetic control of immunoglobulins.

Three γG_2 myeloma proteins of plasma cell tumors induced in the (NZB X BALB/c)F_1 mice have been analyzed for the isoantigens they carry. NZB mice are genotypically $\underline{Ig\text{-}1^e\ Ig\text{-}3^e}$, while BALB/c are $\underline{Ig\text{-}1^a\ Ig\text{-}3^a}$. Two of the myeloma proteins are γG_{2a} globulins. One of these, GPC-7, carries all the isoantigenic specificities of the $Ig\text{-}1^e$ allele while the other, GPC-8, carries all the isoantigenic specificities of the $Ig\text{-}1^a$ allele. Thus, only one of the parental alleles of the mouse in which the tumor arose is expressed in each of these myeloma proteins.

The third myeloma protein, GPC-5, also carries the antigens of only one parental strain (NZB). However, GPC-5, a γG_{2b} globulin, carries only one of the Ig-3 specificities normally associated with γG_{2b} globulins of NZB. Most remarkably, it also carries one Ig-1 specificity normally associated with γG_{2a} globulins of NZB. This is the first analyzed mouse myeloma shown (a) to express some but not all the antigenic specificities normally associated with an allele, and (b) to carry antigenic specificities controlled by two distinct immunoglobulin loci.

Transplantation studies using immunoglobulin marked congenic strains. Over the past three years, we have been breeding two pairs of strains of

L. A. Herzenberg - Immunogenetics and somatic cell genetics. (continued)

mice such that the members of each pair would approach being congenic (having the same genotype) except for the Ig-chromosome region. Skin grafting and lymphoid cell transfer studies have now been carried out with the first pair. The two strains involved are an inbred strain, C3H.SW (CSW), and its congenic partner, CWB/5, which was derived by 5 backcrosses to CSW and 6 brother X sister matings. CSW has the Ig-1^a allele, while CWB has the Ig-1^b allele. These strains are now completely histocompatible for skin. Skin grafts, even after prior sensitization, are permanently accepted in both directions. The production of donor type immunoglobulins by transferred lymphoid cells was obtained with much greater ease than in our previous experiments with allogenic strain cell transfers. However, two barriers to the transferred cells were seen: limited biological space and, what is more likely, a residual histocompatibility antigen(s) for lymphoid cells (although not for skin). These barriers were overcome by limited sublethal X-irradiation and/or by increased numbers of transferred cells. A most exciting prospect is the finding of readily detectable amounts of donor globulins several weeks after transfer of as few as 1,000 spleen cells.

Spleen, bone marrow and fetal liver, but not thymus cells, produce γ-globulins for many weeks after injection into 600R irradiated congenic recipients. As donor type levels increased, host type decreased in some animals to very low levels.

These congenic strains are being used for studies of the ontogeny and regulation of immunoglobulin production and for studies of the genetics of

L. A. Herzenberg - Immunogenetics and somatic cell genetics. (continued)

antibody induction by specific antigens.

The congenic strains have found an important use in showing that fetal liver cells transferred into intact or thymectomized, sublethally irradiated hosts differentiate to produce donor allotype immunoglobulins. However, the thymectomized chimeras are unable to make specific antibodies to at least some antigens while the non-thymectomized controls do make antibodies when challenged.

Backcrossing of CWB/5 to CSW has been continued and a line is now being obtained by inbreeding at the 8th backcross to see whether the histoincompatibility has been bred out. Eleven backcrosses in all have been completed.

The other pair of congenic strains is C57BL/10 ($Ig\text{-}1^b$) and its congenic partner, BTA ($Ig\text{-}1^a$). A line is being developed by inbreeding after 11 backcross generations. It has not yet been tested for cell transfer.

Suppression of allotype production in young animals. Our recent work on the problem of allotype suppression is of importance in understanding the regulation of immunoglobulin production. In the mouse, an antibody directed against an immunoglobulin allotype, Ig-1b, passed from mother to offspring or injected into neonates, suppresses synthesis of immunoglobulin carrying Ig-1b. In allotype homozygotes as well as heterozygotes, the allotype suppression is manifested both by a delay of several weeks in attaining initial detectable allotype levels and a reduction in allotype level continuing into adulthood. There is evidence for a strong intralitter (as opposed to interlitter) correlation of age of onset of immunoglobulin allotype synthesis.

