FEDERAL SECURITY AGENCY
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

APPLICATION FOR RESEARCH GRANT
(Supplemental)

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

Application is hereby made for a grant in the amount of $4360 for the period from September 1, 1954, through August 31, 1955, inclusive (not to exceed 1 year), for the purpose of conducting research on the following subject:

Title of Project: Genetics of Bacteria

Name of Principal Investigator: Joshua Lederberg

Address of Principal Investigator: Department of Genetics
University of Wisconsin
Madison 6, Wisconsin

Title of Principal Investigator: Associate Professor of Genetics

Name of Financial Officer: A. V. Peterson

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 investigations 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.

The University of Wisconsin

Name and Title of Official Authorized to Sign for Institution

A. W. Peterson

Personal Signature

(signed) A. W. PETERSON

(Please Type)
### BUDGET PROPOSED FOR THE YEAR (Supplemental)

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

#### PERSONNEL

<table>
<thead>
<tr>
<th>Position</th>
<th>Requested From P.H.S.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Research Associate (Ph.D.)</td>
<td>$3600</td>
<td>(2) 7400</td>
</tr>
<tr>
<td>Dr. Thomas C. Nelson</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graduate assistant (salary)</td>
<td>500</td>
<td>4000</td>
</tr>
</tbody>
</table>

#### PERMANENT EQUIPMENT

None

#### CONSUMABLE SUPPLIES

<table>
<thead>
<tr>
<th>Item</th>
<th>Requested From P.H.S.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassware and reagents</td>
<td>300</td>
<td>2000</td>
</tr>
<tr>
<td>(additional to primary request)</td>
<td></td>
<td></td>
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</tbody>
</table>

#### TRAVEL

- **Additional for consultations with other workers**
  - **Including scientific meetings in the U.S.**
  - Requested From P.H.S.: $100

#### OTHER EXPENSE

None

#### ESTIMATE OF FUTURE REQUIREMENTS

- The administrative officer 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Requested From P.H.S.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUBTOTAL</td>
<td>4500</td>
<td></td>
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<tr>
<td>OVERHEAD</td>
<td>360</td>
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<tr>
<td>TOTAL FOR THE YEAR</td>
<td>$4860</td>
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**PHS-398**

Rev. 8-51
Public Health Service Support: Show previous and current Public Health Service grants supporting this project.

<table>
<thead>
<tr>
<th>GRANT NUMBER</th>
<th>TITLE OF PROJECT</th>
<th>AMOUNT</th>
<th>PERIOD OF SUPPORT</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREVIOUS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1445-0-02</td>
<td>Genetics of Salmonella</td>
<td>$3780</td>
<td>July 1948 to</td>
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<td>(E-72)</td>
<td></td>
<td>3780</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>4320</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11,880</td>
<td>August 1951</td>
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<tr>
<td>CURRENT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>E-72 (03)</td>
<td>Genetics of Bacteria</td>
<td>4320</td>
<td>Sept. 1951 -</td>
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<td></td>
<td></td>
<td></td>
<td>August 1952</td>
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</table>

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.

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>TITLE OF PROJECT</th>
<th>AMOUNT</th>
<th>PERIOD OF SUPPORT</th>
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<tr>
<td>CURRENT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AEC</td>
<td>Cytogenetic effects of radiations</td>
<td>$1000</td>
<td>3/52 - 2/52</td>
</tr>
<tr>
<td>Chemical Corps</td>
<td>Host-parasite relationships; lysogenicity</td>
<td>6000</td>
<td>7/50 - 1/52</td>
</tr>
<tr>
<td>Institution</td>
<td>Genetics of Bacteria</td>
<td>7000 *</td>
<td>7/51 - 6/52</td>
</tr>
<tr>
<td>&quot; &amp; Rockefeller</td>
<td>Immunogenetics of Bacteria</td>
<td>9000</td>
<td>9/51 - 8/53</td>
</tr>
<tr>
<td>PENDING</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEC</td>
<td>Cytogenetic effects of radiations</td>
<td>2000</td>
<td>3/52 - 2/53</td>
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<tr>
<td>Chemical Corps</td>
<td>Lysogenicity; recombination in bacteria</td>
<td>6000 (proj)</td>
<td>1/52 - 9/53</td>
</tr>
<tr>
<td>Institution</td>
<td>Genetics of Bacteria</td>
<td>6000 *</td>
<td>7/52 - 6/53</td>
</tr>
</tbody>
</table>

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.
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. Cases 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, E72-0(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 recombination 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 topical 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 ultracentrifuged and chemical), Coleman spectrophotometer, analytical balance, shaking and pipetting machines, ultraviolet radiation equipment, a circular Warburg spectrophotometer, de Vries microspectrophotometer, 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 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: 573-584 (with E. L. Tatum)

1947 Gene recombination and linked segregation in E. coli. Genetics 32: 505-523

1950 The selection of genetic recombinations with bacterial growth inhibitors. J. Bact. 60: 221-225


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.


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


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:


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


Prospective candidate for project-associateship on this program: