DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

BETHESDA 14, MD.

NATIONAL INSTITUTES OF HEALTH
OLiver 6-4000

December 8, 1960

In reply refer
to: E-3978

Dr. Joshua Lederberg
Department of Genetics
Stanford University
School of Medicine
Stanford, California

Dear Doctor Lederberg:

The enclosed application for a grant in aid of research will be considered soon by the Allergy and Immunology Study Section and the appropriate National Advisory Council.

Before they do so the members of our reviewing groups would like to have the benefit of your advice, so that they may be more certain about the need for the proposed work, its feasibility, its promise and significance (among other things), and about the competence and past accomplishments of those who are to do the work. If you are willing to offer an opinion or comments about the proposal, please do so simply in a letter addressed to me. In your response, please identify the project. For your convenience a return envelope which requires no postage is enclosed. A response by December 21, 1960 would be appreciated.

The application is a privileged communication, intended to be seen by reviewers only. Please be assured that any comments you offer will be similarly regarded, and that they will be made known only to the members of our advisory groups. The applicant's papers may be returned when you finish with them, or they may be destroyed, but please do not leave them where others may see them.

May I thank you in advance, on behalf of our advisers?

Sincerely yours,

F. W. Appel

Frederick W. Appel, Ph.D.
Executive Secretary
Allergy and Immunology Study Section
Division of Research Grants

Enc.

R'cd date 10-26-60	HEALTH, EDUCATION, AND WELFARE	No. E-3978
Council March/61	PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH	SS A&I (1)
Action	APPLICATION FOR RESEARCH GRANT (A PRIVILEGED COMMUNICATION)	Formerly (NRP: YES)

Application is hereby made for a grant in the amount and for the period stated, for the purpose of conducting research as described herein, in accord with the Agreement signed below.

A. AMOUNT REQUESTED: $ 16,416.00 (Same as total of itemized budget, page 2, item A8.)

B. PERIOD DATES: 5 Mo. 1 Day 1961 Year thru 4 Mo. 30 Day 1962 Year (Normally 12 months. See instructions.)

C. TITLE OF RESEARCH PROPOSAL (Do not exceed 53 typewriter spaces)

Inheritability of Antibody Production by Cultured Mammalian Cells

E. PRINCIPAL INVESTIGATOR:

Name Cohen, Edward P. Telephone No. DU-8-4511 Extension 601

Title Research Fellow Department or Service Medicine

Mailing address of Research office 4200 East Ninth Avenue
Denver 20, Colorado

Institution University of Colorado Medical Center Major Sub Division Allergy & Immunology

F. CO-PRINCIPAL INVESTIGATOR, if any. (Name and title only)

David W. Talmage, M.D.
Professor of Medicine and Microbiology

G. INSTITUTION SPONSORING REQUEST

Name University of Colorado Med. Center

Mail address 4200 East Ninth Avenue
Denver 20, Colorado

Name & title of official authorized to sign application on behalf of institution Robert J. Glaser, M.D.,
Vice-President for Medical Affairs and
Dean of the School of Medicine

H. NAME, TITLE, AND ADDRESS OF FINANCIAL OFFICER:

Mr. J. W. Johnston
Finance Officer
Univ. of Colo. Medical Center
Denver 20, Colorado (462616)

Manner in which check(s) should be drawn:
Finance Officer, The Univ. of Colo.
Medical Center

I. AGREEMENT: It is understood and agreed by the undersigned that any grant received as a result of this application is subject to the following terms: (1) Funds granted as a result of this request are to be expended for research or related purposes as governed by Public Health Service and grantee institution policies; (2) the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes of research; (3) all reports of original investigations supported by the grant shall acknowledge such support; (4) if any invention arises or is developed in the course of the work aided by the grant, the undersigned will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with grantee institution's own policies as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

J. PERSONAL SIGNATURES (in ink)

(1) Principal Investigator _____ _____
(Same as shown in "E" above) (date)