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H-2 antigen of NZB. Mice of the NZB strain develop an "auto-immune" disease with many similarities to the human disease, systemic lupus erythematosus. For transplantation studies, it is useful to know what H-2 type it is. We have shown that the NZB strain has the allele $H\text{-}2^d$ both by hemagglutination and skin grafting studies. Snell's F_1 and component tests were used.

Leonard A. Herzenberg, Ph.D.
Associate Professor

May 23, 1968

Senior Research Associate

Leonore A. Herzenberg	-	suppression of immunoglobulin production, allotypic specificities, antiserum definition.

Postdoctoral Fellows

Ethel Jacobson	-	cell transfer studies with congenic mice.
Alma Luzzati (Research Associate)	-	single cell allotype studies.
Johanna L'age-Stehr (Research Associate)	-	cell transfer studies.

Predoctoral Fellows

Timothy Coburn	-	cell separation studies.
Peter Dolinger	-	antibody avidity studies.
Michel Facon	-	purification and structure studies on immunoglobulins.
Dale Hattis	-	isopyknic centrifugation of immunoglobulins.
Roy Riblet	-	lysozyme-producing mouse tumor and lysozyme polymorphism.
William Clewell (Medical Student)	-	isopyknic centrifugation of immunoglobulins.

Research Assistants

Priscilla Gibbs	-	primarily cell transfer and suppression of immunoglobulin synthesis.
Derek Hewgill	-	primary assay development and antiserum production.

CURRICULUM VITAE - LEONARD A. HERZENBERG

Born: November 5, 1931, Brooklyn, New York

Married: Leonore A. Herzenberg, 1953, 4 Children

Education

1945 - 48	Midwood High School, Brooklyn, New York.
1948 - 52	A. B. Brooklyn College, New York
1952 - 55	Ph. D. California Institutes of Technology, Pasadena, California

Appointments

1955 - 57	Pasteur Institute, Paris, France. American Cancer Society Postdoctoral Fellow
1957 - 59	National Institutes of Health, Bethesda, Maryland Officer, USPHS.
1959 - 64	Stanford University School of Medicine Assistant Professor of Genetics
1964 -	Stanford University School of Medicine Associate Professor of Genetics

Society Memberships

Phi Beta Kappa
Sigma Xi

Major Research Interest

Immunogenetics, somatic cell genetics.

Publications

See the attached sheets.

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis.

Molecular neurobiology. This project consists of basic research into the biochemistry of nerve cells particularly those aspects which differentiate nerve from other types of cells. Current research is therefore concerned with the properties and functions of macromolecules (a) which are specific to nerve cells, (b) whose mutant forms are associated with neurological disease and (c) which although found in other tissues interact specifically with nerve cells. An example of the latter is the Nerve Growth Factor which is an excellent model system for studying growth and differentiation in the nervous system.

The Nerve Growth Factor Protein. The Nerve Growth Factor (NGF) discovered by Levi-Montalcini is a protein which specifically stimulates the growth of functional axons from neurons of the sympathetic and embryonic sensory ganglia. Earlier work showed that the interaction of this protein with the target cell resulted in the stimulation of many biosynthetic pathways of which the stimulation of RNA synthesis appeared to be first in temporal sequence. Because fluorescent antibody to NGF interacted with components within the receptive neuron, it was concluded that part or all of the NGF molecule entered the cell. Nothing else is known about the details of the NGF interaction with the responsive neuron and our approach has been first to characterize the NGF protein itself in greater detail before going on to the metabolic experiments. It turns out that the NGF protein is an unusual protein. It is found in snake venoms and the adult male mouse salivary gland. A new NGF form has been purified forty fold from homogenates of the mouse gland by a procedure involving only gel filtration on Sephadex G-100, DEAE-chromatography and a second gel

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

filtration on Sephadex G-150. In the final product the NGF activity is associated, as judged by a number of physical criteria, with a single protein component comprising over 95% of the total protein of the fraction. It represents 2% of the soluble protein of the gland and 80% of the expressed activity of the gland homogenate. The molecular weight of this new NGF species is approximately 140,000 compared to 20-40,000 for NGF made by the older procedures. The large molecule is stable only between pH 5 and 8. Outside this pH range, it dissociates reversibly into smaller species with no, or considerably lower activity. The products of the complete dissociation are three groups of subunits, one acidic (α), one basic (β) and the third intermediate in net charge (γ), all three having molecular weights of approximately 30,000. The three groups of subunits have been isolated by chromatography on CM-cellulose at low pH and each displays heterogeneity on electrophoresis. Only the β subunit elicits a nerve growth factor type of response in the standard bioassay. It accounts for 25% or less of the original activity and is markedly unstable. However, the original highly active nerve growth factor protein is spontaneously reconstituted when the three subunits are mixed at neutral pH. All three types of subunits are needed for this regeneration; α and γ subunits, while not reacting with each other, combine separately with the β subunits but the resulting complexes have the same low activity and instability as the isolated β subunit itself.

