3/ NIH Similar to application 1959 After Jan 1959 before Dec 1960 for Jan - Dec 1960

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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.

Rx Comp. June 23 1959

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.

Sept 1959

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.

S-38

grants

03

THE UNIVERSITY OF CHICAGO
CHICAGO 37 · ILLINOIS
OFFICE OF THE VICE PRESIDENT · SPECIAL PROJECTS
5801 ELLIS AVENUE

4 October 1962

Dr. Leo Szilard
Hotel DuPont Plaza
1500 New Hampshire Avenue, N. W.
Washington 6, D. C.

Dear Dr. Szilard:

Transmitted herewith are your file copies of application material in connection with the renewal of Grant No. RG-6876-C2 submitted to Public Health Service 28 September 1962.

We will advise as soon as Public Health Service acknowledges that they have received the proposal.

Sincerely yours,

Irene E. Fagerstrom

Irene E. Fagerstrom
Assistant Vice President
(Special Projects)

Enclosures (3)
Proposal pp. 1, 2, 3
Notice of Research Project
Annual Invention Statement

P. S. We have just received notice from Public Health Service that your application was received 1 October 1962.

U. S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

APPLICATION FOR PREVIOUSLY RECOMMENDED YEAR OF RESEARCH GRANT SUPPORT
(A Privileged Communication)

APPLICANT: LEAVE BLANK EXCEPT FOR GRANT NUMBER		
TYPE	PROGRAM	GRANT NUMBER
		CH-00376-04

NOTICE OF RESEARCH PROJECT		INVENTION STATEMENT	
☒ YES	☐ NO	☒ YES	☐ NO

1. TITLE OF RESEARCH PROJECT (limit to 53 letters and spaces)

Quantitative Studies of General Biological Phenomena

2. NAME AND TITLE OF PRINCIPAL INVESTIGATOR OR PROJECT DIRECTOR (Last, first, middle) — DEGREE

Szilard, Leo — Ph.D.
Professor of Biophysics

MAILING ADDRESS

1155 E. 57th Street
Chicago 37, Illinois

TELEPHONE NUMBER

My 3-1601

3. AMOUNT REQUESTED (Should be same as Item 9, Page 2)

$ 27,670

4. DATES OF GRANT PERIOD

FROM 1 Jan 1963

THROUGH 31 Dec 1963

5. NAME AND TITLE, CO-INVESTIGATOR (if any) — DEGREE

None

6. ADDRESS WHERE RESEARCH IS BEING CONDUCTED

The University of Chicago and other similar research centers

7. NAME OF SCHOOL AND DEPARTMENT (or service if applicable)

Division of Physical Sciences
Enrico Fermi Institute for Nuclear Studies

8. NAME AND MAILING ADDRESS OF INSTITUTION SUBMITTING APPLICATION

The University of Chicago
5801 S. Ellis Avenue
Chicago 37, Illinois

9. NAME, TITLE AND MAILING ADDRESS OF FINANCIAL OFFICER TO WHOM CHECKS SHOULD BE MAILED

Mr. A. Wayne Gleeson
Bursar
The University of Chicago
5801 S. Ellis Avenue
Chicago 37, Illinois

10. TYPE OF INSTITUTION

☐ PUBLIC
☐ FEDERAL ☐ STATE ☐ LOCAL
☒ PRIVATE
☒ NON-PROFIT, IRS TAX EXEMPTION NO._____
☐ PROFIT

11. (LEAVE BLANK)

12. TERMS AND CONDITIONS

Any grant awarded on the basis of this application is subject to the following terms and conditions: (1) grant funds are to be expended solely for the research purposes described herein and in the award document and for related purposes; (2) the grant may be revoked in whole or in part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligation was made solely for the purposes authorized in Clause (1); (3) all reports of investigations supported by the grant shall acknowledge such support; and (4) the applicant and principal investigator (project director) agree that, in accordance with Department of Health, Education, and Welfare regulations, 45 C.F.R. Parts 6 and 8, any invention arising out of the activities assisted by the grant shall be promptly and fully reported to the Surgeon General. Whether patent protection on such an invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered in the public interest shall be determined either (a) by the Surgeon General or (b) where a separate formal institutional patent agreement has been reached by the Surgeon General with a nonprofit grantee institution, by such grantee institution in accordance with its own policies.

THE UNDERSIGNED ACCEPT AND AGREE TO THE ABOVE TERMS AND CONDITIONS

13. PRINCIPAL INVESTIGATOR OR PROJECT DIRECTOR (SAME AS ITEM 2)	SIGN IN INK ON ORIGINAL ONLY	DATE
14. OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION Dean Division of Physical	SIGN IN INK ON ORIGINAL ONLY. (Type name and title below signature) W. B. Harrell, Vice President for Special Projects	DATE 9/28/62

PHS-2590
REV. 2-62

FORM APPROVED
BUDGET BUREAU NO. 68-R-249

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 ⟶	GRANT NUMBER
SUMMARY PROGRESS REPORT	GM-06976-04
PRINCIPAL INVESTIGATOR (Name) Leo Szilard	SUMMARY OF ACCOMPLISHMENTS COVERING PERIOD
INSTITUTION The University of Chicago	FROM: 1 Sept 1961 — THROUGH: 17 Sept 1962
TITLE OF PROJECT (Repeat title shown in Item 1 on first page) Quantitative Studies of General Biological Phenomena	

In mammals, and also in the fruit fly, the somatic cells of the female contain two X chromosomes, while the somatic cells of the male contain only one. Accordingly, the cells of the female carry two homologous copies of each sex linked gene, whereas the cells of the male carry only one copy of each. This difference in "dosage" does not usually manifest itself in a phenotypic difference between the male and the female. Recent observations indicate that in the case of mammals, at some point of the embryonal development of the female, one of the two X chromosomes ceases to be functional in the somatic cells. This, on the face of it, could account for the fact that the double dosage of the sex linked genes in the female, as compared to the single dosage of the same genes in the male, does not lead to a difference in the phenotype. However, no such difference in phenotype exists in the fruit fly either, and yet I find that the phenomenon of "dosage compensation", which has been studied in the fruit fly by H. J. Muller, cannot be explained on the assumption that only one of the two X chromosomes is functional in the somatic cells of the female. In those circumstances it is necessary to look for another explanation for "dosage compensation" in the fruit fly. I propose to explain this phenomenon in the fruit fly by assuming that the relevant gene products in the fruit fly are under the control of repressors, in much the same way in which many enzymes are under the control of repressors in bacteria, and by further assuming that in the fruit fly the genes corresponding to the repressors (of those gene products which show "dosage compensation") are located on the X chromosome. These considerations are described in a paper, "The sex chromatin in mammalian cells, 'dosage compensation' in the fruit fly, and enzyme repression in bacteria," which is being circulated in preprint among those interested in this kind of problems.