(2) Authorized official of applicant institution _____ _____
(Same as shown in "G" above) (date)

grm 2 Fo

Mail completed application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

A. BUDGET REQUEST (for the period shown on page 1) E3978

(1) 1. PERSONNEL List all positions, including Principal and Co-investigator. Amounts requested must not exceed proportion of total salary computed from % of time spent.	(2) % time on this project	(3) Requested from PHS (omit cents)
	%	$
Edward P. Cohen	90 %	
	%	
David W. Talmage	25 %	
	%	
Technician	100 %	3774
Perà	%	226
2. PERMANENT EQUIPMENT, itemize (see instructions)		
Ultrathin Sectioning Microtone and related items		$ 2000
Glassware		500
Inverted Microscope		1500
CO_2 Incubator		1000
Small autoclave		450
Double Distiller		400
Dissecting microscope		425
3. CONSUMABLE SUPPLIES, itemize (see instructions)		
		$
Isotopes		1000
Media		1500
Animals		1000
4. TRAVEL, itemize (see instructions)		
Travel to National Scientific meetings		$ 500
5. OTHER EXPENSE, itemize (see instructions)		
		$
6. TOTAL DIRECT COST REQUIREMENTS		$ 14,275
7. INDIRECT COST ALLOWANCE (The administrative official signing this application may request an amount for indirect costs. Review detailed instructions) (Round to low dollar)		$ 2,141
8. TOTAL BUDGET (Same as amount shown in item A, page 1)		$ 16,416

B. ESTIMATE OF SUPPORT REQUESTED FOR THE YEAR FOLLOWING THE BUDGET PERIOD ITEMIZED ABOVE. Applicants for 1-year grants should type the word "None" in space for TOTAL BUDGET shown below.

Personnel	Equipment	Supplies	Travel	Other	Total Direct Cost	Indirect Cost	TOTAL BUDGET
$ 4500	$ 2000	$ 5000	$ 750	$ -	$ 12250	$ 1838	$ 14088

C. ADDITIONAL YEARS OF SUPPORT, beyond the 2 years covered above, if requested. Please show the TOTAL AMOUNTS required for each such additional year, including indirect cost allowance.

3. $ 15,000 4. $ _____ 5. $ _____ 6. $ _____ 7. $ _____

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RESEARCH SUPPORT

List all other research support of the Principal Investigator, including that from own institution, and applications that are pending. Use continuation page if necessary. See instructions.

A. PUBLIC HEALTH SERVICE SUPPORT:

GRANT NUMBER	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
(1) Active or approved:			
EF-11,450	Antibody Production By Mammalian Cell Clones as USPHS Special Postdoctoral Fellow	$8100	September 1, 1960 to August 31, 1961
(2) Applications submitted, awaiting decision:			
	None		

B. ALL OTHER RESEARCH SUPPORT:

SOURCE	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
(1) Active or approved:			
	None		
(2) Applications submitted, awaiting decision:			

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BIOGRAPHICAL SKETCHES

Provide brief sketches for professional personnel already selected who are to be actively engaged in this project. The following format should be used for each person, with Co-investigator (if any) immediately following Principal Investigator, then other professional personnel, lettered consecutively.

A. Principal Investigator: Edward P. Cohen, Research Fellow
(Name and title)

1. Date of birth: Sept. 28, 1932 ; Place of birth: Caldwell, New Jersey ;
Present nationality: United States ; Male ☒; Female ☐.

2. Educational experience:

a. Degrees conferred (Begin with baccalaureate degree. Identify honorary degrees under field.):

DEGREE	INSTITUTION CONFERRING	FIELD(S)	YEAR
M.D.	Washington University (St. Louis)	Medicine	1957

b. Other research training and experience, especially that establishing research qualifications in area covered by this application:

WHERE	NATURE	YEAR
National Institutes of Health	Cell Biology	1958-60
National Institutes of Health	Electron Microscopy	1958-60
Washington University	Immunology	1955-57

3. Fields of present major scientific interest, in order of choice:
Cell Biology, Immunology

4. Supplemental information:

The period spent at the National Institutes of Health was as a Research Associate. This program provided formal courses in basic sciences.