Of the three types of subunits produced by the acid or alkaline dissociation of the 7S species of the mouse nerve growth factor protein, two of them

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

display heterogeneity on electrophoresis. The α subunits contain three major and one minor component and the γ subunits three components. Since each subunit component has a molecular weight around 30,000, they are not all derived from a single 7S nerve growth factor protein. Both individual α and γ subunits are separable by ion exchange chromatography and remain stable after separation. Recombination of any one α and any one γ subunit with the biologically active β subunit produces a 7S species with the physicochemical properties and increased biological activity characteristic of the original preparation of the nerve growth factor protein. The 7S species produced from a common γ but differing α subunits show small differences in electrophoretic mobility which reflect the mobility differences between the α subunits. When a common α but differing γ subunits are used in the recombination the resultant 7S species have the same mobility. Dissociation at either acid or alkaline pH of the 7S species formed from individual α and γ subunits produces only those subunits used in the initial recombination. These results suggest that the nerve growth factor protein preparation contains multiple forms of the 7S species all with the same general subunit composition but differing in the types of subunit they contain. In agreement with this hypothesis, the α subunit composition of the nerve growth factor protein is not constant across its migrating zone on electrophoresis or during elution from ion exchange resin but shows a continuous change from species containing predominantly the α subunits of higher mobility. The finding that the γ subunit composition also varies in the same way suggests that the multiple

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

forms of the nerve growth factor protein may be in equilibrium.

The NGF protein and its subunits have been successfully iodinated with ^{125}I at low levels. These materials will be used to study both the subunit equilibria and, by autoradiographic techniques, the interaction of NGF with the receptive neurons. The nature of the differences between individual subunits of one class, the numbers of polypeptide chains within a subunit and the amino acid sequences and the identity of the non-protein moiety of NGF are all currently under investigation.

The high molecular weight 7S form of NGF exhibits another activity besides its growth promoting properties. It has a similar level of esterase activity on BAEE as does trypsin but a much lower level of proteolytic activity. These enzymatic activities are a property of the γ subunits, the β subunits, which are the only 7S NGF subunits to elicit a response in the bioassay, and the α subunits being inactive. The specific activities of the γ^1, γ^2 and γ^3 subunits are identical and higher than that of 7S NGF. The hydrolysis of BAEE by the γ subunits proceeds in a linear manner in contrast to 7S NGF which shows an initial lag phase. Thus both the biological activity of the β subunits and the enzymatic activity of the γ subunits are altered by their interactions with the other two NGF subunits. Future work will by attempting to find specific inhibition of the γ enzyme activity examine the relationship between enzymatic and biological activity.

Chemical Ontogeny and Polymorphisms of Nervous System Proteins. The major fraction of the protein of the nervous system is imbedded in a matrix

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

with lipid and carbohydrate and because of this inaccessibility has been relatively little studied. An investigation has therefore been made of the conditions required for the solubilization of water insoluble nervous tissue proteins and for their subsequent fractionation. The complete scheme for handling both water soluble and insoluble proteins may be briefly outlined.

Homogenization of whole brain in isotonic sucrose solution releases 15% of the total protein in water-soluble form. Subsequent exposure of the insoluble subcellular pellet to hypotonic buffer solution and ultrasonic treatment liberates an additional 20% of the total protein. Although several major differences were observed, these two aqueous extracts are similar in protein composition, being resolved into 50 distinct bands. Efficient dispersion of the insoluble residue remaining after osmotic shock, in buffer containing the non-ionic detergent Triton X-100 solubilizes an additional 34% of the total protein. By electrophoresis in the presence of Triton, this extract was fractionated into 10-15 protein bands, 3 or 4 of which were specific to this fraction and were profoundly influenced by the ratio of protein to detergent in the system. Electrophoresis of such extracts in the presence of sodium lauryl sulfate improves the resolution greatly, giving 20 clearly defined protein bands. Extraction of the Triton X-100-insoluble residue with the anionic detergent sodium lauryl sulfate results in solubilization of 21-31% of the total brain protein. By electrophoresis in the presence of sodium lauryl sulfate, this extract was fractionated into 40 distinct protein bands, the resolution of which are dependent upon the detergent concentration in

