September 11, 1959

Dr. Alan T. Waterman
Director
National Science Foundation
1951 Constitution Avenue
Washington 25, D.C.

Dear Dr. Waterman:

Transmitted herewith are two (2) signed copies and eighteen (18) additional copies of an application for support of research entitled "A Quantitative Study of the Molecular Bases of General Biological Phenomena" submitted on behalf of Dr. Leo Szilard, Professor of Biophysics, The University of Chicago. The proposed period of research is five years and the estimated cost is $101,291.

The proposal is endorsed on behalf of the University by Professor William H. Zachariasen, Dean, Division of Physical Sciences, and by William B. Harrell, Vice President in Charge of Business Affairs.

Your favorable consideration of the enclosed application will be appreciated.

Very truly yours,

William B. Harrell
Vice President

Enclosures
(Proposal - 20)

cc: Dr. L. Szilard ✓ w/cy proposal
Dean W. H. Zachariasen

THE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

Proposal to the National Science Foundation

for a Research Grant

in Support of

"A QUANTITATIVE STUDY OF THE MOLECULAR BASES

OF GENERAL BIOLOGICAL PHENOMENA"

Prepared by

Dr. Leo Szilard
Principal Investigator

Proposed Starting Date: February 1, 1960

Proposed Duration: Five Years

Estimated Cost: $101,291

Approved: _____
William H. Zachariasen
Dean, Division of Physical Sciences

_____
William B. Harrell
Vice President - Business Affairs

1 September 1959

THE UNIVERSITY OF CHICAGO

It is proposed that the National Science Foundation award a five-year grant for "A Quantitative Study of the Molecular Bases of General Biological Phenomena" to be performed by Dr. Leo Szilard under a traveling professorship program. The proposed support would cover salary, travel and other expenses (as detailed in the budget attached), with the understanding that Dr. Szilard would be relieved (a) of any teaching duties at The University of Chicago, and (b) of any obligation to be in Chicago at a specific time, in order to have full freedom for pursuit of research interests at any institution of learning where work is in progress in which Dr. Szilard desires to participate. The following material is presented in support of the proposal:

(a) Research Plan and Supporting Data;

(b) Curriculum Vitae of Leo Szilard;

(c) Partial bibliography of Dr. Szilard listing some of his papers in physics and publications in biology; and

(d) Budget Estimates.

This proposal has also been presented to the National Institute of Health, Public Health Service.

Research Plan and Supporting Data

The purpose of the proposed study is to gain insight into certain general biological phenomena rather than to try to understand the functioning of specialized biological structures (such as, for instance, of the nerve fiber, of the muscle fiber, or of the specialized sense organs). I am particularly interested in those general biological phenomena where it may now be possible to gain insight on a molecular basis into quantitative relationships which can be checked against data obtained from available observations or experiments as yet to be made. The proposed work would take as its starting point preliminary theoretical studies which I carried out in the past three years.

At the University of Chicago, I am holding a Research Professorship. I have neither any teaching duties nor any fixed obligations to be in Chicago at certain fixed periods of time. This freedom has enabled me in the past three years to spend considerable time at various laboratories away from Chicago. It is my understanding that, under the grant here requested, I would have full freedom to move about wherever my research interests may take me. It is anticipated that I may spend nine months of the year away from my home, at various laboratories where work may be pursued in fields in which I am interested.

The problem of aging has interested me for a few years, but not until August of last year was I able to find a workable approach to this problem. At that time, I was able to formulate a theory which leads to quantitative predictions that are capable of being tested by experiments. (Proc. Natl. Academy of Sci., 45: 30-45, 1959). Attached is a copy of a one-page article by John Lear, which appeared in England, in The New Scientist, and which, even though it is not entirely correct, gives an intelligible summary of the paper.

Below I list a number of other problems with which I dealt during the past three years in lectures or in extended conversations with interested colleagues at institutions other than The University of Chicago. Under the grant requested, I would endeavor to pursue these problems further.

(1)

1a) The molecular basis of induced enzyme formation in micro-organisms.

1b) The molecular basis of antibody formation in mammals.

(2)

The gene-protein problem.

(3)

The question whether in general the competent form of the gene has an inherent stability which has not hitherto been taken into consideration in discussing the role of mutations in evolution.

I shall now proceed to indicate which of the above-listed problems I have given sufficient attention to be able to appraise the likelihood that they may yield significant results in the foreseeable future. With respect to each of these problems, I shall try to indicate, whenever possible, what particular approach I would propose to adopt.

AD (1)

In the past three years I have given some thought to the molecular basis of the formation of inducible enzymes in micro-organisms, and I have ended up by postulating a "model" which appears to be capable of resolving the paradoxes and which appears to be consistent with the experimental facts known so far. I assume that an enzyme molecule is formed on a specific enzyme forming site and remains at first attached to that site by a chemical bond. No further enzyme molecules can be produced at that site until this chemical bond is broken. This bond may be ultimately broken by a universal enzyme present in the cell.

The rate of production of a particule enzyme would be determined by the extent to which the attached enzyme molecule itself sets up a steric hindrance for the universal enzyme. Also, small molecules present in the cell may act as specific repressors for a particular enzyme because they combine reversibly with the attached enzyme molecule, and as long as they are so combined, they set up a steric hindrance for the universal enzyme.

