FEDERAL SECURITY AGENCY
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

APPLICATION FOR RESEARCH GRANT

(Supplemental)

(LEAVE BLANK)
E-72 (C&S)
M&I (3)

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
DIVISION OF RESEARCH GRANTS
Bethesda 14, Maryland

Rec. 2-18-52

Date June '52 Council

Application is hereby made for a grant in the amount of $ 4860 for the period

from September (Month) 1 (Day) 1952 (Year) through August (Month) 31 (Day) 1953 (Year)

inclusive *(not to exceed 1 year)* for the purpose of conducting a research project on the following subject:

(Give only brief descriptive title)

TITLE OF PROJECT: Genetics of Bacteria

NAME OF PRINCIPAL INVESTIGATOR	TITLE OF PRINCIPAL INVESTIGATOR
Joshua Lederberg	Associate Professor of Genetics

ADDRESS OF PRINCIPAL INVESTIGATOR
Department of Genetics
University of Wisconsin
Madison 6 Wisconsin

NAME OF FINANCIAL OFFICER TO WHOM CHECK SHOULD BE MAILED	TITLE OF FINANCIAL OFFICER
A. W. Peterson	Vice President, Business & Finance

ADDRESS OF FINANCIAL OFFICER
Bascom Hall
University of Wisconsin
Madison 6, Wisconsin

AGREEMENT

It is understood and agreed by the applicant: (1) That funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or 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 set forth in this application; (3) that all reports of original investigatons supported by any grant made as a result of this request shall acknowledge such support; (4) that if any patentable discoveries or inventions are made in the course of the work aided by any grant received as a result of this application, the applicant will, in consideration of such grant, refer to the Surgeon General of the Public Health Service, for determination, the question of whether such patentable discoveries or inventions shall be patented and the manner of obtaining and disposing of the proposed patents in order to protect the public interest.

NAME OF INSTITUTION: The University of Wisconsin

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION *(Please Type)*: [redacted]

PERSONAL SIGNATURE (This agreement must carry the actual signature of the official whose name appears on the line above.): (signed) A. W. PETERSON

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Form Approved
Budget Bureau No. 68-R249.4

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These dates to be the same as those given on page 1.

BUDGET PROPOSED FOR THE YEAR (Supplemental) through

NOTE: Under column entitled "OTHER" indicate funds presently available or anticipated from other sources including own institution.

	BUDGET	
	REQUESTED FROM P.H.S.	OTHER
PERSONNEL *(Itemize all positions by indicating type; names of professional personnel, if selected.)*		
Research Associate (Ph.D.) Dr. Thomas C. Nelsen is under consideration. Whether another candidate will be considered if Dr. Nelsen comes here under other auspices has not yet been decided. Full-time	$ 3600	(2) 7400
Graduate assistant (contribution to salary)	500	4800
PERMANENT EQUIPMENT *(Itemize)*		
None		
CONSUMABLE SUPPLIES *(Itemize)*		
Glassware and reagents (additional to primary request)	500	2000
TRAVEL *(State purpose)*		
Additional, for consultations with other workers (including scientific meetings in the U.S.)	100	
OTHER EXPENSE *(Itemize)*		
None		
SUBTOTAL	4500	
NOTE: The administrative official signing this application may add for overhead an amount not to exceed 8 percent of the operating costs, i.e. 8 percent of the subtotal. — OVERHEAD	360	
TOTAL FOR THE YEAR	$ 4860	

ESTIMATE OF FUTURE REQUIREMENTS

Estimate of future requirements applies to funds needed from the Public Health Service for the years subsequent to the period proposed at the top of this page. The blanks at the right provide space for requesting four additional years of support; any amounts entered should include "overhead" if such is to be requested. Do not leave any of these spaces blank—enter one of the following as applicable: The amount needed, "not applicable," "unknown" or "none". FOR FURTHER INFORMATION: See detailed instructions accompanying application forms.

1 ~~$ 9180~~ (RG) (This item is identical with that appearing on the primary request, and is simply restated)

2 ~~$ 9180~~ (RG)

3 ~~$ 9180~~ (RG)

4 Unknown

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PUBLIC HEALTH SERVICE SUPPORT: Show previous and current Public Health Service grants supporting this project:

GRANT NUMBER	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
PREVIOUS 1445-0-02 (E-72)	Genetics of Salmonella	$ 3780 3780 4320 11,880	July 1948 to August 1951
CURRENT E-72 (C3)	Genetics of Bacteria	4320	Sept. 1951 - August 1952

ALL OTHER SUPPORT: Excluding Public Health Service, but including that from own institution, list support from other sources for this project. If none, so indicate.