Prepared for the Science Information Exchange.

Not for publication or publication reference.

U. S. Department of

HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Submit with completed Application to: Division of Research Grants, National Institutes of Health, Bethesda 14, Md.

TITLE OF PROJECT: Quantitative Studies of Memory

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS or PROJECT DIRECTORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project. Include day-month-year of birth of principal investigators.

Leo Szilard
Professor of Biophysics
Enrico Fermi Institute of Nuclear Studies
The University of Chicago, 5801 S. Ellis Ave., Chicago 37, Illinois

NAME AND ADDRESS OF APPLICANT INSTITUTION:

SUMMARY OF PROPOSED WORK — (200 words or less — Omit Confidential data.)

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

In my paper (Proc. Nat. Acad. Sci. 46, 293, 1960), I postulated a simple biochemical mechanism upon which the "memory" may be based which manifests itself in the secondary antibody response. Professor Herbert Anker suggested in a "Letter" to Nature that memory in the central nervous system might perhaps be based on the same biochemical mechanism. A set of postulates has been formulated which would have to hold if this particular memory mechanism accounts for the phenomena of memory that manifest themselves in the central nervous system. It is proposed to examine whether it may be possible to account on the basis of these postulates for remembering a "sequence" and whether a memory trace, which corresponds to a sequence, could be localized in individual neurons.

SIGNATURE OF PRINCIPAL INVESTIGATOR or PROJECT DIRECTOR: Division of Physical Sciences Leo Szilard

Identify the Professional School (medical, dental, public health, graduate, or other) with which this project should be identified:

SCHOOL

INVESTIGATOR — DO NOT USE THIS SPACE

PHS-166
REV. 12-61

FORM APPROVED
BUDGET BUREAU NO. 105-R002.

(5-38) D

September 9, 1962

(Preprint.)

The sex chromatin in mammalian cells, "dosage compensation" in the fruit fly, and enzyme repression in bacteria.* (1962)

By Leo Szilard

The Enrico Fermi Institute
For Nuclear Studies

The University of Chicago

In the past two years evidence has been rapidly accumulating in favor of the view that in the somatic cells of adult female mammals only one of the two X chromosomes is functional, and that the inactive X chromosome condenses to form the sex chromatin masses which are visible in certain types of somatic cells of the female. Apparently in the course of the embryonic development of the female, at some point or other, one of the X chromosomes becomes suppressed. Which of the two X chromosomes becomes suppressed seems to be determined by a random process; in one clone of somatic cells the X chromosome derived from the mother is suppressed, while in another clone, of the same type of cells, the X chromosome derived from the father is suppressed. Thus sex linked coat-colour mutants

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*Work performed under a Research Grant of the National Institutes of Health.

are variegated in the heterozygote, with alternating patches of normal and mutant colour, i.e., such heterozygotes are a mosaic of two different kinds of cell. This would then explain the appearance of the tortoiseshell cat, which carries "yellow" on one X chromosome and "black" on the other.

H. J. Mueller has noted and stressed the fact that a sex linked gene, which determines eye colour in the fruit fly, is normally present in two doses in the female but only in one dose in the male and that, nevertheless, the eye colour of the male and the eye colour of the female is the same.(1.) He postulated the existence of some special mechanism, "dosage compensation," which would operate specifically in the case of genes located on the X chromosome. This mechanism is responsible for the fact that even though the female carries two of the corresponding sex linked genes and the male carries only one, the concentration of a gene product in the somatic cell may be the same for the male and the female. He discussed, in particular, the case of a mutant sex linked gene, affecting eye colour, called "apricot." In the normal fruit fly the eye colour may be saturated, but in the case of the "apricot" mutant the eye colour of a male fruit fly can be shown to be roughly proportional to the number of "apricot" genes carried by the individual fly.

In Man the concentration of the product of a sex linked gene is the same in the male, which carries only one dose of it, as in the female which carries two doses. This has been shown in the case of the enzyme glucose -6- phosphate dehydrogenase.(2.) But it has been also shown(3.) that in the heterozygote female, which carries a competent and an incompetent allele of the gene, the cells in the blood behave as though they consisted of a mixture of two types of cells: one containing the normal amount of enzyme glucose-6-phosphate dehydrogenase, and the other devoid of this enzyme. Accordingly, in the case of Man, there would be no need to postulate the existence of a mechanism providing for "dosage compensation" for sex linked gene products; rather, the phenomena observed would be accounted for by the postulate that in the case of Man (and other mammals) only one X chromosome is active in the somatic cells of the adult female.

It would seem, however, that this postulate must not be extended to the fruit fly, because the behavior of the eye colour in "apricot" fruit flies cannot be explained by postulating that only one X chromosome is functional in the somatic cells of the female fly. If only one X chromosome were functional, then we would have to expect the same eye color in a female which carries one dose of "apricot" on each of its two X chromosomes, as well as one extra dose of "apricot", as in a male which carries one dose of

"apricot" on its single X chromosomes, as well as one extra dose of "apricot". This conclusion is inescapable, because if only one of the two X chromosomes is functional in the somatic cells of the female, then both the cells of the female and the cells of the male carry two functional doses of "apricot" and therefore their eye colours ought to be the same. This conclusion is not born out by the experiments; rather, H. J. Muller found that the eye colour of such a male is darker than the eye colour of such a female - in the ratio of 4 to 3.

In these circumstances one may ask whether Muller's observations of eye colour in the fruit fly require that we postulate ad hoc some special mechanism which would account for them, or whether "dosage compensation" in the fruit fly can be understood on the basis of known mechanisms.

It is known that in bacteria the production of many enzymes is under the control of certain corresponding repressors and that the gene for the repressor is different from the gene for the enzyme. On the basis of what is known, it is reasonable to assume that in bacteria the concentration of an enzyme, which is under the control of a repressor, is determined by the ratio in the cell of the number of genes for the enzyme and the number of genes for the corresponding repressor. We shall refer to this ratio as the "determining ratio." If we generalize from what we know about bacteria to the somatic cells of the

fruit fly and if we further postulate that the genes corresponding to the repressor of a sex linked enzyme, which shows "dosage compensation," is located on the X chromosome, then we can account for the observed facts concerning the eye colours in the fruit fly as follows:

A homozygote "apricot" female carries two "apricot" genes and two genes for the corresponding repressor, while an "apricot" male carries one "apricot" gene and one gene for the corresponding repressor. In either case the "determining ratio" is 1; hence such a female and such a male have the same eye colour.

An "apricot" male, which carries an extra dose for "apricot," carries one gene for the corresponding repressor; and the "determining ratio" therefore is 2. A homozygote "apricot" female, which carries an extra dose for "apricot" has three "apricot" genes and two genes for the corresponding repressor; the "determining ratio" is therefor 3 over 2. Accordingly, we should expect the ratio of the eye colour of the male and female to be 4 to 3, and this is the ratio actually observed by Muller.

Even though the phenomena of the suppression of the excess X chromosome might universally hold for mammalian cells, this does not necessarily mean that the mechanism of dosage compensation which, operates in the fruit fly, does not also operate in mammals. This mechanism might very well operate in somatic cells during the embryonic

development, up to the point when one of the two X chromosomes of the female becomes suppressed in these cells.

References:

1. Muller, H. J., Extension of the Preservation of Genetic Adaptation, Harvey Lecture, Feb. 18, 1948 (The Harvey Lectures, Series XLIII, 1947-48, 1950, Charles C. Thomas, Springfield, Ill.)

2. Grumbach, M. M., Marks, P. A., Morishima, A., Lancet, 1962, i, 1330.

3. Buetler, E., in "Metabolic Basis of Inherited Diseases" (edited by J. B. Stainbury) p. 1031, New York, 1960.

(17)

LEO SZILARD

Honors

Aug. 14, 1922 — Ph.D. cum laude, University of Berlin

Nov. 24, 1941 — Fellow, American Physical Society

Aug. 6, 1945 — Certificate of Appreciation, U.S. War Department, Manhattan District. Signed by Secretary Stimson.

Jan. 2, 1952 — Popular Mechanics' Half Century Hall of Fame (50 Americans, 1901-1951)

May 2, 1952 — Member, Society of the Sigma Xi

May 12, 1954 — Fellow, American Academy of Arts and Sciences

Feb. 27, 1960 — Humanist of the Year, American Humanist Association

March 1960 — Einstein Gold Medal of the Lewis & Rosa Strauss Memorial Fund

Apr. 27, 1960 — Living History Award, Research Institute of America (Quarter century 1935-1960)

May 18, 1960 — Atoms for Peace Award

Apr. 25, 1961 — Elected to membership, National Academy of Sciences

Oct. 8, 1961 — Honorary Doctor of Humane Letters, Brandeis University

1957 to 1963 — ~~American delegate to the~~ Participant in Pugwash meetings

1970 — Crater on far side of moon named SZILARD by International Astronomical Union

Oxford

c/o Liebowitz
420 Riverside Drive
New York City

January 13th, 1939

Professor F.A. Lindeman
Christ Church
Oxford, England

Dear Professor Lindeman:

Three months have now passed since, acting on an impulse, I cabled you that I am postponing my sailing for an indefinite period on account of the international situation, and that I should be grateful if my further absence could be considered as leave without pay. I sent you this cable after Czechoslovakia was forced to accept the Berchtesgaden demands, and it must have reached you at a moment when many people believed that there was an immediate danger of general war. You may have therefore thought that this assumed danger prompted me to postpone my sailing, and you may then have wondered why I did not return to Oxford after the Munich agreement, that is if you gave any thought to my continued absence at a time when urgent political and defense questions must have been claiming most of your thoughts.

It seems to me that the Munich agreement created, or at the very least demonstrated, a state of international relations which now threatens Europe and in the long run will threaten the whole civilized world. This cannot fail to claim the attention of all of us, and, if the situation is to be improved, the active cooperation of many of us. I greatly envy those of my colleagues at Oxford who in these circumstances are able to give their full

attention to the work which has been carried on at the Clarendon Laboratory and who are able to do so without offending their sense of proportion. To my great sorrow I am apparently quite incapable of following their example.

Since my collaboration in the work, for which you were good enough to win the support of Imperial Chemical Industries, would be of little value unless Itgave the work my full attention, it seems best in the circumstances that I should not embark upon it. This being so, I do not feel that I am entitled to keep any payments which Imperial Chemical Industries may have made to me under the new agreement, i.e. after January 1st of last year. I should be grateful if you could perhaps communicate on this subject with Dr. Slade and tell him how very thankful I am for the help I had from Imperial Chemical Industries in the past, and how very much I regret that the deterioration of the international situation which occured while I was abroad, makes it impossible for me to collaborate in the work which Dr. Slade kindly consented to support. If Dr. Slade wishes me to refund payments made to me after January 1st of last year, I shall be very glad to do so. In this case Dr. Slade will have to let me know the amount which actually has been paid to my account, and also to what account and under what heading he wishes me to transfer this amount.

It seems to me that those who wish to continue to dedicate their work to the advancement of science would be well advised to move to America where they may hape for another ten or 15 years of undisturbed work. I myself find it very difficult, though, to elect such "individual salvation", and I may therefore return to

England if I can see my way of being of use, not only in science, but also in connection with the general situation. It is hardly necessary to state that, if I shall be in England and if you want me to do so, I shall be most happy again to cooperate with those who work in the Clarendon Laboratory. It may be best, however, that I should not receive financial support from the Laboratory, as such financial support is bound to be linked with fixed obligations which I would rather avoid.

For the time being, I do not yet see my way of being of use in England in connection with the general situation, though I see certain potential possiblities in this respect. In view of these I am at present not looking for a "job" on this side of the Atlantic. Perhaps I shall have an opportunity to talk to you about all this if I shall visit England in a not too distant future.

Naturally I regret that it will not be possible for me to collaborate in building up apparatus for the new Clarendon Laboratory. I trust that the spirit of inflation, which must necessarily accompany any armament race such as is on at present, will at least make it possible for you to obtain the funds necessary for carrying on research in the new laboratory.

Please excuse the three months' delay of this letter. Immediately after the Munich agreement it did not seem possible for me to have a sufficiently balanced view, and I had to allow some time to elapse before I was able to write without bitterness of this event.

With kind regards to all, I am,

yours very sincerely,

(Leo Szilard)

D Slsbk 2

Columbia University
in the City of New York

DEPARTMENT OF PHYSICS

April 6, 1939

Dr. Leo Szilard,
Pupin Physics Laboratories,
Columbia University.

Dear Dr. Szilard:

I told you that I would write you a letter to put on record my invitation to you to be a guest of the Department of Physics until June 1, 1939 to work on certain researches with Dr. Zinn and to have the privileges that are appropriate for a guest in our laboratory. Laboratory keys have already been issued to you, and I enclose with this a card by the use of which you can obtain a key to the outer door of the building by calling at Room 111 Low Memorial Library so that you may have access to the laboratory at times when the outer door is closed. The key obtained with this card is to be returned on leaving the building.