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

the electrophoresis system. The four sequential protein extracts obtained under appropriate conditions with isotonic sucrose, hypotonic buffer, Triton X-100 and sodium lauryl sulfate represent protein pools of finite size and together account for at least 90% of the total brain protein. By electrophoretic analysis the majority of the proteins in the water soluble pools are similar but those in the Triton and sodium lauryl sulfate extracts differ not only from the water soluble proteins but from each other.

These procedures have been employed for investigation of the quantitative and qualitative changes in brain proteins during the ontogenetic development of the mouse. The most pronounced accumulation of proteins occurs duringthe initial 2 or 3 weeks after birth, with the greatest increases taking place among the detergent-soluble proteins, which account for a steadily increasing proportion of the total protein, indicating an increased synthesis of membranous and structural proteins. After the first few postnatal weeks, the changes in the protein content of brain are much less pronounced, occurring at a greatly diminished rate. During this time, the brain weight approaches a constant adult level, as do the quantities of protein solubilized by aqueous, Triton X-100, and 0.1% sodium lauryl sulfate solutions. However, the 1% sodium lauryl sulfate extract continues to increase markedly in protein content throughout the life span of the mouse, reaching a value of 20% of the total protein by 2 years after birth. These quantitative changes are accompanied by concurrent changes in the electrophoretic composition of each fraction, indicated by the appearance or disappearance of specific proteins or

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis. (continued)

shifts in their relative proportions, which occur at specific, although not identical, stages of development. The periods during which these changes in brain protein occurred correlate well with the known temporal sequence of brain histological and electrophysiological maturation and of behavioral development, implying a possible interrelationship of these phenomena.

E. M. Shooter - Molecular neurobiology, genetic control of hemoglobin synthesis (continued)

Personnel

Senior (on sabbatical leave)

E. Glassman, Professor of Genetics
University of North Carolina - Molecular neurobiology (1968-69)

Sarane T. Bowen, Associate Professor of
Biology, San Francisco State - Genetic, developmental and structural aspects of hemoglobins

Postdoctoral

M. Baker - Nerve growth factor - physical properties and relationship of enzymatic and biological activities

H. R. Fisk - Nerve growth factor - molecular composition and equilibria, interaction with responsive neurons

C. Louis - Physical and chemical properties of membrane proteins and their interaction with lipid

T. Waehneldt - Micro methods for characterizing membrane proteins, sub-cellular and developmental aspect with neurological mutants

Predoctoral

K. Borden - Specific acidic proteins in nerve cells

S. Morris - Turnover of brain proteins, axonal flow and synaptic membrane production

R. Perez - Developmental aspects of brain proteins

A. R. Piltch - Nerve Growth Factor - Chemical characterization of differences between subunits and sequence work

A. Smith - Nerve Growth Factor - Molecular composition and interactions, metabolic effects

P. Goodall (Medical student) - Physical chemistry of Hb-A and its subunits and of mutant hemoglobins.

C U R R I C U L U M V I T A E

Eric M. Shooter

Born: April 18, 1924, Mansfield, Nottingham, England.

Married: Elaine Arnold (Born Dec. 22, 1924) Newhall, Burton-on-Trent.
Children: Annette (Born Nov. 18, 1956) Redhill, Surrey, England.

