

THE LOUIS BLOCK FUND FOR BASIC RESEARCH AND ADVANCED STUDY

GRANT PROPOSAL

FROM: Leo Szilard
(Department or individual)

TITLE OF PROJECT: Study of the formation of adaptive enzymes in bacteria
and the formation of antibodies in mammals

PROPOSED PERIOD: October 12, 1957 - ^{Sept 30th} ~~October 12~~, 1958

PROPOSED TOTAL BUDGET: \$4500.

SUBMITTED TO THE BOARD FOR CONSIDERATION AT ITS MEETING

OF: October 12, 1957

SIGNATURE OF PROPOSER: _____

APPROVED BY CHAIRMAN: _____

I. General Statement

What funds are needed.

A) I have developed during the past six months, which I spent in Chicago, certain notions concerning the formation of adaptive enzymes in bacteria and the formation of antibodies in mammals. 2) The funds requested are needed to enable me to keep in close contact with a number of laboratories outside of Chicago (in addition to those with which I may keep in contact in Chicago, ~~such as experiments to be considered by~~ *such experiments as the laboratories of* Dr. David W. Talmage and Dr. Herbert Anker);

a) in order to develop further these notions, and

b) in order to make arrangements -- if possible -- for the performance of certain basic experiments that appear necessary, in the light of these notions.

The funds ~~requested~~ *will* requested ~~would~~ be spent for travelling expenses within the United States, for secretarial services both in Chicago and away from Chicago, and conceivably for the cost of reprints and excess pages of a paper that might be published in the Proceedings of the National Academy of Sciences.

Travelling expenses outside of the United States would be limited to a few weeks' stay in Cambridge, England, for the purpose of consultation with a group now assembled there by F.H.C. Crick (which includes from the United States: Hoagland, Dulbecco, Benzer, and Streisinger); and a few weeks' stay in Paris for *the purpose of* consultation with Jacques Monod (Institute Pasteur) and his group. These expenses will not include travel to and from Europe. (The latter expenses will be borne in the case of the first trip by the German Chemical Society, and in the case of a possible second trip, within the year, by the French Atomic Energy Commission.)

B) Possibilities of outside support.

The ~~results obtained~~ ^{conclusions by me} during the past six months are the outcome of my digesting material which I have assembled during a preceding period of roving among different laboratories in the United States. I undertook this roving at my own expense, as an experiment in preparation for a roving assignment that was expected to be set up by the National Science Foundation.

Such an assignment was proposed to the National Science Foundation by five institutions, which included the California Institute of Technology, the Rockefeller Institute, and the University of Chicago. These five institutions filed a grant application with the National Science Foundation. While the officers of the Foundation who handled this application were in favor of this grant, they were not able to get it passed by the Divisional Committee and therefore suggested to me that I withdraw the application. This I have done. They indicated to me their willingness to accomplish ^{essentially} the same objective in a different ^{way} form, and suggested that I file another grant application to cover, for a five-year period, part of my salary, travel expenses and secretarial services.

It would not seem advisable, ~~however,~~ to file such an application until some of the ~~results~~ ^{conclusions} based on the last six-months' work are published or available in publishable form. Part of these results will be incorporated in an invited paper that I shall present on October 7th at the Berlin meeting of the German Chemical Society. Upon my return from Europe I might then file a grant application with the National Science Foundation, if ~~3~~ upon consultation with the officers of the Foundation, ~~2~~ they favor taking such a step at that time.

II. Proposed budget

\$1500-\$2,000. out of the requested \$4500. are expected to be spent for secretarial services at the University of Chicago, and \$2500.-\$3,000. are expected to be spent for travel expenses and secretarial services away from Chicago. Of these travel expenses, \$300. are expected to be spent in Paris and Cambridge, England.

(New page)

III. Description of Project

The kinetics of the induction of the enzyme, β -galactosidase, has been studied for a number of years mainly at the laboratory of Jacques Monod (Institut Pasteur) in Paris, and more recently also by Aaron Novick and Milton Weiner in Chicago. Bacteria, like E. coli, produce this enzyme at a rate which depends on the inducer concentration. After much experimentation it became possible to study the kinetics of induction of this enzyme under conditions that permitted an interpretation of the observed results. It was then found that, if the inducer is added to a growing bacterial culture of E. coli, the bacteria will start producing the enzyme at the full rate, ~~(that depends on the inducer concentration)~~, ^(corresponding to) almost immediately upon the adding of the inducer. Accordingly it seemed that studying the kinetics of enzyme induction will not give us much insight into the mechanism of ~~how~~ ^{through which the} the rate of enzyme production is controlled ~~by~~ the bacterium.