In certain bacteria there are a great number of enzymes which catalyze biochemical steps along what we may call "stray" biochemical pathways. A number of normal metabolites are degraded along such pathways. A great majority of these enzymes appear to be inducible by the substrate of the enzyme. I assume that the rate of production of these inducible enzymes is normally repressed by small molecules which are capable specifically to combine with the enzyme and which, by specifically combining with the attached enzyme molecule, prevent the enzyme from leaving the specific enzyme forming site. The substrate of such an inducible enzyme may be assumed to be a chemical analogue of the repressor of the enzyme.

Accordingly, I assume that the substrate induces the enzymes in two ways: It induces the enzyme by competing with the repressor for the attached enzyme molecule and it induces the enzyme by competing with the precursors of the repressor for enzymes which lie on the biochemical pathway leading to the formation of the repressor. Under such conditions the substrate must of necessity enhance the formation of the enzyme provided that the cell itself does not abundantly convert the substrate into the repressor.

I am inclined to believe that the tools now at hand may permit us to determine to what extent the above described model of induced enzyme formation may be correct or to what extent it would have to be modified in order to become acceptable.

Further, I am inclined to believe that the mechanism of antibody formation in mammals could probably be elucidated fairly rapidly also if concrete models were formulated that were capable of being experimentally tested, particularly if one were to study the antibodies formed to artificial haptens rather than to the natural haptens of foreign proteins.

There are a number of models for antibody formation that one might be tempted to propose but most of these can be eliminated on the basis of the facts so far established. The number of the remaining possible models is not very large. If they are described sufficiently concretely then they could be scrutinized effectively in short order.

I shall illustrate what I have in mind by singling out one particular model. I have selected it as the first model to be scrutinized because it does not postulate any mechanisms involved in antibody formation which would go substantially beyond the mechanisms which may be presumed to be involved in the formation of inducible enzymes in micro-organisms.

Obviously we cannot at this time exclude the possibility that there may be involved in antibody formation mechanisms which go beyond those involved in the formation of inducible enzymes, nor even can we be certain at this time that there is more than a superficial resemblance between antibody formation in mammals and induced enzyme formation in micro-organisms.

I am inclined, however, tentatively to postulate as a basic tenet that antibody formation in mammals and inducible enzyme formation in micro-organisms have one important feature in common, which is as follows: Just as a repressor molecule can specifically combine with an enzyme molecule which is still attached to its specific enzyme forming site, so an antigen molecule can, in certain circumstances, specifically combine with an antibody molecule which is still attached to the specific antibody forming site. This basic tenet does by no means define a concrete model and it is possible to base two models, very different in nature, on the same tenet.

Which of these two models shall be given preference? The answer to this question depends upon whether we shall be forced to say that the so-called secondary, or anemnestic, response to the injection of an antigen requires us to assume that the specific antibody forming site is modified by the antigen. Because I am reluctant to assume that this is the case until I may be forced to do so, I shall discuss here of the two alternative models the one which gets by without such an assumption.

In discussing this "simple" model I shall limit myself to the formation of antibodies in the response to the injection of a soluble antigen into the rabbit. Further, I shall limit myself to an antigen which consists of a foreign protein (which is antigenic in the rabbit) to which there is coupled an artificial hapten

in rather low abundance. We shall have to distinguish here between antibody formed to the artificial hapten and antibody formed to the natural haptens of the foreign protein. By "antibody" we shall always mean combining antibody which need not be capable of precipitating the antigen.

In the following I shall list as an "intelligent guess" phenomena which we may expect to characterize the formation of such an antibody to the artificial hapten in the rabbit. It should be comparatively simple experimentally to verify whether or not these phenomena in fact exist. Assuming here that they do, we must then demand that our model for antibody formation account for all of them. The phenomena postulated are as follows:

1. To the first injection of the antigen the rabbit responds with a production of a certain amount of antibody to the artificial hapten.

2. If one permits a period of, say four weeks, to elapse, and if then the antigen is injected for a second time there is a greatly enhanced formation of antibody (secondary or anemnestic response) to the artificial hapten.

3. Following the second, third or fourth injection of the antigen there will be a production of antibody to the artificial hapten sustained long after the antigen has been presumably eliminated from the system.

4. If the antigen is injected into a new-born rabbit which cannot form antibodies, there will result an immune paralysis and for a period of time the rabbit will not form antibody to the artificial hapten in response to the injection of the antigen.

The "simple" model I propose is the following: There are in the cells of the lymphatic system present a very large number of genes responsible for the formation of enzymes that catalyze chemical reactions along stray biochemical pathways. Normally the rate of production of all these enzymes is repressed by small molecules present in the cell which specifically combine with the attached enzyme molecule and prevent its leaving the specific enzyme forming site. In the cells of the lymphatic system there are also present - according to the views here adopted - various mutant forms of the above mentioned genes and these mutant genes produce protein molecules - (which are related to the corresponding enzymes) - the antibodies. An antibody molecule resembles the related enzyme molecule sufficiently to be able to combine with the substrate of the enzyme but the antibody enzyme lacks the catalytic activity of the enzyme. We may assume that the repressor which hinders the formation of an enzyme hinders the formation of the related antibody also.