Sincerely yours,

George B. Pegram

GBP:H

George B. Pegram

F

S.b.s bk fldr #1

CLASS OF SERVICE DESIRED			
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Send the following message, subject to the terms on back hereof, which are hereby agreed to

To Lindemann _____ 19____

Street and No. Christ Church

Place Oxford

Have on account of international
situation with great regret postponed
my wedding for an indefinite period
& would be very grateful if you
could consider absence as leave
without pay Hope work busy Hope
Please communicate my sincerely kind
good wishes to all in these days of
grave decisions Archie

Sender's address for reference

Sender's telephone number

In 1946 he became a professor of Biophysics and studied problems of molecular biology such as bacterial genetics, protein synthesis and antibody formation. Together with Aaron Novick, he developed the "Chemostat", a device to grow bacterial populations in a stationary state. He also developed a theory of aging and a theory of Memory and Recall. He remained professor at the University of Chicago until 1964 when he joined the Salk Institute of Biological Studies as Resident Fellow.

Full time
Consultant to Basic Research
Program, National Institute
of Mental Health, PHS.

May 19, 1958 - 59 (? Dec 31)

Published
Bio 9 (Jan 1959
subm. Nov 24, 1958)

Bio 10 (publ. Sept 26, 1959)

Grant from Rockefeller
Foundation

Bio 8, Nov 20, 1955
subm. May 31, 1955

bbs 23

METALLURGICAL LABORATORY PERSONNEL — FEBRUARY 16, 1942

Arthur H. Compton, Project Leader

Samuel K. Allison, Experimental Chief

Enrico Fermi, Research Coordinator

Eugene P. Wigner, Theoretical Chief

Gregory Breit, Information Chief

R. L. Doan, Laboratory Director

J. C. Stearns, Personnel Chief

H. W. Byers, Procurement Chief

Norman Hilberry, Administrative Asst.

Haydn Jones, Shop Facilities
L. Szilard

RESEARCH ASSOCIATES:

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Seymour Bernstein
Lyle B. Borst
Alvin C. Graves
Elizabeth R. Graves
William P. Jesse
W. R. Kanne
Philip G. Koontz
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J. H. Manley
Alan C. G. Mitchell
Henry W. Newson
Louis A. Slotin
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Martin D. Whitaker
Ernest O. Wollan
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Theodore F. Petry
Glenn Sprankle

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D. E. Schilling, Chief
Alexander Glon
John Moriarity
Walter L. Peterson

STENOGRAPHERS:

Anne Hultgren
Dorothy Johnson
Pearl Margolis
Opal L. MacDonald
Pauline McGrath
Gertrude Nissenbaum
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Katherine Tracy

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Lillian Sexton

SHOP:

T. J. O'Donnel, Chief
John Costa
S. W. Dietze
Clyde R. Emery
Joseph Getzholtz
Richard Gluck
John P. Larsen
Casmer Lesnieski
Joseph Novak

February 19,1942

Compton, Hilberry, Wheeler, Doan.

J. C. Stearns

Outline of chemical work pertaining to our project.

Szilard - Material chief.

I. Analysis and control.

Rodden)
)
)(Beverly)
)
Scribner)

Furman

Bricker

Mack (Spectroscopist)

Hoffman - Bureau of Standards.

II. Princeton Absorption Experiment.

Cowan

Chicago chemist (Inorganic graduate student)
(See Schlesinger or Warren Johnson)

III. Chemical Development.

Suggest Spedding of Iowa State.

McCoy (UO_2)

Brown , Jura.

Chemical engineer
Pilot plant for separation of rare earths.
Use of cyclotron at St. Louis.

IV. Chemical Engineering.

Murphree
Thiele.

V. Consultants.

Metallurgy - Archer, Benbow.
Chemistry - Schlesinger, Brown.

J. C. Stearns

February 19,1942

Compton, Hilberry, Wheeler, Doan.

J. C. Stearns

Outline of chemical work pertaining to our project.

Szilard - Material chief.

I. Analysis and control.

Rodden)
)
)(Beverly)
)
Scribner)

Furman

Bricker

Mack (Spectroscopist)

Hoffman - Bureau of Standards.

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Metallurgy - Archer, Benbow.
Chemistry - Schlesinger, Brown.

J. C. Stearns

III 6s 7A

(1)

October 2, 1946

I was born in 1898 in Budapest, Hungary and studied Electrical Engineering at the Institute of Technology in Budapest, at the Institute of Technology in Berlin, Charlottenburg and subsequently Physics at the University of Berlin where I obtained a Doctor's Degree in Physics (Dr. Phil.), in 1922.

From about 1925 to 1933 I was attached to the teaching staff at the University of Berlin as Privatdotent for Physics. In 1934 I started working in the field of nuclear physics, first, as a guest at St. Bartholomew's Hospital in London and until 1938 at the Clarendon Laboratory at the University of Oxford. In March, 1939, I started work as guest of the Physics Department at Columbia University on uranium and from November, 1940 until February, 1942 I was a member of the staff at the National Defense Research Division of Columbia University. From February, 1942 until April, 1946 I served as Chief Physicist with the Metallurgical Laboratory at the University of Chicago.

L. Szilard

bbs 9

May 13th, 1946

Dr. Leo Szilard,
Metallurgical Laboratory,
Chicago, Illinois

Dear Dr. Szilard:

The contract #W-7401-eng-37 covering the operation of the Metallurgical Laboratory terminates on June 30, 1946 and it will not be possible, therefore, to renew your contract with the Metallurgical Laboratory; and I am unable to offer you a position in the new Argonne National Laboratory.

May I take this opportunity to express the appreciation of the Metallurgical Laboratory for your very valuable contributions to its success. Your foresight and initiative were largely responsible for obtaining support for the original atomic energy program and your work on piles and your vision for new types of piles have been important in the development of the research program of the Laboratory.

I know that you will find interesting work to do in which you will continue to work for the safety and welfare of the nation and I wish you every success in it.

Sincerely yours,

Farrington Daniels, Director
Metallurgical Laboratory

FD:FA

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Received Date	6-25-59
Council Assigned	Nov '59
Action	

Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH

Mail Completed Application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

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	RG-6876 M
	S.S.S. (1)
Formerly	

APPLICATION FOR RESEARCH GRANT

Date June 23, 1959

Application is hereby made for a grant in the amount of $26,735.00 (omit cents) for the period from January (month) 1, (day) 1960 (year) through December (month) 31, (day) 1960 (year), inclusive for the purpose of conducting a research project entitled (Limit to 53 typewriter spaces).