Somewhat more penetrating ~~theoretical~~ considerations, which I made in the past six months, show however that the kinetics of enzyme induction may give us an insight into the mechanism involved, after all. The

precise meaning of this statement is as follows: *P* Dr. Aaron Novick and I have developed several years ago a new method for experimenting with growing cultures of bacteria. This method (based on a gadget we have called the Chemostat) permits us to slow the rate of protein synthesis, and thereby to ~~xxxx~~ reduce the growth rate of the bacteria up to a factor of ten. *I am able to reduce -* For any mechanism of enzyme induction one may propose ~~I can~~ *I can* ~~theoretically deduce~~ how the level of inducible enzyme maintained in the bacterium will change, if we first grow the bacteria fast and then lower the growth rate by, say, a factor of two. After such a change in the growth rate, the enzyme level will reach a new steady state in which the enzyme level may be -- depending on the mechanism assumed -- identical, higher, or lower than in the steady state at the fast growth rate. Moreover, if the two enzyme levels are identical in the two steady states, then I can theoretically deduce -- for each particular model ~~for the~~ *of* ~~mechanism~~ of enzyme induction -- whether ~~during~~ *during* the transition period, *which* ~~that follows~~ the lowering of the growth rate, the enzyme level remains unchanged, whether it first falls and then rises or whether it first rises and then falls.

The theoretical predictions ~~can~~ be checked by the appropriate experiment for which the Chemostat furnishes us with a convenient tool. Accordingly within a comparatively short period of time -- say, a year -- it might be possible to discover the "right" mechanism for enzyme induction, *by the process of elimination - noting the "wrong" ones.*

But even without performing any new experiments, we may postulate on the ground of general considerations that the "right" mechanism for enzyme induction must obey a principle which may be called "the principle of growth-rate independence of enzyme ratios." This principle need not hold strictly, but a bacterium using *↑* for the regulation of the level of its enzymes a mechanism that would grossly violate this principle would be at

a disadvantage in nature where it must grow ^{as fast as possible} under a great variety of nutritional conditions. On this basis alone, I was able to eliminate some of the mechanisms that one might be tempted to propose for enzyme induction.

Guided by this principle, I have been able to gain some insight into the likely mechanisms through which bacteria ^{might} ~~may~~ control the level of the different enzymes which they contain. These mechanisms appear to be able to account for the general characteristics of both the phenomenon of enzyme induction (which has been known for at least two decades), and the equally striking phenomenon of enzyme repression (which has been known for just over a year). Moreover, by allowing myself to be guided by the principle of growth-rate independence, I have been led to adopt a mechanism for enzyme induction which appears to be capable of accounting also for the phenomenon of antibody formation in mammals -- in response to the first injection of an antigen.

The study of adaptive enzyme formation in bacteria ~~can give us~~ cannot give us much guidance, however, for explaining the general characteristics of antibody formation in response to the second injection of the same antigen; i.e. the so-called anamnestic response. Neither can it give us much guidance for explaining the general characteristics of the phenomenon of immune tolerance (that may be evoked by injecting antigen into a new-born rabbit or by maintaining a certain level of the antigen, over a certain period of time, in the embryo).

It ~~appears possible to~~ ^{appears possible to} explain these phenomena also by assuming quite plausible ^{additional} mechanisms. There are, however, several ^{such} plausible mechanisms and their discussion must remain ~~highly~~ speculative in the absence of certain basic experiments on antibody formation which have been left undone. On the basis of the notions, to which I have been led through the study of

adaptive enzyme formation in bacteria, I believe ^{that} I am in a position to say ~~that~~ these experiments are. There appears to be reasonable hope that, if ~~it is possible to arrange for~~ these experiments ^{are} ~~to be~~ performed, we may gain insight into the ~~true~~ mechanisms ^{that account} ~~of antibody formation that~~ ~~are~~ responsible for the phenomena of anamnestic response and immune tolerance.