Permanent Address: Department of Genetics
Stanford University School of Medicine
Palo Alto, California

1942-45	Natural Sciences Tripos (Part II in Chemistry) University of Cambridge
1942	Exhibitioner of Gonville & Caius College, Cambridge
1943	Minor Scholar of same.
1945	B.A. (Cantab.)
1945-46 and 1946-49	Research under Professor Sir Eric Rideal in the Department of Colloid Science, Cambridge and the Davy Faraday Laboratory of the Royal Institution, London (Proteins of the ground nut).
1949	M.A. (Cantab.)
1950	Ph.D. (Cantab.)
1948-50	Postdoctoral Fellowship with Dr. J. W. Williams, Department of Chemistry, University of Wisconsin, Madison, and partly with Dr. D. E. Green, Enzyme Institute, University of Wisconsin. (Enzymes of the electron transport system).
1950-53	Senior Scientist in charge of Biochemistry, Brewing Industry Research Foundation, Nutfield (Proteins and enzymes of barley and other brewing materials).
1953-63	Lecturer in Biochemistry, Department of Biochemistry, University College, London with Professor Ernest Baldwin. (Molecular biology of normal and abnormal haemoglobins; protein-ion interactions of ribonuclease).
1961-62	U.S.P.H.S. International Fellow, Department of Biochemistry, Stanford University School of Medicine, with Professor R. L. Baldwin (Replication of DNA).
1963-68	Associate Professor of Genetics, Stanford University School of Medicine (Molecular Neurobiology). Head of Neurobiology Group, Lt. Joseph P. Kennedy, Jr. Laboratories for Molecular Medicine.
1964	D.Sc. University of London (awarded for distinguished work in the field of Biochemistry).
1968-present	Professor of Genetics, Stanford University School of Medicine.

J. Lederberg and E. C. Levinthal - Relevance of Current Program of Instrumentation Research Laboratory to Problems of Molecular Biology.

The activities of the Instrumentation Research Laboratory are largely supported by NASA in connection with their interest in an automated biological laboratory for the exploration of the planets. This has related to other biological interests in this department and elsewhere in the medical school in the areas of mass spectrometry, cell separation and classification, and the general question of computor managed instrumentation.

A. Mass Spectrometry

The connection to mass spectrometry is not too surprising since the same reasons that make mass spectrometry a powerful tool for biological explorations carried out remotely on a planet forty million miles distant from the earth make it a most sensitive and selective method for analyzing organic molecules important in problems of molecular biology germane to modern medicine. It is a potentially powerful method for investigating the unknown structure of unknown molecules in nerve tissue that form the engram which is part of the process of memory, as well as Martian surface material. Cytochemistry via mass spectrometry is still a distant and challenging goal. However, significant progress has been made during this period. In addition to building a base for further advances our efforts have yielded results of present value.

The problem and the program can be subdivided into serpaate areas of concern. First, there is the question of volatilizing the molecules of interest. This can be approached by means of chemical modification of the class of molecules under investigation. We have successfully applied this concept to problems of resolution and identification of optical isomers of amino acids using the combination of a gas chromatograph and mass spectrometer. Molecules of biological interest are characterized by asymmetries at one or more of the carbon atoms incorporated in the molecule. From the viewpoint of the exobiologist this statement is the basis of the well-known significance of

optical activity as a clue for the recognition of life. The preparation of volatile diastereoisomers, their separation by gas chromatograph and their further identification by a mass spectrometer provides a method important to both terrestrial and extraterrestrial biology. The general concept is elucidated in the papers enumerated in the bibliography . Initially the technique was applied to the high sensitivity scanning of amino acids for optical activity while other work has demonsted the general applicability of the method. A second but much more difficult method, which has the advantage that it is more directly applicable to the goal of cytochemistry, could conceivably utilize electron, heavy particle or photon beam energy. We have investigated the use of both heavy particles and laser photon beams. In the case of the former, we had useful but discouraging results. In the latter case our present results show some real promise.

A second subdivision of this effort addresses itself to acquiring basic data on the mass spectra of a large number of monomers of biological interest. A report covering the work on amino acids has already been published. Work on nucleotides and related products is in progress.

Computer control of mass spectrometers describes the third categorization of the program. Full advantage of a mass spectrometer as a biological tool can only be achieved when the instrument is under computer control. The typical processes of calibration and optimization of operating parameters are sufficiently complex that they require automation if it is desired to analyze a large number of spectra in a short period of time. We have designed and built a very effective system for computer operation of both a time-of-flight and quadrupole mass spectrometer. This system has been and is continuing to be used for biological research purposes. The system is being elaborated to

to provide a more sophisticated level of control. In addition, work is underway to achieve some degree of control of high resolution mass spectrometers than use magnetic filters.