Let us now consider an antigen composed of a "foreign protein" to which

is coupled an artificial hapten which happens to be a chemical analogue of one of the numerous repressor molecules present in the cells of the lymphatic system. When such an antigen penetrates across the membrane of the lymphatic cell the artificial hapten will compete with the precursors of the repressor for those enzymes contained in the cytoplasm which lie on the biochemical pathway leading to the formation of the repressor. Accordingly the antigen will enhance the formation of antibodies which are capable of specifically combining with the artificial hapten.

Up to this point there is a close parallel maintained to the induction of an enzyme in bacteria by the substrate of the enzyme, but at this point the analogy ends. In bacteria the substrate which combines with an enzyme molecule from the repressor and thereby enhances the formation of the enzyme, whereas we assume that if the artificial hapten of the antigen combines with an antibody molecule attached to the antibody forming site it does not act as an inducer but rather as a repressor. The antigen molecule may set up a steric hindrance just as would the repressor molecule itself.

We shall assume here, for the sake of argument, that the antibody forming sites are contained within the nucleins of the lymphatic cell and are thus to some extent protected by the nuclear membrane from being too easily reached by the antigen. To the extent as such protection is incomplete and antigen molecules combine specifically with antibody molecules attached to their specific antibody forming sites, the antigen causes immune paralysis. Such immune paralysis may last for a few weeks after the free antigen has disappeared from the cell.

The simple model here presented explains the immunological phenomena, spelled out above, as follows:

1. When our antigen is first injected into a rabbit there are two things going on simultaneously. The artificial hapten of the antigen combines specifically with certain enzyme molecules contained in the cytoplasm of the cell and thereby enhances the formation of antibody which is capable of specifically combining with the artificial hapten. While this is going on a certain amount of antigen may penetrate across the nuclear membrane and a certain fraction of the antibody molecules which are attached to the specific antibody forming sites will specifically combine with the artificial hapten of the antigen and the antibody forming sites involved will then be prevented from producing antibody. Thus we have at the same time an enhancement of antibody formation accompanied by partial immune paralysis and therefore we obtain a subdued antibody response.

2. If we wait for a few weeks after the first injection, and inject the same antigen for the second time into the rabbit, the antibodies contained

within the nuclear membrane will protect the antibody forming site from being reached by the antigen. Accordingly on this occasion there will be no or little immune paralysis and we will obtain an almost unrestrained antibody response.

3. After repeated injections of the antigen there might be strong antibody production sustained long after the antigen disappears because the antibody may be expected specifically to combine with the corresponding repressor and thereby to reduce the concentration of the free repressor within the antibody forming cell.

4. When an antigen is injected into a new-born rabbit which is not yet capable of forming antibodies, the antigen may reach a high concentration within the nuclear membrane and according to the views here presented, immune paralysis will result. Such immune paralysis may be expected to disappear, several weeks after all free antigen has been eliminated from the antibody forming cell, because the antigen molecules combined with attached antibody molecules may dissociate off.

A concrete model of the kind given above leads to experiments which might in short order either lend strong support to the model or indicate that the model is wrong. In the case of the "simple" model outlined above strong support for the model might come from the following type of experiment. A rabbit may be repeatedly injected with natural foreign protein (to which there has not yet been coupled the artificial hapten.) Subsequently an antigen consisting of the foreign protein to which is coupled the artificial hapten, is injected in such a "pre-immunized" rabbit and the production of antibody which is capable of specifically combining with the artificial hapten is determined. If it is then found that much more such antibody to the artificial hapten is produced in the pre-immunized rabbit than in the non-pre-immunized control rabbit, this would lend strong support to the simple model given above. At least the experiment would then indicate that the secondary response is not based on a modification by the antigen of the specific antibody forming site.

Should experiments force us to say that in order to explain the secondary response we must assume that the antigen does modify the specific antibody forming site, then we may contemplate two alternatives:

(a) The antigen molecule which is combined with the antibody molecule that is attached to the antibody forming site may break the antibody forming site, and the antibody forming site might restitute with a smaller or greater deletion. The antibody forming site thus modified would in a number of cases give rise to more copious production of antibody and this would have to account for the secondary response. If, however, the antibody site

is broken too frequently, then it may be finally destroyed and this will have to account for immune tolerance.

(b) Alternatively we may assume that when the antigen combines with an antibody molecule attached to the antibody forming site, it may induce the antibody forming site in the same sense as certain physical agents may induce a lysogenic phage in bacteria. The antigen would thus cause a reproduction of the specific antibody forming site in the cytoplasm. Immune tolerance would be due to the combination of the antigen molecules with antibody molecules attached to the antibody forming sites contained in the cytoplasm.

The simple theory which I have described above is difficult to reconcile with a recent experiment of Jean Marie Dubert (CR Vol. 243-2, page 1939, 1956). Even though this experiment was performed on only four rabbits and perhaps not in a way that is most adequate for our purposes, it still serves as a warning to caution. I might be wrong in believing that the time has arrived for singling out concrete models and to scrutinize them one by one. Perhaps we ought to wait with such an approach until we are in a position to say for certain whether or not the antigen molecule lends any assistance to the antibody in the folding process and plays a part in determining its tertiary structure. I am rather convinced, however, that with a little more cooperation between different laboratories interested in this general problem and by putting a much greater stress on experiments which utilize artificial haptens it should be possible to elucidate the mechanism of antibody formation in the foreseeable future.