B. David W. Talmage, M.D., Professor of Medicine and Microbiology
(Name and title)

1. Date of birth: Sept. 15, 1919 ; Place of birth: Kwangju, Korea ;
Present nationality: United States ; Male ☒; Female ☐.

2. Educational experience:

a. Degrees conferred (Begin with baccalaureate degree. Identify honorary degrees under field.):

DEGREE	INSTITUTION CONFERRING	FIELD(S)	YEAR
M.D.	Washington University (St. Louis)	Medicine	1944
B.S.	Davidson College		1941

b. Other research training and experience, especially that establishing research qualifications in area covered by this application:

WHERE	NATURE	YEAR
University of Pittsburgh	Ass't Research Prof. Pathology	1951-52
University of Chicago	Assoc. Prof. Medicine	1952-59
University of Colorado	Professor of Medicine & Microbiology	1959-

3. Fields of present major scientific interest, in order of choice:
Immunology, Allergy

4. Supplemental information:

RESEARCH PLAN AND SUPPORTING DATA

Details of the proposed plan and other necessary data should be typed (single spaced) in accord with the outline below, which is suggestive only. See instructions. Please continue numbering pages in sequence for entire application. Additional continuation sheets, if needed, may be requested from the Division of Research Grants.

1. **RESEARCH PLAN**
   - A. Specific Aims - Provide a concise statement of the aims of the work immediately proposed, and relate these to your long-term goal.
   - B. Method of Procedure - Give details of your research plan, including how results will be analyzed. For each specific aim mentioned in "A" show how your plan is expected to fulfill the aim.
   - C. Significance of this Research - Explain why the results of the proposed work may be important.
   - D. Facilities Available - Describe the general facilities at your disposal. List the major items of permanent equipment.
2. **SUPPORTING DATA**
   - A. Previous Work Done on this Project - Describe briefly any work you have done to date that is particularly pertinent.
   - B. Results Obtained by Others - Summarize important results to date obtained by others on this problem, citing publications. Select no more than five.
   - C. Personal Publications - Cite your most important publications on this or closely related work. List no more than five.
   - D. Justification of Budget - Defend itemized budget for the initial period (A, page 2) where you feel it necessary, and delineate reasoning basic to budget estimates for continuation years.

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1. RESEARCH PLAN

A. Specific Aims — Until recently, the investigation of the phenomenon of antibody synthesis had been indirect, based on levels of circulating antibody, correlated with cytomorphologic changes in specific cell types.

To explain the mechanism of antibody synthesis three theories have been formulated. These theories differ in: 1) the role ascribed to antigen in the variation of the antibody producing potential of cells and 2) the inheritability of this potential. Thus, antigen is thought to induce changes in antibody forming potential which depend on persistence of antigen and are therefore not inheritable (Haurowitz and Pauling); antigen is thought to produce inheritable changes in antibody producing cells (Burnet and Fenner, Schweet and Owen, Szilard); or inheritable changes are thought to occur in antibody producing cells in the absence of antigen (Talmage, Burnet and Lederberg).

Since the work of Nossal and Lederberg, and Cohn, Lennox and Attardi, the investigation of the mechanism of antibody synthesis has moved to a direct cellular level through the use of highly sensitive techniques of antibody detection and the isolation of single cells. Thus far, these two groups have found that a single differentiated cell synthesizes only one (Nossal) or in some cases two (Cohn) antibodies. Although both groups agreed that cells varied in their antibody producing potential, there is no conclusive evidence that antigen plays a role in inducing this variation.

It is now possible to isolate and cultivate single mammalian cells in a plating technique similar in principle to bacterial cloning. The resultant clones represent a genetically identical strain of cells. An additional untried experimental approach is the in vivo cultivation of a genetically uniform cell population with intraperitoneal millipore filter chambers. The purpose of this

project is to investigate the inheritability of antibody producing potential in cultured mammalian cells.

Method of Procedure - The proposed plan of investigation will utilize basic in vitro cell culture techniques and in vivo millipore filter chambers. Both of these experimental methods allow the serial propagation of cells. The spleen cells of animals hyperimmunized with one or multiple antigens may be so cultivated and a determination of the maintenance of the antibody producing potential made. The restimulation of these cells will be attempted, to the same or a different antigen.