Fourthly, there is the question of data retrieval for subsequent computer analysis. The ultimate goal of a two dimensional micro-description of the distribution of molecules in a tissue by means of their mass spectra presents formidable problems of data handling. The bandwidth requirements are at least an order of magnitude greater than color video. High bandwidth data retrieval and buffer storage are required. We have not directly confronted this problem. General advances in the technology of high speed solid state switching devices and information storage methods lend some hope for the future. We have, however, made some modest steps. We have implemented an interface system for a direct data link from a high resolution mass spectrometer to an IBM 360/50 computer.

The fifth and last subdivision of the program really represents most clearly the ultimate goal for which the previously described efforts provide the technological tools. Ultimately the spectra acquired must be analyzed. This requires computer manipulation of chemical hypotheses. This poses a problem in both artificial intelligence and organic chemistry. A great deal of progress has been made in this direction. Most of this research is supported by the Advanced Research Projects Agency of the Office of the Secretary of Defense, Contract No. SD-183, and carried out in collaboration with Professor E. Feigenbaum of the Department of Computer Science.

J. Lederberg and E. C. Levinthal - Relevance of Current Program of Instrumentation Research Laboratory to Problems of Molecular Biology.

While the high resolution mass spectrometer is perhaps the most capable single instrument for organic structural analysis, the sheer volume of its signal output poses formidable problems of data reduction and data analysis. These problems would be multiplied by the number of samples that would need to be processed by the micro-scanning mass spectrometer which is our ultimate goal. At one level, the problem is the identification of mass numbers with compositional formulas. However, no mass spectral signal is free of noise and great effort must then be spent to obtain an accurate determination of mass to ultimate resolution. Much of this effort is wasted when it does not answer a concrete question, i.e., which of a set of possible compositions is indicated by a given measurement. Even for all compositions, the corresponding mass numbers are not continuously distributed; they are rather the discrete set of numbers calculated from linear integral sums of nuclidic masses, and represented in the tables.

The tabulations and calculation programs (see bibliography) are the first step in a control program for the mass spectrometer. As soon as the peak is identified within a given mass neighborhood, the competing possibilities should be computed, then weighted in accordance with any other available information. This allows the experimental problem to be restated as a choice among competing possibilities, and the signal information need be accumulated only long enough to lead to a meaningful choice among them.

In solving a structure, the chemist hypothesizes a series of trial structures, then matches them with the data (in this case a mass spectrum, but this can be generalized to any data set) and accepts or rejects his trial

solutions, usually part by part, in a structure. Much of this tedious effort could be emulated or at least assisted by the computer in a program we call "mechanized induction".

For this purpose, a language has been devised for representing chemical structures in easily computable form; "Dendral '64". The development of this language required the filling of a surprising gap; the systematic application of simple topological principles to the field of chemical graphs - that is, a symbolic representation of organic molecules. Existing notations were found to be quite defective as the chemist already knows too well from his difficulties with nomenclature (other organizations like Chemical Abstract Service also recognize the problem and are working on it, but tend to compromise topological rigor for the benefit of established traditions in notation). At any rate, with the help of some theorems on canonical forms of trees, and on Hamilton circuits of planar maps (for acyclic and cyclic structures respectively), a complete system has been worked out. This gives an algorithm by which the computer can generate an exact list of all isomers in a given composition.

By itself this is a futile approach to any but the simplest problems, since the number of possible isomers quickly exceeds the range of a fast computer. Heuristic and symbiotic methods are therefore called for whereby the computer emulates or cooperates in the use of human problem solving techniques in searching wisely selected parts of the space of possible solutions. Professor Edward Feigenbaum and Dr. Richard Watson of the Computer Science Department participated in a cooperative effort to program efficient displays of structural ideas for conversational interaction with the computer. This

J. Lederberg and E. C. Levinthal - Relevance of Current Program of Instrumentation Research Laboratory to Problems of Molecular Biology.

was a step to evaluate the chemists problem solving heuristics incorporating them in the machine program. The effort was limited at present by the existing computer facilities (a PDP-1 machine) with inadequate displays.

A complete description of the current status is given in an article by J. Lederberg and E. A. Feigenbaum. The following is the abstract of that paper.

"A computer program for formulating hypotheses in the area of organic chemistry is described from two standpoints: artificial intelligence and organic chemictry. The Dendral Algorithm for uniquely representing and ordering chemical structures defines the hypothesis-space; but heuristic search through the space is necessary because of its size. Both the algorithm and the heuristics are described explicitly but without reference to the LISP code in which these mechanisms are programmed. Within the program some use has been made of man-machine interaction, pattern recognition, learning, and tree-pruning heuristics as well as chemical heuristics which allow the program to focus its attention on a subproblem and to rank the hypotheses in order of plausibility. The current performance of the program is illustrated with selected examples of actual computer output showing both its algorithmic and heuristic aspects. In addition some of the more important planned modifications are discussed."