AD (2)

In the past few years I have taken some interest in the gene-protein problem and I was particularly interested in estimating the rate at which one enzyme forming site may be capable of producing the corresponding enzyme molecule. I circulated a memorandum on this subject to a small group of interested colleagues (including Alexander Rich and Sidney Brenner) for the purpose of obtaining their criticisms of the considerations presented. Because of the unresolved difficulty that has arisen in connection with the observed great differences in guaninadenine ratios in the DNA of the different families of micro-organisms, I have for the present reached a deadlock in this work and I am not able to appraise the chances of its making substantial progress in the foreseeable future.

Rather than to engage in speculation, it appears at this point more advisable to make use of the recent discovery that bacillus subtilis is capable of undergoing transformation. This opens up the possibility to study under very favorable conditions transformation where bacillus subtilis is the recipient

and unrelated families of bacteria, which have a different guanin-adenine ratio are the donors. Both transformation and abortive transformation may be studied under such circumstances. I assume that work along these lines will be pursued at a number of places, and I propose to follow such work closely and bide my time.

AD (3)

In discussing the phenomenon of mutation, one generally assumes that this phenomenon and its significance for evolution may be appraised on the basis of the following tenets:

"Each gene produces a specific protein, in many cases a protein which has specific enzymatic activity. Each gene can mutate to noncompetence, which means that its product, if any, is devoid of its specific enzymatic activity. Each gene can mutate to incompetence independently of any other gene. A gene which has mutated to incompetence can undergo a back mutation to competence. In a micro-organism, there will be in general no selection pressure operating against the mutant, incompetent, form of a gene if that gene produces an enzyme that is not necessary for maximal growth rate under the particular conditions of culture. In general, the mutations of a gene to incompetence are more frequent than the back mutations of the incompetent form of the gene to competence."

Because of the importance of these tenets for the theory of evolution, it would be of some value to test their validity. With the means which are now at hand, such a test should at present be possible.

Let us consider, for example, the enzyme system involved in a synthesis of the amino acid tryptophan. If the bacterium is grown in a chemostat in the presence of tryptophan, mutants which have lost their ability to synthesize tryptophan will not be at a selective disadvantage. In the presence of mutagens which increase the mutation rate by some large factor without too much killing, it should be possible to establish a mutational equilibrium in the chemostat. (We disregard here for the sake of argument the possibility that population changeovers may hinder the establishment of the mutational equilibrium.)

In the mutational equilibrium one may then determine what fraction of the bacterial population has retained the capability of growing in the absence of tryptophan. Since a large number of enzymes are involved in the synthesis of tryptophan, on the basis of the above quoted tenets one would expect the fraction of the population capable of growing in the absence of tryptophan to be very small.

There exists, however, a remote possibility that the competent form of gene might possess an inherent stability, so that in a mutational equilibrium in the absence of selection the fraction of the population containing the gene in its competent form is substantially larger than one would a priori assume.

In the past few years I have discussed this possibility with George Streisinger, Sidney Brenner, and Mat Meselson. It would be my intention to arrange for experiments to be performed along these lines at some suitable laboratory.

In order to indicate what kind of persons I would expect to take an interest in some of the problems which I would wish to pursue, I am presenting below a list of names. To the names of those with whom I had some communication on the subject named I have affixed a star.

Re: Induced enzyme formation in micro-organisms.

AARON NOVICK* - Institute of Molecular Biology, The University of Oregon

BORIS MAGASANIK* - Cambridge, Mass.

WERNER MAAS* - Department of Microbiology, New York University Medical School

MELVIN COHN* - Stanford University

SIDNEY BRENNER - MRC Unit for Molecular Biology, Cavendish Laboratory, Cambridge, England

BRUCE AMES* - NIH, Bethesda, Maryland

JACQUES MONOD* - Pasteur Institute, Paris

ARTHUR PARDEE* - The Virus Institute, University of California, Berkeley

FRANCOIS JACOB* - Pasteur Institute, Paris

Re: Antibody formation.

ED LENNOX* - Department of Microbiology, New York University Medical School

MELVIN COHN - Stanford University

HOWARD GREEN* - Department of Pathology, New York University Medical School

COLIN M. MAC LEOD - University of Philadelphia, Philadelphia

Re: Inherent stability of competent genes.

MAT MESELSON* - California Institute of Technology

Re: The gene-protein problem.

MAUREY FOX* - The Rockefeller Institute, New York

F. H. C. CRICK* - MRC Unit for Molecular Biology, Cavendish Laboratory, Cambridge, England.

ALEXANDER RICH* - MIT, Cambridge, Mass.

In the following I list a number of institutions where conditions might be favorable for the experimental pursuit of some of the problems in which I am interested:

The Department of Microbiology, New York University Medical School, New York City (Head of Department - Bernard Horecker)

The California Institute of Technology, Pasadena, Calif. (Heads of Divisions - George Beadle and Linus Pauling)

MRC UNIT for Molecular Biology, Cavendish Laboratory, Cambridge, England (Director - N. F. Mott)

The Pasteur Institute, Paris (Heads of Divisions - Jacques Monod and Andree Lwoff)

The Department of Pathology, New York University Medical School, New York City (Head of Department - Stetson)

Stanford University (Departments of Joshue Lederberg and Arthur Kornberg)

Curriculum Vitae of Leo Szilard

I was born in Budapest, Hungary, in 1898. I went through officers' school there during the first World War and studied engineering there.