TITLE OF PROJECT: Quantitative Studies of General Biological Phenomena

Principal Investigator	Co-Principal Investigator, if any:
Name Leo (First) SZILARD (Middle) (Last)	Name (First) (Middle) (Last)
Title Professor of Biophysics	Title
Dept. Enrico Fermi Institute for Nuclear Studies	Dept.
School	School
University or Institution The University of Chicago	University or Institution
Street Address 5801 South Ellis Avenue	Street Address
City and State Chicago 37, Illinois	City and State
Name, Title and Address of Financial Officer: Albert F. Cotton, Bursar, The University of Chicago, 5801 South Ellis Avenue, Chicago 37, Illinois	Check to Be Drawn as Follows: The University of Chicago

Page 3 omitted-no entries (RG)

AGREEMENT

It is understood and agreed by the applicant: (1) That funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes set forth in this application; (3) that all reports of original investigations supported by any grant made as a result of this request shall acknowledge such support; (4) that, if any invention arises or is developed in the course of the work aided by any grant received as a result of this application, the applicant institution will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with its own policies, as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

NAME OF INSTITUTION: The University of Chicago

ADDRESS: 5801 South Ellis Avenue

CITY AND STATE: Chicago 37, Illinois

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION *(Please Type)*: W. H. Zachariasen, Dean, Division of Physical Sciences

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

(use ink)

Bpc

PRIVILEGED COMMUNICATION

PHS 398

Form Approved

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RG 6876

PROPOSED BUDGET for the period shown on page 1

NOTE: Under column entitled "OTHER" indicate funds presently available or anticipated from other sources, including those from own institution.	PERCENT OF TIME TO BE SPENT ON THIS PROJECT	BUDGET REQUESTED FROM PHS (Omit Cents)	BUDGET OTHER U. of Chicago
PERSONNEL: Itemize All Positions, Indicating Type, Percent of Time To Be Spent On This Project and Names of Professional Personnel Selected.		$	$
Leo Szilard, Professor of Biophysics, serving on a regular academic appointment (based on 9 months' service to the University but paid in 12 monthly installments)	100%	11,000.00	4,000.00
Teachers' Annuity and Social Security (6.8% of above salary)		748.00	272.00
PERMANENT EQUIPMENT (See instructions reference itemization of equipment)		$	$
CONSUMABLE SUPPLIES (Itemize)		$	$
TRAVEL (State Purpose)		$	$
For travel and subsistence while away from home		7,500.00	
OTHER EXPENSE (Itemize)		$	$
Secretarial services and office expenses while away from home base		4,000.00	

NOTE: The administrative official signing this application may add an amount for indirect costs.

IMPORTANT

Review detailed instructions before computing indirect cost allowance.

SUBTOTAL (DIRECT COSTS)	$ 23,248.00
INDIRECT COSTS PHS PARTICIPATION ADJUST TO LOW DOLLAR	$ 3,487.00
TOTAL BUDGET (OMIT CENTS)	$ 26,735.00

ESTIMATE OF FUTURE YEARS REQUESTED FROM PUBLIC HEALTH SERVICE

ADD'L YEARS	PERSONNEL	EQUIPMENT	SUPPLIES	TRAVEL	OTHER	SUBTOTAL (DIRECT COSTS)	INDIRECT COST ALLOWANCE	TOTAL
1st	$	$	$	$	$	$	$	$
2nd								
3rd		SEE PAGES 2A and 2B.						
4th								

If additional years requested are not contemplated enter "NONE" under total for first additional year.

ESTIMATE OF FUTURE YEARS REQUESTED FROM PUBLIC HEALTH SERVICE

Add'l years	Personnel	Travel	Other	Subtotal (Direct costs)	Indirect Cost Allowance	Total
1st	$11,748.00	$7,500.00	$4,000.00	$23,248.00	$3,487.00	$26,735.00
2nd	11,748.00	7,500.00	4,000.00	23,248.00	3,487.00	26,735.00
3rd	12,561.00	7,500.00	4,000.00	24,061.00	3,609.00	27,670.00
4th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00
5th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00
6th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00
7th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00
8th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00
9th	15,000.00	7,500.00	4,000.00	26,500.00	3,975.00	30,475.00

The above estimates are based on the following premises:

Dr. Szilard's salary rate is set above at $15,000 per annum. According to University policy, Dr. Szilard is scheduled to be retired on October 1, 1963. From the suggested starting date of the grant - January 1, 1960 - to the date of retirement, it is proposed that provision for his salary and teachers' annuity and social security benefits be divided between NIH and the University as follows:

	NIH	University	Total
1. Salary			
Orig. year of proposed grant	$11,000	$4,000	$15,000
First add'l year	11,000	4,000	15,000
Second add'l year:	11,000	4,000	15,000
Third add'l year:			
1 Jan. - 30 Sept. 1960	8,250	3,000	11,250
1 Oct. - 31 Dec. 1960	3,750	-	3,750
Total Third Year	$12,000	$3,000	$15,000
2. Teachers' Retirement and Social Sec. Benefits (6.8% of sal.)			
Orig. year of proposed grant	$ 748	$ 272	$ 1,020
First add'l year	748	272	1,020
Second add'l year	748	272	1,020
Third add'l year:			
1 Jan. - 30 Sept. 1960	561	204	765
1 Oct. - 31 Dec. 1960	-	-	-
Total Third Year	$ 561	$ 204	$ 765
3. Total of Items 1 and 2			
Orig. year of proposed grant	$11,748	$4,272	$16,020
First add'l year	11,748	4,272	16,020
Second add'l year	11,748	4,272	16,020
Third add'l year:			
1 Jan. - 30 Sept. 1960	8,811	3,204	12,015
1 Oct. - 31 Dec. 1960	3,750	-	3,750
Total Third Year	$12,561	$3,204	$15,765

ESTIMATE OF FUTURE YEARS REQUESTED FROM PUBLIC HEALTH SERVICE (continued)

It is assumed that Dr. Szilard may spend 9 months of the year away from his home, visiting other laboratories, including laboratories in Europe, and that he will need for travel and subsistence $7,500.00 per year. It is estimated that he will have to spend, while away from home, on secretarial services, including the rental of office equipment, an average of $4,000.00 a year. This estimate is based on the assumption that Dr. Szilard will need the services of a secretary hired in different locations on a temporary basis averaging between 20 and 30 hours a week over a period of 40 weeks per year, at rates from $2.50 to $4.00 per hour.

No salary items have been included for professional personnel other than Dr. Szilard. It is assumed that Dr. Szilard may pursue certain experimental aspects of his project in collaboration with professional personnel, employed by other institutions where Dr. Szilard may hold no administrative responsibility. Alternatively, The University of Chicago may employ professional personnel for Dr. Szilard's project and may apply - as the need arises - for additional grants.

Under the grant requested, I would endeavor to pursue these problems further.

- -

List of problems:

(1)

1a) The possibility of explaining the general phenomenon of aging on the basis of random inactivation of chromosomes of the somatic cells.