THE LOUIS BLOCK FUND FOR BASIC RESEARCH AND ADVANCED STUDY

GRANT PROPOSAL

FROM: LEO SZILARD
(Department ~~of~~ ^{or} individual)

TITLE OF PROJECT: Study of the formation
of adaptive enzymes
in Psychobrya and the formation
of antibodies in mammals
PROPOSED PERIOD: Oct 12 157 ~~June~~ Oct 2/57

PROPOSED TOTAL BUDGET: \$ 4500

SUBMITTED TO THE BOARD FOR CONSIDERATION AT ITS MEETING
OF: Oct 12 157

SIGNATURE OF PROPOSER: L. Szilard

APPROVED BY CHAIRMAN: _____

~~In accordance with the regulations of the Board, all requests to the Fund should reach the Board members not later than ten days prior to its meeting. In order to facilitate this, twenty copies of each proposal should be in the hands of the Dean of the Division not later than twelve days before the meeting of the Board. One copy must be signed by the person submitting the proposal.~~

*Author
Talmadge } This
 } must
 } meet
 } the or later*

I General Statement
What funds are needed.

I have developed during the past six months, ~~during~~ ^{spent} which I ~~stayed~~ in Chicago, certain notions concerning the formation of adaptive enzymes in bacteria and the formation of antibodies in mammals. ²⁾ The funds requested

are needed to enable me to keep in close contact with a number of laboratories outside of Chicago (~~as well as in Chicago~~) ^{in addition to those} with which I shall ^{my} keep in contact in Chicago.

- a) ^{to be considered by Dr. S. O. Schramm, W. D. H. Murray and Dr. Herbert Auer.} In order to develop further these notions, and
- b) ^{such as experiments} In order to make arrangements -- if possible -- for the performance of certain basic experiments that appear necessary, in the light of these notions.

The funds requested would be spent for travelling expenses within the United States, for secretarial services both in Chicago and away from Chicago, and conceivably for the cost of reprints and excess pages of a paper that might be published in the Proceedings of the National Academy of Sciences.

Travelling expenses outside of the United States would be limited to a few weeks' stay in Cambridge, England for the purpose of consultation with a group ^{now being} assembled there by F. H. C. Crick (which includes from the United States; Hoagland, Dulbecco, Benzer, and Streisinger); and a few weeks' stay in Paris for consultation with Jacques Monod (Institute Pasteur) and his group.

^{These expenses will not include travel to and from Europe} (Travel expense ~~from and to~~ ^{from and to} to and from Europe ^{and from Europe} will be borne in the case of the first trip ^{to Europe} by the German Chemical Society, and in the case of ^{the} ^{second} ^{trip} ^{by} the French Atomic Energy Commission.)

Possibilities of outside support.

The results obtained during the past six months are the outcome of ^{my} digesting ~~the~~ the material which I have assembled during a preceding period of roving among different laboratories in the United States. I undertook this roving at my own expense as an experiment in preparation for a roving assignment that was expected to be set up by the National Science Foundation.

Such an assignment was proposed to the National Science Foundation by five institutions, which included the California Institute of Technology, the Rockefeller Institute, and the University of Chicago. These five institutions filed a grant application with the National Science Foundation. While ~~some~~ ^{some} of the officers of the ~~National Science~~ ^{National Science} Foundation who handled this application were in favor of this grant, they were not able to get it passed by the ~~Committee of the Division~~ ^{Divisional}, and therefore suggested to me that I withdraw the application. This I have done. They indicated to me their ~~desire~~ ^{willingness} to accomplish the same objective in a different form, and suggested that I file another grant application which might ~~assume~~ ^{to cover} for a five-year period part of my salary, travel expenses and secretarial services. ~~I intend to file such an application on the basis of the results of my work during the past six months when this work is published. A preliminary report on this work will be presented at the meeting of the German Chemical Society in Berlin on October 7th. Details of my proposed activities for the period of October 1st of this year to October 1st of next year are described in the attached appendix.~~

It would not seem advisable, however, to file such an application until some of the results based on the last six-months' work are published or available in publishable form. Part of these results will be incorporated in an invited paper that I shall present on October 7th at the Berlin meeting of the German Chemical Society. Upon my return from Europe I might then file a grant application with the National Science Foundation if, upon consultation with the officers of the Foundation, they favor taking such a step at that time. ~~The attached appendix gives additional information concerning the nature of the work that is involved.~~

II. Proposed budget

out of
of the requested \$4500., \$1500-\$2,000. are ~~anticipated~~ ^{expected} to be ~~needed~~ ^{spent} for secretarial services at the University of Chicago, and \$2500-\$3,000. are expected to be spent for travel expenses and secretarial services away from Chicago. Of ~~the~~ ^{these} travel expenses, \$300. are expected to be spent in Paris and Cambridge, England.