In 1920 I left Hungary to continue my engineering studies in Berlin. However, the attraction of physics proved to be too great. Einstein, Planck, Von Laue, Schroedinger, Nernst, Haber, and Franck were at that time all assembled in Berlin and attended a journal club in physics which was also open to students. I switched to physics and obtained a Doctor's degree in physics at the University of Berlin under Von Laue in 1922. My thesis /(1) - see attached list of publications/ showed that the Second Law of Thermodynamics covers not only the mean values, as was up to then believed, but also determines the general form of the law that governs the fluctuations of the values.

Subsequently, I was a research worker in one of the Kaiser Wilhelm Institutes in Berlin and later joined the teaching staff of the University of Berlin (as Privatdozent) where I remained until 1933. Of the papers (1 - 4) published during this period, some are experimental, and some are theoretical. The last one (4) established the connection between entropy and information which forms part of present day information theory.

In 1933 I went to England. I considered at that time becoming a biologist, and A. V. Hill said that he would find a position for me as a demonstrator in physiology. It occurred to me, however, just then that a nuclear chain reaction might be possible if we could find an element that would emit neutrons when bombarded by neutrons. Artificial radioactivity was discovered a few months later by Joliot and seemed to provide an important new research tool in nuclear physics. This decided me to move into nuclear physics.

In the summer of 1934 I started work as a guest in St. Bartholomew's Hospital in London and this work resulted in the establishment of the Szilard-Chalmers Reaction (5) and the discovery that slow neutrons are emitted by beryllium if the beryllium is exposed to gamma rays of radium (6). In 1939, after the discovery of the fission of uranium, the use of these slow neutrons from beryllium made it possible to see that uranium emits neutrons when bombarded by neutrons; the fast neutrons emitted by uranium could be easily distinguished from the bombarding slow neutrons.

In 1935, after a visit to New York, where I spent a few months as research associate at New York University, I accepted a position at the Clarendon Laboratory, Oxford University. During this period I worked in the field of nuclear physics (8-11). In 1938 I came to America under arrangement with Oxford University, which permitted me to spend half my time in the United States. I was in the United States during the time the Munich Agreement was negotiated. After Munich I decided to stay in the United States on a full-time basis, and I resigned at Oxford.

In January 1939 I learned of the discovery of fission. It seemed important to find out at once if neutrons are emitted in that process, for in that case a chain reaction in uranium had to be regarded as a serious possibility. I, therefore, asked the permission of Columbia University to work there as a guest and perform an experiment in order to settle this question. This experiment (jointly performed with Walter Zinn) led to the discovery of the neutron emission of uranium, upon which the chain reaction is based (12, 13). The same discovery was made independently at about the same time by Fermi and Anderson, as well as by Joliot and his group.

In July, 1939, I recognized that a chain reaction might be set up in a system composed of graphite and uranium. Because of the serious consequences of this possibility, it seemed that this was a matter in which the government ought to take an interest. I, therefore, went to see Professor Einstein to enlist his help in approaching the government. After several consultations, in which E. P. Wigner and Edward Teller participated, Einstein wrote a letter to President Roosevelt; and in response to this letter, the President appointed a committee under the chairmanship of the Director of the National Bureau of Standards.

In February 1940 I described the chain-reacting uranium-graphite system in a paper I sent to the Physical Review (February, 1940). For reasons of secrecy, this paper was not published.

In November of 1940 a government contract was given to Columbia University for the development of the graphite-uranium system, and I became a member of Columbia University's National Defense Research Staff. Early in 1942 our group was moved to the University of Chicago; and on December 2, 1942, the chain reaction system was put into action.

Recently a patent was granted to the Atomic Energy Commission on the chain-reacting graphite-uranium system, jointly in the names of Enrico Fermi and myself.

In 1943 I became a naturalized citizen of the United States.

In October, 1946, I joined the staff of the University of Chicago as Professor of Biophysics in the Institute of Radiobiology and Biophysics. This institute never grew as originally intended, it had a succession of directors, and it was recently dissolved. I remained on the staff of the University of Chicago as Professor of Biophysics and was transferred to The Enrico Fermi Institute for Nuclear Studies.

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When in 1946 I was faced with the task of converting myself into a biologist, I teamed up with Dr. Aaron Novick, who was at that time a physical chemist. I had known him from his work in the uranium project. We both got our training in biology through summer courses, such as Delbrück's course in Cold Spring Harbor in bacterial viruses, and Van Niel's course in bacterial biochemistry at Pacific Grove. Novick and I worked as a team until the Institute of Radiobiology and Biophysics was dissolved.

When we started out, we tried to understand a striking phenomenon just then discovered by A. Kelner, who showed that bacteria killed by ultraviolet light can be reactivated by shining visible light at them (17). A detailed analysis of the phenomenon enabled us to interpret it in terms of a "poison" that is produced by ultraviolet light and is decomposed by visible light. This interpretation was at first controversial due to Dulbecco's work on light reactivation of ultraviolet killed bacterial viruses, but has in the meantime become widely accepted. My own interest in the subject waned when I could not convince myself that we were dealing with a phenomenon that serves a useful biological purpose in the life of the bacteria.