J. Lederberg and E. C. Levinthal - Relevance of Current Program of Instrumentation Research Laboratory to Problems of Molecular Biology.

B. Cell Separation

The work on cell separation has proceeded to the point where there are now three instruments capable of carrying out worhwhile biological experiments. The first of these is a volumetric cell separator, the second is a high speed fluorescent cell separator and the third is a cell separator and identifier developed by the Watson Labs of IBM under the direction of Dr. L. A. Kamentsky. While the ultimate goal of the Instrumentation Laboratory is the possible application of these principles to the biological exploration of the planets, our present efforts are directed toward current biological and medical problems. This has made it possible for us to gain the active participation of other scientists here at the Medical School. These have included Professor H. S. Kaplan, Executive Head of the Radiology and Radiotherapy; Dr. I. L. Weissman, Department of Radiology; Professor George Hahn, Department of Radiology; Professor Leonard Herzenberg, Department of Genetics; Professor R. Kallman, Department of Radiology.

Initially, we have established criteria for cell types which could be used as assay material while developing the prototype instruments leading to uses of instruments designed for cell separation in experiments that are biologically significant.

These criteria are:

1) Cells of at least two major size categories.

2) Cells which are not adherent to one another and which can be maintained in single cell suspension.

3) Cells which can be reproducibly obtained and maintained with good viability.

4) Cells which can be divided into subclasses according to biological function, subclasses which might be related to cell volume or cell fluorescence. For these reasons, we chose to work on thoracic duct populations of lymphocytes, which are:

1) Divided into at least 3 size subclasses.

2) Naturally obtained in single cell suspension and do not form clumps of 2 cells or greater.

3) Can be maintained in a viable state with appropriate media for as long as 24 hours.

4) Have at least two significant biological criteria for size-subclass separation:

a) DNA synthesis: Large and medium thoracic duct lymphocytes are in a continuous cycle of cell division. At least 80% of their cell cycle is devoted to DNA synthesis, and therefore short-term incubation of thoracic duct lymphocyte population with radioactive DNA precursors will selectively label large and medium cells. Small lymphocytes only rarely divide.

b) Small lymphocytes are "immunologically competent cells", whereas populations of large and medium lymphocytes are not. We have developed rapid and easy methods for testing immunological competence.

c) Both of the above assays detect these biological functions in viable cells only. Therefore, since we have a system (described previously) in which only viable cells fluoresce, we can test the fluorescent cell separator's action also.

The following are a few of the medically and biologically significant experiments we hope to perform:

1) Test purified cell populations for cell-cell interactions in the induction, development, and execution of the immune response (in conjunction with Professor L. Herzenberg and Dr. I. Weissman).
   a) Isolation of antigen-processing cells by phagocytosis of fluorogenic substrate.
   b) Isolation of immunologically competent cells by size.
   c) Isolation of antibody-forming cells by size following adherence of large antigenic particles.
2) Isolation of cells in mitosis by size criteria in order to establish cell lines in vitro which are synchronously cycling. These will be useful to determine the actual cellular and molecular events which determine the differential sensitivity of cells to radiation and certain drugs as a function of their place in the cell cycle (in conjunction with Prof. George Hahn, Department of Radiology).
3) Detection and isolation of cancer cells in the blood stream (metastases) in order to determine the type of cancer therapy most appropriate for the patient (in conjunction with Prof. H. S. Kaplan, Executive Head of Radiology and Radiotherapy Prof. R. Kallman, Radiology Department).

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4) Isolation and testing of cell types in Hodgkins Disease, (a cancer of the lymph node system) in order to determine:

   a) The malignant cell, its biochemistry and radiosensitivity

   b) The cell type (in these patients) responsible for widespread immunological deficiency, and how this deficiency is maintained in conjunction with Professor H. S. Kaplan.

5) Isolation and testing of the cell type in the bone marrow theoretically designated as the "stem" cell, which is responsible for redevelopment of normal blood cell types following irradiation In conjunction with Dr. Weissman.

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