SOURCE	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
CURRENT			
AEC	Cytogenetic effects of radiations	$ 1000	3/51 - 2/52
Chemical Corps	Host-parasite relationships:lysogenic'y	6000	7/50 - 1/52
Institution	Genetics,of Bacteria	7000 *	7/51 - 6/52
* & Rockefeller	Immunogenetics of Bacteria	9000	9/51 - 8/53
PENDING			
AEC	Cytogenetic effects of radiations	2000	3/52 - 2/53
Chemical Corps	Lysogenicity; recombination in bacter.	8000(pror)	1/52 - 9/53
Institution	Genetics of Bacteria	6000 *	7/52 - 6/53

*Exclusive of investigator's salary. Incl. current equipment costs

RESEARCH PLAN AND SUPPORTING DATA

On the continuation pages provided give details of the proposed plan and other necessary data in accordance with the outline below. Number each page, the first continuation page being page 4. Additional continuation pages, if needed, may be requested from the Division of Research Grants. See detailed instructions before preparing this portion of the application.

1. RESEARCH PLAN
   - A. Specific Aims—Provide a concise statement of the aims of the proposed work.
   - B. Method of Procedure—Give details of your plan of attack.
   - 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. PREVIOUS WORK DONE ON THIS PROJECT
   Describe briefly any work you have done to date that is particularly pertinent.
3. PERSONAL PUBLICATIONS
   Cite your most important publications on this or closely related work. List no more than five.
4. RESULTS OBTAINED BY OTHERS
   Summarize pertinent results to date obtained by others on this problem, citing publications deemed pertinent. Select no more than five.
5. BIOGRAPHICAL SKETCHES
   Provide brief sketches for *All* professional personnel selected who are to be actively engaged in this project.

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Justification for continuation of support.

As has been pointed out in previous applications, this research program is developing in a relatively new field. It may be many years before new theoretical advances in bacterial genetics can be translated into specific improvements in medical practice. Continued support is requested simply in order to permit the continued development of our experimental program on a long-range basis. Some specific problems have been solved, at least partially, but as many others arise out of these solutions.

Justification for supplemental support.

Initial requests for research support from the Public Health Service were at the rather modest level of about $4000 per annum. This grant, applied primarily to work on Salmonella transduction, was sufficient to enable one graduate student to assist in this research, and initially, to help provide some of the durable apparatus needed. For some years, little substantial progress could be reported from this project, and there might have been some question whether even the modest investment would be recovered. During the last year, however, the picture has changed completely to give experimental findings of considerable general interest. Clues of similar import have developed in studies with E. coli. Further expansion of our work on these subjects appears to be desirable. The supplemental grant would permit the assignment of a more mature research worker (a post-doctoral associate) to collaborate on these problems, the details of which are presented in the appended progress report. Fortunately, this step would coincide with the provision of increased laboratory space by the University of Wisconsin so that facilities for an expanded staff will be available.

Research Plan.

This project is already in progress, and its objectives and approaches are most profitably discussed in terms of the findings already and currently investigated. These are summarized in the appended Progress Report for the current grant, E72-C(3).

A. Specific aims. These may be restated as a deeper understanding of the mechanisms by which specific traits of bacteria are regularly transmitted from generation to generation, and conversely the mechanisms of bacterial variation. So far, Escherichia coli and Salmonella typhimurium have been studied as type organisms for the occurrence of genetic recombination, and already two contrasting mechanisms have been found: sexual fusion and reduction in E. coli; another and new mechanism in Salmonella, transduction. Immediate objectives in this long-term study are given in part D of the Progress Report.

B. Method of procedure. Please see Progress Report, parts B and D.

C. Significance of research. Please see Progress Report, part C.
The mechanisms of bacterial variation and the characteristics we are investigating (drug-resistance; enzyme patterns; antigenic structure) are fundamental to clinical bacteriology, chemotherapy, vaccine preparation, and typical and diagnostic epidemiology.

D. Available facilities: a well equipped microbiological research laboratory with chemical benches, incubators, refrigerator and fume hood. The equipment includes several centrifuges (including multispeed and chemical), Coleman spectrophotometer, analytical balance, shaking and pipetting machines, ultraviolet radiation equipment, a circular Warburg manometric apparatus, de Fonbrune micromanipulator, lyophil apparatus, and a well appointed setup for critical microscopy (including darkfield and phase-contrast) and photomicrography. It should be pointed out, however, that this type of work requires, for the most part, little elaborate equipment compared to personnel needs. For special purposes, the facilities of the Enzyme Research Institute and of other university departments have been made available.