Next, we turned our attention to the study of bacterial viruses in the assumption that viruses may prove to be much simpler than bacteria. We obtained some interesting results (18) but decided to shift after a while to the study of bacteria.

The two phenomena in which we were initially interested were a) mutations and b) the formation of adaptive enzymes which promised to provide a tool for the study of protein synthesis. We were dissatisfied, however, with the methods that were available for the study of these phenomena. It seemed to us necessary to study bacterial populations in the growing condition in a stationary state, i.e. we thought we ought to use a continuous flow device We developed such a device, which we called a "Chemostat". In this particular device the rate of growth of the bacteria can be changed by changing the concentration of one of the growth factors of our choosing which we make the controlling growth factor.

We started out by using the "Chemostat" for the study of mutations and obtained quite unexpected results at the very outset. It turned out, for instance, that the rate at which certain mutations occur does not change when we change the rate at which the bacteria divide; we could vary the rate of growth within a wide range without changing the rate at which these mutations occurred. We found one family of compounds - purines - which may cause an about tenfold increase in the mutation rate of bacteria without any appreciable killing. And we also found antimutagens, which in very small contractions will fully counteract the effect of purine-type mutagens.

In a bacterial population maintained in the "Chemostat" there occur evolutionary changes (19) and one strain of bacteria is replaced by a mutant strain, which can grow faster in the conditions prevailing in the growth tube of the "Chemostat". We observed successive evolutionary steps of this sort in each experiment of sufficiently long duration and were able to analyze the phenomenon.

After the dissolution of the Institute of Radiobiology and Biophysics I did not maintain a laboratory. In the last few years my interests centered mainly on quantitative studies of general biological phenomena. Among these were the molecular bases of induced enzyme formation and of the formation of antibodies in mammals. My last published paper (25) attempts to give a quantitative theory of the process of aging which should be applicable to mammals.

PARTIAL BIBLIOGRAPHY OF LEO SZILARD
(with annotations)

A. Physics

(1) Zeitschrift für Physik, 1925, p. 753, 32. This paper extends the application of thermodynamics to the derivation of the laws of thermodynamical fluctuations. It was accepted as dissertation by the University of Berlin.

(2) Zeitschrift für Physik, 1925, p. 688, 33. - jointly with H. Mark. This paper reports experiments which revealed anomalous scattering of X-rays.

(3) Zeitschrift für Physik, 1926, p. 743, 35. - jointly with H. Mark. This paper reports experiments on polarizing X-rays by reflection on crystals.

(4) Zeitschrift für Physik, 1929, p. 840, 35. This paper evaluates the increase of entropy which is connected with operations of an intelligent being on a thermodynamical system if these operations are controlled by measurements of variables which are subject to thermodynamical fluctuations. This paper was accepted as Habilitationsschrift by the University of Berlin.

(5) "Chemical Separation of the Radioactive Element from its Bombarded Isotope in the Fermi Effect" -- jointly with Chalmers. Nature, p. 462, 134, 1934. This paper demonstrates a generally applicable process (Szilard-Chalmers reaction) for the concentration of a radioactive element produced by neutrons if the element has to be separated from a mass of a stable element with which it is chemically isotopic.

(6) "Detecting Neutrons Liberated from Beryllium by Gamma Rays," p. 494, 134, 1934. Nature.
This paper describes the discovery of radium-beryllium photo neutrons which, being of low energy, represent a useful tool in nuclear research. They were used later in the discovery and investigation of neutron emission of uranium on which a chain reaction is based.

(7) "Liberation of Neutrons from Beryllium by X-Rays" -- jointly with a group of six others, p. 880, 134, 1934. Nature. Using X-rays in place of gamma rays the threshold for the emission of photo neutrons from beryllium is determined by varying the voltage of an X-ray tube and is found to be somewhat above 1.5, and well below 2 m.e.v.

(8) "Radioactivity Induced by Neutrons" -- jointly with Chalmers, p. 98, 135, 1935. Nature. In this paper a neutron induced radioactive period of about 3-1/2 hours is reported in Indium which does not fit in with the explanations found for other radioactive periods. In a later paper it is shown that it is due to an excited Indium nucleus which is isomeric with stable indium nucleus 115.

(9) "Absorption of Residual Neutrons," p. , 136, 1935. Nature.
This paper reports the discovery of neutron resonances at low energies, gives an estimate of their energies, and states that the energies can be measured by observing the absorption of the residual neutrons in boron or lithium.

(10) "Gamma Rays Excited by Capture of Neutrons," p. 323, 139, 1937 -- jointly with Griffiths. Nature. This paper reports on the observation of gamma rays emitted by a number of odd elements which are strong neutron absorbers. The counts observed per absorbed neutron were found to be within 15 per cent identical for all these elements.

(11) "Radioactivity Induced by Nuclear Excitation" -- jointly with Goldhaber and Hill, p. 47, 55, 1939. Phys. Rev. In this paper the previously reported period in indium is investigated and the conclusion is reached that it is due to nuclear excitation of the stable indium isotope 115.