III - Description of Project
 APPENDIX

The kinetics of the induction of the enzyme, β -galactosidase, has been studied for a number of years ^{mainly} at the laboratory of Jacques Monod (Institut Pasteur) in Paris, and more recently also by Aaron Novick and Milton Weiner in Chicago. Bacteria, like E. coli, produce this enzyme at a rate which depends on the inducer concentration. After much experimentation it became possible to study the kinetics of induction of this enzyme under conditions that permitted an interpretation of the observed results. It was then found that if the inducer is added to a growing bacterial culture, ^{of E. coli,} the bacteria will start producing the enzyme at ^{the full} ~~a~~ ^{rate that depends on the inducer concentration} certain rate almost immediately upon the adding of the inducer. ^{referred} Accordingly it ~~appeared~~ that studying the kinetics of enzyme induction will not give us much insight into the mechanism of how the rate of enzyme production is controlled in the bacterium.

Somewhat more penetrating theoretical considerations, which I made in the past six months, show however that the kinetics of enzyme induction may give us an insight into the mechanism involved after all. The precise meaning of this statement is as follows: Dr. Aaron Novick and I have developed several years ago a new method for experimenting with growing cultures of bacteria. This method, (based on a gadget we have called the Chemostat) permits us to slow the rate of protein synthesis, and thereby to reduce the growth rate of the bacteria up to a factor of ten. For any mechanism of enzyme induction one may propose I can theoretically deduce how the level of inducible enzyme maintained in the bacterium will change, if we first grow the bacteria fast and then lower the growth rate by, say, a factor of two. After such a change in the growth rate, the enzyme level will reach a new steady state ⁱⁿ which ^{depending on the mechanism assumed} may be identical, higher, or lower than the steady state at the fast

(in the new enzyme level)

growth rate. Moreover, if the two enzyme levels are identical in the steady states, then ~~in the case of~~ ^{for} each particular model for the mechanism of enzyme induction ^{that} I can theoretically deduce whether, during the transition period ~~following~~ the lowering of the growth rate, the enzyme level remains unchanged, whether it first falls and then rises or whether it first rises and then falls.

~~In the case of each proposed model~~ ^T the theoretical predictions can be checked by the appropriate experiment for which the Chemostat furnishes us with a convenient tool. Accordingly within a comparatively short period of time ^{in a year} it ~~should~~ ^{might} be possible to ~~eliminate the "wrong" mechanisms and to pick out the "right" mechanism for enzyme induction.~~ ^{discover}

But even without performing any new experiments, we may postulate on the ground of general considerations that the "right" mechanism for enzyme induction must obey a principle which may be called "the principle of growth rate independence of enzyme ratios." This principle need not be ~~obeyed~~ ^{held} strictly, but a bacterium using for the regulation of the level of its enzymes a mechanism that would grossly violate this principle would be at a disadvantage in nature where it must grow under a great variety of nutritional conditions. ^{On this basis alone I am able to eliminate}

^{note some of the mechanisms that one might be tempted to} Guided by this principle, I have been able to gain some insight into the likely mechanisms through which bacteria may control the level of the different enzymes which they contain. These mechanisms appear to be able to account for the general characteristics of both the phenomenon of enzyme induction (which has been known for at least two decades), and the equally striking phenomenon of enzyme repression (which has been known for just over a year). Moreover, ^{I have been led to adopt a} ~~the mechanisms for enzyme induction to which I have been led,~~ ^{which} by allowing myself to be guided by the principle of growth-rate independence, appear to be capable of accounting also for the phenomenon of antibody formation in mammals ^{in response to the} first injection of an antigen.

to prepare for enzyme induction.

The study of adaptive enzyme formation in bacteria ^{can} give us no ^{much} guidance ^{however} for explaining the ~~general~~ general characteristics of antibody formation in response to the second injection of the same antigen; i.e. the so-called anamnestic response. Neither ^{can it} do they give us ^{much} guidance for explaining the general characteristics of the phenomenon of immune tolerance (that may be evoked by injecting antigen into a new-born rabbit or ^{by} maintaining a certain level of the antigen, over a certain period of time, in the embryo).

It ~~appears possible to~~ ^{could} explain these phenomena ^{also by assuming} ~~also through~~ ^{quite} plausible mechanisms, ^{but} ~~but~~ there are ^{several} ~~several~~ plausible mechanisms and their discussion must remain high ^{ly} speculative in the absence of certain basic experiments ~~relating to the general characteristics of~~ antibody formation which have been left ~~undone~~. On the basis of the notions to which I have been led through ^I the study of adaptive enzyme formation in bacteria, I believe I am in a position to say what these experiments are. There appears to be reasonable hope that, if it is possible to arrange for these experiments to be performed, we ^{will} ~~will~~ gain insight into the ^{mechanism} ~~mechanism~~ of antibody formation ^{that is} ~~which is~~ responsible for the ~~general characteristics of~~ the phenomena of anamnestic response and immune tolerance.