1b) The possibility of explaining the differences of the rate of aging of individuals on the basis of the genetic inheritance of the individual.

1c) The decline of the fertility of women with age.

1d) The change with age in the number of live embryos and the number of corpus lutea in pregnant mice.

1e) The relationship between the age of the bull and the "permissible reduction" in the number of spermatozoa used for insemination, as a function of the age of the bull. The "permissible reduction" is here defined as the fraction of the spermatozoa contained in one ejaculate, which must be inseminated, in order to achieve a pregnancy with some fixed probability q. The value of q may be chosen more or less arbitrarily, provided only that q is sufficiently small compared to 1; q might be, for instance, between 1/2 and 1/4.

1f) The relationship between the age of the mother and the frequency of congenital malformations which are due to an abnormal chromosome number in the affected individual.

1g) The possible relationship between the malignancy of a mammalian cell and its abnormal chromosome number.

(2)

The possibility of disentangling whether various factors which enhance the killing effect or the mutagenic effect of ionizing radiation on micro-organisms or mammalian cells act by enhancing the production of the chromosomal lesions or whether they act by repressing the restitution of such lesions.

(3)

3a) The molecular basis of induced enzyme formation in micro-organisms.

3b) The molecular basis of antibody formation in mammals.

(4)

4a) The basic phenomenon involved in delayed hypersensitivity and tissue compatibility.

4b) The role of immunological defense mechanisms of mammals in delaying or preventing the onset of a malignancy.

(5)

The gene-protein problem.

(6)

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.

(7) The higher functions of the brain.

(8) The problem of sleep.

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, when possible, what particular approach I would propose to adopt.

AD (1)

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.

This theory explains the difference between the longevity of individuals on the basis of the number of defective genes of a certain class which they have inherited. The class of genes which is involved consists of those genes which are essential for the life of the somatic cell and which we may, therefore, designate as "vegetative genes". Mutant incompetent forms of such vegetative genes are recessive cell lethals, to which I shall refer below as "faults".

In order to check the theory by experiments, one may expose a population of mice to ionizing radiation and observe the life expectancy of the adult offspring. I assume that a number of "vegetative genes" essential for the life of the somatic cells of mammals is about the same as the number of genes important for the lives of micro-organisms, which may be estimated to be about 3,000. Russell has observed the frequency with which a given dose of X-rays produces mutations in mice, and, if his results are taken at their face value and are extrapolated to man, then (on the basis of the assumption of 3,000 vegetative genes) the life-shortening of the adult offspring of parents who have been exposed to X-rays would amount to about three days per rep. An experimental test of the theory along these lines may take five to six years and would require rather large facilities. It might be possible to arrange for such experiments in collaboration with one of the sites of the AEC.

We may now perhaps go further and tentatively assume that the random inactivation of a whole chromosome (which the theory postulates to be the elementary step in the process of aging), consists in the irreversible destruction of the chromosome. Random chromosome breaks, for instance, which do not restitute, might lead (even in the cells of non-dividing tissues) to the irreversible destruction of the functioning of both chromosome pieces. Assuming that this occurs at the same rate in somatic cells and oogonia as well as spermatogonia, we may then draw certain conclusions from the theory which could be experimentally tested, perhaps in a comparatively short time.

If a spermatozoon (containing a haploid set of chromosomes) carries a chromosome that has suffered an aging hit, and were to fertilize an ovum, the resulting embryo would be abnormal and, in the great majority of cases, not viable. The

fraction of spermatozoa which are "defective" in this sense, should increase with the age of the male. Accordingly, with increasing age of the male, it would more and more frequently happen that an ovum is "spoiled" by being fertilized by a defective spermatozoon, unless we assume that a defective spermatozoon loses out, under natural conditions of fertilization, in competition with spermatozoa which contain an intact haploid set of chromosomes. We may either assume (a) that a spermatozoon which comes from a spermatogonium that has suffered an aging hit will lose out in the competition, or else, we may assume (b) that a spermatozoon which contains a haploid set of chromosomes which has suffered an aging hit, will lose out in the competition.

The "permissible reduction" in the number of spermatozoa contained in one ejaculate, as defined under 1e), may be predicted on the basis of the theory by assuming either (a) or (b), and the prediction may be tested experimentally, for instance in the case of cattle, where artificial insemination is standard operational practice. Because the cost of experiments with cattle might prove to be prohibitive, it might perhaps be necessary to work out a satisfactory technique for artificial insemination for a suitable rodent.

In the female there are initially present many oocytes, and it is possible that the kind of selection postulated above for the fertilizing spermatozoon operates with respect to the mature ovum also. A mature ovum would then be expected to contain an intact haploid set of chromosomes just as the spermatozoon, which is capable of reaching the ovum, may be expected to contain an intact haploid set of chromosomes.

If no such selection operates with respect to the ovum, then the theory leads us to predict that the probability that a human ovum may give rise to the viable embroyo must fall off with the age of the mother, by a factor of $(e)^{1/12}$ per year. A straightforward comparison of such a prediction with the available facts is rendered difficult in the case of man because the frequency of intercourse falls off with increasing age of the couple. It should be possible, however, to compare the number of corpus lutea in pregnant mice with the number of live embroyos contained in the uterus. The litter size in the mouse falls off with the age of the female to about one-half, at the age at which the mouse ceases to have further litters. However, the mouse undergoes "silent" pregnancies beyond that age, apparently caused by the inability of the mouse to deliver if the number of live embroyos is too small. Accordingly it would be necessary to compare the number of live embroyos with the number of corpus lutea in "silent" pregnancies occurring in old mice.

If there is selection operating against the mature ovum lacking a functional chromosome, then the litter size ought to fall off with age more slowly than the theory would otherwise predict.

In the case of the spermatozoon at least, we have no choice but to assume that a strong selection does operate against the fertilization of an ovum by a spermatozoon that lacks one functioning chromosome. This does not mean, however, that this selection operates with hundred percent efficiency. It could well be that in a few percent of the cases the selection fails and an ovum is fertilized by a spermatozoon which lacks a functioning chromosome. The resulting embroyo may then be expected to suffer early fetal death. The frequency of such defective

embroyos should increase with the father's age.

On the basis of considerations of this kind I am led to raise the question whether a substantial fraction of embroyos suffering early fetal deaths lacks a chromosome. It is my intention to arrange for an investigation aimed at elucidating this point. Should it prove difficult to get hold of human embroyos suitable for the purposes of such an investigation, then one may have to turn to mice. In this case non viable embroyos found in "silent" pregnancies of old mice would represent the object of choice.