(12) "Instantaneous Emission of Fast Neutrons in the Interaction of slow Neutrons with Uranium" -- jointly with Zinn, p. 799, 55, 1939. Phys. Rev. In this paper the discovery of the neutron emission of uranium is reported. It is estimated that two neutrons are emitted per fission. The neutrons from uranium are made visible on an oscillograph screen. As primary neutrons, radium-beryllium photo neutrons were used which, because they are slow, can be easily distinguished from the fast neutrons emitted by uranium. This discovery which was made independently, and about the same time, by Fermi and Anderson, as well as Joliot and his co-workers, indicated the feasibility of a sustaining nuclear chain reaction.

(13) "Emission of Neutrons by Uranium" -- jointly with Zinn. p. 619, 56, 1939. Phys. Rev. Detailed report of above mentioned experiments, number of neutrons per fission measured as 2.3.

(14) "Neutron Production and Absorption in Uranium" -- jointly with Anderson and Fermi. p. 284, 56, 1939. Phys. Rev. This paper reports an investigation on the possibility or impossibility of a chain reaction in a uranium-water system. It is estimated that 1.5 neutrons are emitted for every thermal neutron which is absorbed by uranium.

Dr. Szilard's part in bringing about of the first nuclear chain reaction; in the design of the first nuclear reactor (atomic pile) are described, in the Official Report: Atomic Energy for Military Purposes, Henry D. Smythe, 1945, Princeton University Press, pages 34, 47, etc.

B. BIOLOGY

(17) A. Novick and Leo Szilard - EXPERIMENTS ON LIGHT-REACTIVATION OF ULTRA-VIOLET INACTIVATED BACTERIA. Proceedings of the NATIONAL ACADEMY OF SCIENCES. Vol. 35, No. 10, pp. 591-600.

(18) Aaron Novick and Leo Szilard - VIRUS STRAINS OF IDENTICAL PHENOTYPE BUT DIFFERENT GENOTYPE. Science, January 12, 1951, Vol. 113, No. 2924, pp. 34-35.

(19) Aaron Novick and Leo Szilard - EXPERIMENTS WITH THE CHEMOSTAT ON SPONTANEOUS MUTATIONS OF BACTERIA. Proceedings of the NATIONAL ACADEMY OF SCIENCES. Vol. 36, No. 12, pp. 706-719, December, 1950.

(20) Aaron Novick and Leo Szilard - DESCRIPTION OF THE CHEMOSTAT. Science, December 15, 1950. Vol. 112, No. 2920, pp. 715-716.

(21) Aaron Novick and Leo Szilard - EXPERIMENTS ON SPONTANEOUS AND CHEMICALLY INDUCED MUTATIONS OF BACTERIA GROWING IN THE CHEMOSTAT. Cold Spring Harbor Symposia on Quantitative Biology. Vol. XVI, 1951.

(22) Aaron Novick and Leo Szilard - ANTI-MUTAGENS. Nature, Vol. 170, p. 926, November 29, 1952.

(23) Aaron Novick and Leo Szilard - EXPERIMENTS WITH THE CHEMOSTAT ON THE RATES OF AMINO ACID SYNTHESIS IN BACTERIA. Dynamics of Growth Processes. Princeton University Press, pp. 21-32, 1954.

(24) Maurice S. Fox and Leo Szilard - A DEVICE FOR GROWING BACTERIAL POPULATIONS UNDER STEADY STATE CONDITIONS. Journal of General Physiology 39, p. 261-6, 1955.

(25) Leo Szilard - ON THE NATURE OF THE AGING PROCESS. Proc. Nat. Academy of Sciences, Vol. 45, pp. 30-45, 1959.

The first of these papers (#17) investigates a phenomenon discovered by A. Kelner after the war, who showed that bacteria "killed" by ultraviolet light can be revived by shining visible light on them. Experiments designed to analyze the phenomenon are described in this paper; they lead to the conclusion that the ultraviolet light produces a "poison" which can be inactivated by light and that this "poison", if present when, subsequent to irradiation, the bacteria divide, will cause both death and mutations.

The second paper (#18) describes the discovery that, when a bacterium is infected simultaneously with two related viruses which differ from each other both in genotype and phenotype, the virus population emerging from the bacterium contains a class of viruses which have the genotype of one and the phenotype of the other.

The papers #19 to #23 describe a new way of studying bacteria by maintaining a bacterial population in a stationary (exponentially growing) state indefinitely and controlling the growth rate by controlling the rate of supply of an essential growth factor. An apparatus is described in these papers which will conveniently accomplish this and which is designated as the Chemostat.

In studying mutations in bacteria or the formation of adaptive enzymes in bacteria inaccurate, and, therefore, misleading results are frequently obtained by studying bacterial cultures in flasks in which the number of bacteria increases exponentially, and the use of the Chemostat is frequently indicated in studies of this sort.

In the papers #19 to #22, the Chemostat is used in the study of mutations. It turns out that the rate at which mutations occur in a growing bacterial population under the conditions studied is not proportional to the rate at which cell division occurs, rather the mutation rate is constant per

unit time independent of the rate at which the culture is growing. There is found one group of compounds, all purine derivatives, of which caffein is one, which greatly increases the mutation rate without having an appreciable killing effect on the bacteria.

There is another group of compounds described in these papers, all of them ribosides of purines which in small quantities will completely counteract the action of the above mentioned purine type mutagens and also reduce the rate of spontaneous mutations.