I. GENERAL STATEMENT.

A. Brief statement telling what funds are needed for (research salaries, equipment and supplies, etc.)

B. Listing of personnel. (Ignore if proposal involves only purchase of equipment)

C. In order to be considered by the Board, it must be shown that outside support cannot be obtained. Please indicate what attempts have been made to secure outside support, and what related support exists. If related support has recently been terminated please indicate.

D. Term of project

II. PROPOSED BUDGET.

A. Salaries

B. Equipment and supplies

C. Other

D. Total

III. DESCRIPTION OF PROJECT.

In this supporting statement, include background, history, relevant bibliography, and qualifications of personnel. Attach additional pages if needed.

Mr. Zilov

DIVISION OF THE PHYSICAL SCIENCES
Office of the Dean

Important

August 28, 1957

To the Members of the Faculties
Division of the Physical Sciences

Gentlemen:

The Autumn Quarter meeting of the Board of the Louis Block Fund for Basic Research and Advanced Study is scheduled for Saturday, October 12, 1957. In accordance with the regulations of the Board, all requests to the Fund should reach the Board members not later than ten days prior to the meeting, namely, October 2, 1957. It is requested that twenty (20) copies of each proposal be sent to my office, Eckhart 111, not later than October 1, so there will be ample time to forward copies to all members of the Board before the ten day limit expires.

At the March 9th meeting of the Board a form to be used for submitting proposals was approved. Copies of this form, to be used as a guide for preparing proposals, may be obtained from the departmental chairmen or from Eckhart 111.

Sincerely,

Warren C. Johnson

Warren C. Johnson, Dean
Division of the Physical Sciences

SECTION'S I & III

OPERATING RULES AND REGULATIONS FOR THE ADMINISTRATION OF THE LOUIS BLOCK

FUND FOR BASIC RESEARCH AND ADVANCED STUDY

I - PROPOSALS

1. Content - All proposals for financial support or assistance from the Fund shall be in writing and shall set forth briefly (a) the amount sought, (b) the purpose for which the support or assistance is sought, (c) the manner in which the grant, if made, will be expended, (d) the period for which the grant is requested, (e) the amount and source of other support presently available and being used in connection with the proposal, and (f) such other information as is pertinent and will enable the Board to pass upon the merits of the proposal and to determine its eligibility for support under the terms and conditions governing the use of the Fund.

2. Submission - Each Dean shall solicit proposals from the faculty of his Division, and all proposals received shall be screened by a committee of the Division by which the proposals are received, which shall consist of the three (3) members of the Board of such Division. Each Dean shall submit to the Board at least ten (10) days before a meeting, all proposals received by him and, in addition, the recommendation of his screening committee. The Secretary of the Board shall acknowledge receipt of each proposal to the Dean submitting same, and shall make a record of the receipt and disposition of such proposal in a book maintained by him for that purpose.

3. Board Action - All proposals for financial support or assistance must be approved by the Board at a regular or special meeting of the Board. The Board shall review and take action upon each proposal submitted to it at the earliest practicable date. The Board may request additional information in any case where it is not prepared to act on the basis of the information submitted. The action taken by the Board with respect to each proposal, together with a brief statement of the basis for the action, shall be recorded in the minutes of the meeting at which the action was taken, and the Dean submitting the proposal shall be advised in writing by the Secretary of the Board of the action taken.

4. Notification - The appropriate Dean shall notify each faculty member submitting a proposal of the action taken by the Board at the meeting where it is reviewed by the Board. In the cases where proposals are approved the Chairman of the Board shall notify the Comptroller of the action taken and shall deposit a signed copy of said notification with the Secretary of the Board.

III - PRIORITY OF EXPENDITURES FROM FUND

Until changed by Board action, the following list of priorities shall govern expenditures from the Fund:

1. Salary increases for first-rate men on the staffs of the Divisions.

2. New appointments of outstanding individuals, if possible, on a sharing basis with ordinary University funds; it being the intention that in the case of such appointments the University will increasingly absorb the costs of the appointments.
3. Provision of funds for equipment to give support to young research men not supportable by outside funds.