AD (2)

The theory predicts that if a population is exposed, generation after generation, to the same dose of ionizing radiation, senescence will set in progressively earlier and earlier, and finally a new mutational equilibrium is reached concerning the number of faults per person. According to the theory, if the number of faults is doubled, senescence will set in about 15 years earlier.

From the point of view of maintaining the longevity of the human race, it would be important to know whether the mutations produced by ionizing radiation occur less frequently if the dose of ionizing radiation is given at a lower rate. That this should be the case did not at first appear a priori probable; yet, such a result is not inconceivable. We know that ionizing radiation, in addition to producing chromosomal lesions in proliferating mammalian cell cultures (which may occasionally restitute with the deletion of one or several genes) also produces a physiological effect which manifests itself by causing a lag in cell division. This physiological effect may be expected to depend on the dose rate. It is conceivable that the production of mutations by ionizing radiation falls off with the dose rate, not because the dose rate affects the production of chromosomal lesions but, rather, because it affects - via the physiological effect - the probability that the chromosomal damage may restitute with or without delection of a gene.

Accordingly, it would appear to be important to disentangle the factors which determine whether or not a chromosomal lesion is produced and the factors which determine the probability that chromosomal lesion will restitute with or without gene delection. Perhaps the tools are now at hand that may enable us to disentangle these two factors. Thus, for instance, Szybalski has shown that if in a proliferating cell culture one incorporates a certain chemical analogue of thymidine into the DNA and then exposes the culture to X-rays, the killing of cells by the radiation is enhanced. In cases of this sort it should be possible to determine whether the enhanced killing is due to an increased production of chromosomal lesions or to decreased restitution of the lesions produced. It is uncertain, however, whether an adequate analysis of phenomena of this sort could be carried out on animal cell cultures. It is conceivable that one may have to use a different biological material where more powerful techniques are available, such as for instance the technique developed by Kim Atwood for analyzing radiation damage in neurospera.

AD (3)

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 attached to the enzyme forming site protects the attached 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.

An experiment somewhat along these lines was made by Jean Marie Dubert (CR 243-2, p. 1939, 1956) which is difficult to reconcile with the simple model described above. Even though this experiment was made with only four rabbits and might also be inadequate for other reasons from the point of view of our purposes, it still represents a warning to caution. It might be that it is too early to attempt to formulate concrete models and to check them one by one. Perhaps we ought to wait with such a procedure until we can say with certainty whether the antigen assists the antibody in folding, and whether the antigen may thus determine the tertiary structure of the antibody.

I am inclined to believe that given a somewhat greater cooperation among various laboratories who are interested in these problems and with a much greater stress placed on the use of artificial haptens, the basic mechanism of antibody formation could be elucidated in the foreseeable future.

If it were possible to gather some 15 to 20 of the younger men who have recently moved into this field, or who are about to move into it, for a leisurely conference lasting for about three weeks, it would be possible to reach a considerable clarification of what experiments would need to be done in order to achieve rapid progress. One would hope that at the end of such a conference most participants would know just what experiments they themselves would want to do and also just how they would have to do these experiments in order to be convincing to the others. If something of this sort could be arranged and perhaps repeated after two years, it is possible that we may gain within five years substantial insight into the mechanisms of antibody formation in mammals. I had occasion to explore whether a proposal to hold such a conference would be welcomed by men who would be desirable participants, and it is my intention to arrange for such a conference if it is possible to obtain the required financial support.

AD (4)

Concerning the basic phenomenon involved in delayed hypersensitivity and tissue compatibility, as well as the role of immune mechanisms, in delaying or preventing the onset of a malignancy in a mammal, I have so far not been able to make appreciable progress. Dr. George Klein, of the Laboratory of Tumor Biology, of the Karolinska Institute, Stockholm, has asked me to visit his laboratory to discuss such problems. I propose to spend several weeks there, and, subsequently, I might perhaps be in a better position to appraise the chances of obtaining significant results in this field.

AD (5)

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 guanin-adenine 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 (6)

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 microorganism 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.

AD (7)

I have some interest in the higher functions of the brain but have not so far been able to pursue this interest. It is my intention to utilize my contacts with the National Institute of Mental Health to deepen my at present rather rudimentary knowledge of this field. I would be particularly interested in setting up experiments aimed at finding out something about the structure of memory.

AD (8)

Very little is known about the nature of sleep. I do not believe that this problem is of very great basic theoretical interest, but I am interested in it because of its very great practical importance. It appears reasonable to believe that the mechanism which forces us to sleep evolved at a stage of man's development when during darkness the most useful activity that was possible was to sleep. Today, however, if it were feasible to put the mechanism which induces sleep out of action, or, alternatively, to keep the mechanism which induces wakefulness in operation 24 hours a day, man's useful life could be prolonged by about one-third. This is probably the single most important gain in extending active life that might be accomplished within the foreseeable future. It would be my intention to pursue this subject further if there opens up an opportunity to do so effectively.

- -

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: The problem of aging.
H. J. MULLER* - University of Indiana
JOSHUA LEDERBERG* - Stanford University
GEORGE BEADLE - California Institute of Technology
KIM ATWOOD* - University of Chicago

Re: Induzed 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, 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 MAC LEOD - University of Philadelphia
HERBERT ANKER* - The University of Chicago

Re: Inherent stability of competent genes.
MAT MESELSON* - California Institute of Technology

Re: Delayed hypersensitivity, tissue compatibility, and the tumor problem.
HILARY KOPROVSKI* - The Wistar Institute, Philadelphia
GEORGE KLEIN* - Laboratory for Tumor Biology, Karolinska Institute, Stockholm
JIM WATSON - Harvard, Cambridge, Mass.

Re: Higher functions of the brain and the problem of sleep.
ROBERT B. LIVINGSTON* - The National Institutes of Health

Re: Killing and mutagenic effect of ionizing radiation on mammalian cells.
MORTIMER ELKIND* - NIH, Bethesda, Maryland
KIM ATWOOD - The University of Chicago
RENATO DULBECCO - 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, 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 Institute of Molecular Biology, The University of Oregon, Eugene, Ore. (Director - Aaron Novick)

The National Institute of Mental Health, Bethesda, Md. (Scientific Director - Robert B. Livingston)

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

The Wistar Institute, Philadelphia (Director - Hilary Koprovski)

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

The Laboratory for Tumor Biology, Karolinska Institute, Stockholm (Director - George Klein)

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 Joshua Lederberg and Arthur Kornberg)

The Oak Ridge National Laboratory, Knoxville, Tenn. (Director - Alvin Weinberg

16 [illegible]

S-38 D

To be inserted on page 2 of Progress Report 01

Statement of Accomplishment covering period January 1, 1960 to September 30, 1960

A model for the control of the rate of production of repressible enzymes has been developed and this model is described in detail in "The Control of the Formation of Specific Proteins in Bacteria and in Animal Cells", Proceedings of the National Academy of Sciences, Volume 46 p.277 (March) 1960. This model assumes that in bacteria the repressor controls the rate of formation of the enzyme by the enzyme forming site, rather than the rate of formation of the enzyme forming site itself. Experiments which are at present being conducted in a number of different laboratories, with which the author maintains contact, might elucidate, within a year, whether this "premise" is correct.