In paper #23, the Chemostat is used to study the biosynthesis of amino acids in bacteria and the regulatory mechanisms which are involved in it. The bio-synthetic apparatus of the bacteria respond to amino acid concentrations in the medium, which are exceedingly low. For instance, a bacterium which can make arginine and will do so if there is no arginine in the medium, will stop making arginine if an arginine concentration of 10^{-9} ga/ce is maintained in the medium in the Chemostat. (Novick and Szilard - unpublished.)

One way of studying such regulatory mechanisms is based on the use of a mutant which is blocked in the synthesis of an amino acid--in our case Tryptophane--and which pours out into the medium a "precursor" of that amino acid. Paper #23 utilizes such a mutant. In the absence of Tryptophane in the medium, a precursor of Tryptophane is poured out by the mutant into the medium at a rate which is independent of the growth rate of the bacteria. In the presence of Tryptophane this "precursor" is <u>not</u> poured out by the bacteria. It is conceivable that this indicates a general phenomenon of regulation through a negative feed-back of the final product at one of the <u>early</u> steps of the metabolic pathway leading to Tryptophane.

In paper #24, there is described a device called a breeder. In this device bacteria may be grown in a continuous flow of nutrient. The flow of the nutrient is controlled by the turbidity of the bacterial culture and the growth is not limited by a growth factor, as is the case in the "Chemostat."

This device was developed in order to study mutations in bacteria under conditions of growth at the maximal rate, and such a study was carried out by Maurice S. Fox.

Paper #25 develops a theory of the basic process of aging. According to the theory, the elementary step in the process of aging consists in the random inactivation of whole chromosomes. The differences in the longevity of individuals are attributed to the difference of the number of recessive cell lethals they have inherited.

THE UNIVERSITY OF CHICAGO

Proposal for
"A Quantitative Study of the Molecular
Bases of General Biological Phenomena"
Professor Leo Szilard

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Proposed Budget Estimates - Five Years
Commencing 1 February 1960

As will be noted in the following budget estimates, Dr. Szilard's total annual salary rate is set at $15,000. According to University policy, Dr. Szilard is scheduled to be retired on October 1, 1963. From the suggested starting date of the proposed grant - 1 February 1960 - to date of retirement, it is proposed that the provision for salary and teachers annuity and social security benefits be divided between the National Science Foundation and The University of Chicago, as shown. Subsequent to 1 October 1963 there would be no participation in direct costs by the University; however, there would be some contribution for indirect costs (not shown in budget) in connection with administration of the proposed grant.

	Participation by		
	NSF	University	Total
A. First Year - 1 Feb. 1960 - 31 Jan. 1961			
1. Salary - Dr. L. Szilard	$11,000	$ 4,000	$15,000
2. Teachers Retirement and Social Security Benefits (6.8% of Item 1.)	748	272	1,020
3. Travel and Subsistence	2,000	--	2,000
4. Secretarial Services and Office Expense	3,000	--	3,000
5. Total Direct Costs	$16,748	$ 4,272	$21,020
6. Indirect Costs (15% of Item 5.)	2,513	--	2,513
7. Total Costs - First Year	$19,261	$ 4,272	$23,533
B. Second Year - 1 Feb. 1961 - 31 Jan. 1962			
(Same as First Year) Total Costs - Second Year	$19,261	$ 4,272	$23,533
C. Third Year - 1 Feb. 1962 - 31 Jan. 1963			
(Same as First and Second Years) Total Costs - Third Year	$19,261	$ 4,272	$23,533

Continued on following page.

	Participation by NSF	University	Total
D. Fourth Year - 1 Feb. 1963 - 31 Jan. 1964			
1. Salary - Dr. L. Szilard			
a. 1 Feb. 1963 - 30 Sept. 1963(a)	$ 7,334	$ 2,666	$10,000
b. 1 Oct. 1963 -31 Jan. 1964	5,000	--	5,000
Total Salary	$12,334	$ 2,666	$15,000
2. Teachers Retirement and Social Security Benefits - 1 Feb. 1963 - 30 Sept. 1963(a)	499	181	680
3. Travel and Subsistence	2,000	--	2,000
4. Secretarial Services, etc.	3,000	--	3,000
5. Total Direct Costs	$17,833	$ 2,847	$20,680
6. Indirect Costs (15% of Item 5.)	2,675	--	2,675
7. Total Costs - Fourth Year	$20,508	$ 2,847	$23,355

(a) 8 months - 1 Oct. 1963 Date of retirement for Dr. Szilard.

E. Fifth Year - 1 Feb. 1964 - 31 Jan. 1965			
1. Salary - Dr. L. Szilard	$15,000	$ --	$15,000
2. Travel and Subsistence	2,000	--	2,000
3. Secretarial Services, etc.	3,000	--	3,000
4. Total Direct Costs	$20,000	--	20,000
5. Indirect Costs (15% of Item 4.)	3,000	--	3,000
6. Total Costs - Fifth Year	$23,000	--	$23,000

SUMMARY

First Year	$19,261	$ 4,272	$23,533
Second Year	19,261	4,272	23,533
Third Year	19,261	4,272	23,533
Fourth Year	20,508	2,847	23,355
Fifth Year	23,000	--	23,000
Total	$101,291	$15,663	$116,954

1 September 1959