2. Previous work. This has been summarized in greater detail in previous applications and progress reports.

With E. coli K-12, Tatum and Lederberg, and Lederberg have investigated the mechanism of genetic recombination. This has been interpreted as a consequence of a sexual process, occurring at a frequency too small to be detectable by direct microscopic study (about 1 per million vegetative cells). Because of the low frequency, selective methods are required to detect the recombinants. For this purpose, nutritional mutants have been particularly useful, but alternative techniques using inhibitors are also available. The best evidence for the sexual basis of recombination has been the isolation of diploid hybrid cells which later segregate the parental markers. The interpretation of these cells as heterozygotes has been verified by single cell pedigree studies (Zelle and Lederberg).

In the earlier work, studies were confined to derivatives of strain K-12. Subsequently, a screening method was developed that has permitted about three percent of E. coli isolates from various sources to be characterized as interfertile.

Previous work with Salmonella has been confined to a nutritional survey.

3. Personal publications.

1947 Gene recombination in the bacterium Escherichia coli. J. Bact. 53: 673-684 (with E. L. Tatum)

1947 Gene recombination and linked segregation in E. coli. Genetics 32: 505-525

1950 The selection of genetic recombinations with bacterial growth inhibitors. J. Bact. 59: 211-215

1951 Single cell isolations of diploid heterozygous E. coli. J. Bact. 61: 351-355. (with M. R. Zelle)

1951 Prevalence of E. coli strains exhibiting genetic recombination. Science 114: 68-69

4. Results obtained by others. The basic experimental findings of this work have been confirmed in several laboratories. Additional contributions may also be cited as follows:

a. Confirmation that the agent of recombination in E. coli is not filtrable.
b. and c. Further linkage studies and application to drug-resistance
d. Kinetic studies on the frequency of recombination
e. Stimulation of recombination by pre-treatments with UV.

a. Davis, B.D. 1950 Nonfiltrability of the agents of genetic recombination in E. coli. J. Bact. 60: 507-508

b. Newcombe and Nyholm 1950 The inheritance of streptomycin resistance and dependence in crosses of E. coli. Genetics 35: 603-611

c. Cavalli, L.L. and Maccacaro, G.A. 1950 Chloramycetin resistance in E. coli, a case of quantitative inheritance in bacteria. Nature 166:991-2.

d. Nelson, T.C. 1951 Kinetics of genetic recombination in E. coli. Genetics 36: 162-175

e. Clark, J.B. et al. 1950 The stimulation of gene recombination in E. coli J. Bact. 59: 575-579

See also "Papers in microbial genetics: bacteria and bacterial viruses" selected by J. Lederberg. University of Wisconsin Press, Madison, 1951.

5. Biographical sketches.

Principal Investigator:

Lederberg, Joshua. b. Montclair, N.J., 1925. B.A. Columbia 1944. Medical School, Columbia 1944-46; Ph. D. (microbiology) Yale 1947. Fellow, Jane Coffin Childs Fund for Medical Research, 1945-46. University of Wisconsin: Asst. Professor of Genetics 1947-1950; Assoc. Prof. 1950———. University of California, Berkeley: Visiting Assoc. Prof. Bacteriology 1950.

Affiliated Personnel (Salaries from other non-institutional sources):

Lederberg, Esther M. (nee Zimmer) b. New York City, 1922. B.A. Hunter 1942 . M.A. Stanford 1946 . Ph. D. Wisconsin 1950. Scholar, N.Y.Bot.Gard. 1941-42. Res. Asst. (Carnegie) at N.I.H. 1942-43. Junior Biologist (P-1) N.I.H. 1943-44. P.H.S. Predoctoral Research Fellow, N.C.I., 1947-49. University of Wisconsin: University Fellow 1949-50; Project Associate 1950———.

Skaar, Palmer David. b. Mishawaka, Ind., 1923. B.A. Indiana 1947. Ph.D. Indiana 1952(pending). University of Wisconsin: Project Associate 1951——.

Prospective candidate for project-associateship on this program:

Nelson, Thomas Clifford. b. Columbus, O., 1925. B.S. Queens College, N.Y. 1946 M.A. 1946 Ph.D. 1951 Columbia. Lecturer in Biophysics, Columbia 1947-1949. Guggenheim Research Fellow, California Institute of Technology, 1950-51. Assistant Professor of Biology, Vanderbilt U., 1951--. Publications: see section 4d above.