To
Be
taken
to
ALTSERT
on Fri

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: April 1, 1958 to March 31, 1959

PROPOSED TOTAL BUDGET: $4500.

SUBMITTED TO THE BOARD FOR CONSIDERATION AT ITS MEETING OF: March 8, 1958

SIGNATURE OF PROPOSER: _____

APPROVED BY CHAIRMAN: _____

February 25, 1958

I. General Statement

A) What funds are needed.

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. 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 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 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 consultations with Jacques Monod (Institut 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 conclusions reached 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, 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 to file such an application until some of the 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 upon consultation with the officers of the Foundation 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.

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 (a rate determined by the inducer concentration) 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 through which the rate of enzyme production is controlled by the bacterium.

Somewhat more penetrating 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. I am able to deduce -- for any mechanism of enzyme induction one may propose -- 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 of enzyme induction -- whether during the transition period, which 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 prediction 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 eliminating 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 a mechanism for the regulation of the level of its enzymes 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 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 tentatively 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 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 explain these phenomena also, by assuming quite plausible additional mechanisms. There are, however, several mechanisms conceivable and the choice of one of them must remain 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 what these basic experiments are. There appears to be reasonable hope that, if these experiments are performed, we may gain insight into the mechanisms that account for the phenomena of anamnestic response and immune tolerance.

m

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 to September 30, 1958

PROPOSED TOTAL BUDGET: $4500.

SUBMITTED TO THE BOARD FOR CONSIDERATION AT ITS MEETING OF: October 12, 1957

SIGNATURE OF PROPOSER: _____

APPROVED BY CHAIRMAN: _____

September 30, 1957

I. General Statement

A) What funds are needed.

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. 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 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 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 consultations with Jacques Monod (Institut 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 conclusions reached 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, 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 to file such an application until some of the 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 upon consultation with the officers of the Foundation 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.

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 (a rate determined by the inducer concentration) 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 through which the rate of enzyme production is controlled by the bacterium.

Somewhat more penetrating 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. I am able to deduce -- for any mechanism of enzyme induction one may propose -- 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 of enzyme induction -- whether during the transition period, which 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 prediction 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 eliminating 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 a mechanism for the regulation of the level of its enzymes 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 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 tentatively 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 <u>first</u> injection of an antigen.

The study of adaptive enzyme formation in bacteria 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 explain these phenomena also, by assuming quite plausible additional mechanisms. There are, however, several mechanisms conceivable and the choice of one of them must remain 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 what these basic experiments are. There appears to be reasonable hope that, if these experiments are performed, we may gain insight into the mechanisms that account for the phenomena of anamnestic response and immune tolerance.

m