A clone of cells may be investigated in a similar manner and a determination of the antibody producing potential of this group of cells with identical inheritance made.

If stimulation of cells with antigen in vitro does not lead to measurable antibody production (the usual experience of other investigators) the response of such cells to antigen after transfer to a millipore chamber or an isologous tolerant host will be tested.

The experiments outlined above require sensitive techniques for antibody detection. Initially the labeled antigen binding techniques of Farr and a modification recently developed in this laboratory will be tried. These can detect as little as 0.01 ug antibody N per ml. In order to test the antibody produced by small numbers of cells, a still more sensitive technique, that of phage neutralization, will be applied.

As an important corollary, the persistence of the antigen may be investigated utilizing a highly sensitive technique involving electron microscopy. The protein ferritin contains up to 23% of its weight as iron. This molecule gives a characteristic pattern in the electron microscope and a single molecule may thus be observed. The intracellular presence of the ferritin antigen (or its absence) may be seen in antibody producing cells.

Significance of this Research - The nature of the antibody response and in particular the inheritability of the phenomenon has important implications in our understanding of cell genetics (somatic cell differentiation) and the mechanisms of protein synthesis. Insight may be gained into a host of diseases presumed to be caused by an altered or misdirected antibody response.

Facilities Available - The Department of Allergy and Immunology has ample facilities to successfully carry out the outlined project. The Department of Anatomy has promised the use of a Norelco electron microscope. However, preparative facilities for ultrathin sections will have to be provided by us. There is an animal room and caretaker at our disposal. For cell culture, a complete laboratory has been assured.

(LEAVE BLANK)

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2. Supporting Data

A. Previous Work Done on this Project - During my two years as a research associate at the National Institutes of Health in Bethesda, I worked under the direct supervision of Dr. Harry Eagle and collaborated as junior investigator on two of his projects in mammalian cell physiology. In addition, I designed an operational, simplified mammalian cell chemostat and carried out experiments which described the characteristics of cell growth under these conditions. (See Publications, below). Working under the direction of Dr. David B. Scott and Dr. Marie U. Nylen at the National Institute of Dental Research during this same two year period, I completed a study of the degenerative changes occuring in cultured mammalian cells under conditions of amino acid deprivation as seen with the electron microscope, (see publications, below). As a medical student, I completed a thesis under the direction of Dr. Melvin Cohn entitled, "The Production of Isotopically Labeled Antibodies to Insulin".

B. Results Obtained by Others - The important work in this field which has direct implications to the project has been done by:

1. Nossal, who demonstrated that single cells synthesize one antibody, Brit. J. Exp. Path., 40:118, 1959.

2. Cohn, Lennox and Attardi, who demonstrated that single cells synthesize one or in some cases two antibodies, Bact. Rev., 23:213, 1959

Both of these groups used isolated cells in nongrowing conditions from previously hyperimmunized animals. The inheritability of antibody synthesis was not investigated.

3. Algire et al (Annals N.Y. Acad. Sci. 64:1009, 1957) demonstrated that intraperitoneal millipore filter chambers supported the growth of cells.

4. T. Mahinodan showed that cells in these chambers produce easily detectable amounts of antibody. (Personal communication).

5. Smith, Metzger et al (Proc. Soc. Exp. Biol. & Med., 104:336, 1960) have demonstrated that a single ferritin molecule may be seen in the electron microscope.

C. Personal Publications -

1. A Simplified Mammalian Cell Chemostat: Characteristics of Cell Growth in Continuous Culture, E. P. Cohen and H. Eagle. Federation Proceedings, 19:426, 1960 (Part II). Submitted, Journal of Experimental Medicine, October, 1960.

2. Microstructural Changes Induced in Mammalian Cell Cultures by Omission and Replacement of a Single Essential Amino Acid, E. P. Cohen, D. B. Scott, M.U. Nylen, Experimental Cell Research, In Press.