The above-quoted paper also assumes that the repressor can attach itself to the enzyme and it is shown that accordingly the cell might have two stable states, a state in which the enzyme level is high and a state in which the enzyme level is low. The validity of this assumption does not depend on the above-mentioned "premise" and the assumption might provide the key to the understanding of a certain type of differentiation, discussed in the paper.

A second paper "The Molecular Basis of Antibody Formation", Proceedings of the National Academy of Sciences, Volume 46, p.293 (March) 1960 discusses the possibility that antibody formation - in the primary response - is based on this type of differentiation, triggered by the injection of an antigen into the rabbit. This theory can account for a number of phenomena listed in the paper, including the phenomenon of immune tolerance of the new-born rabbit. The explanation of immune tolerance is, however, again based on the "premise" that the repressor controls the rate at which the protein - in this case the antibody - is formed by the specific protein forming site. If future experiments should show that this "premise" is

wrong, then the theory of immune tolerance would have to be modified and it is not as yet clear whether a satisfactory modification of the theory would be possible, in that contingency.

A theory for the dependence of the sex ratio at birth on the age of the father has been presented in "Dependence of the Sex Ratio at Birth on the Age of the Father", Nature, Volume 186 pp.649-650, (May) 1960, which is based on a theory of ageing previously presented by the author (Proc. Nat. Acad. Sc. 45,32. 1959). The theory accounts for the decrease in the ratio of boys to girls, with increasing age of the father, on the ground that a spermatogonium in which the X-chromosome suffers an "ageing hit" may not continue to give rise to sperm, whereas a spermatogonium in which the Y-chromosome suffers an "ageing hit" may continue to give rise to sperm.

The End

S-38

Grant 02

Sept. 61.

(1) An experimental method has been devised and a theory of the experiment developed which should make it possible to determine the dose of radiation which would raise the mutation rate to twice the value of the spontaneous mutation rate. The method consists in exposing a population of mice to ionizing radiation and subsequently determining, among the first generation off-spring, the proportion of females whose off-spring shows an abnormal sex ratio. The method is described in a paper dated March 10, 1961 "Induction of Mutations in Mammals by Ionizing Radiation", which is being privately circulated to those interested in this type of problem.

(2) In my paper (Proc. Nat. Acad.Sci. 46, 293, 1960), I postulated a simple biochemical mechanism upon which the "memory" may be based which manifests itself in the secondary antibody response. Herbert Anker suggested in a "Letter" to Nature that memory in the central nervous system might perhaps be based on the same biochemical mechanism. A set of postulates has been formulated which would have to hold if this particular memory mechanism accounts for the phenomena of memory that manifest themselves in the central nervous system.

Professor

It is proposed to develop further the theory on enzyme repression, presented in Proc. Nat. Acad. Sci. 46, 277, 1960 and the related theory on antibody formation, presented in Proc. Nat. Acad. Sci. 46, 293, 1960. Recent experimental evidence leads to the assumption that in bacteria messenger RNA molecules, rather than the ribosomes carry the information for the specific proteins which are formed. Since the repressors cannot chemically recognize the specific messenger RNA molecules, this raises the question of how the repressors can control the rate of formation of the messenger RNA molecules. The possibility that the repressors exercise such a control indirectly, so to speak, through a negative feedback mechanism, will be examined. Further, it will be examined to what extent the concept of the messenger RNA, and the control of its rate of formation through a negative feedback mechanism might permit an explanation of immune tolerance in the new-born rabbit, which is radically different from the explanation given in my paper quoted above.

It is proposed to develop further a theory on enzyme repression, presented in Proc. Nat. Acad. Sci. 46, 277, 1960 and a related theory on antibody formation, presented in Proc. Nat. Acad. Sci. 46, 293, 1960. In their present form both of these theories are based on the "premise" that the repressor controls the rate at which the protein forming site forms a specific protein, rather than the rate at which this site itself is formed. X The theory on antibody formation is based on the postulate that the repressor is capable of combining with the specific antibody and it explains on the basis of this postulate - which is independent of the above-mentioned "premise" - the production of antibody in the primary response. The theory leans, however, on this "premise" for the explanation of immune tolerance in the new-born rabbit. It is proposed to evaluate experiments which are in progress (in a number of different laboratories) in order to determine if the above-mentioned "premise" may have to be abandoned. With this possibility in mind, conceivable alternative explanations of immune tolerance will be explored.

02 orig 5 52

INDUCTION OF MUTATIONS IN MAMMALS BY IONIZING RADIATION

by

Leo Szilard

The Enrico Fermi Institute
for Nuclear Studies
The University of Chicago
Chicago 37, Illinois

Our purpose here is to describe a project aimed at determining the magnitude of the mutagenic effect of ionizing radiation in mammals, such as mice. For the purposes of this project, we must distinguish between mutations which have been inherited by a given generation of a population, and mutations which have been acquired by that generation. The acquired mutations may have spontaneously arisen in the animal during its embryonic development or after birth, and they may have been induced by exposing the animal to ionizing radiation, either during its embryonic development or after birth.

The proposed project is limited to the study of recessive lethal mutations which are carried by an X chromosome, i.e., it is limited to the so-called sex linked recessive lethals.

Both the acquired and the inherited sex linked recessive lethals should manifest themselves in a reduction of the proportion of males in the brood, at birth. This effect may be expected, however, to be very small. Thus, for example, if

½% of the X chromosomes in the ova carry an inherited or acquired sex linked recessive lethal, then the proportion of the males in their brood would thereby be reduced only by $\frac{1.7}{1000}$. Rather than to rely on this effect, we shall adopt a method of study which appears to be more promising. Our method can provide us with reliable information concerning the abundance of the sex linked recessive lethals that have been inherited by the female population. This may be seen as follows:

Let us consider a population of 10,000 female mice and assume, for the sake of argument, that a small proportion of these females, say 1%, have inherited a sex linked recessive lethal. In a sample of 10,000 female mice there would, then, be about 100 females who have inherited a sex linked recessive lethal.

A female mouse which has inherited a sex linked recessive lethal will, on the average, have 33 males in a brood of 100. Accordingly, we should expect to find in our sample of 10,000 females, about 50 females who have 33, or less than 33, males in a brood of 100.

The rest of the female mice which have not inherited a sex linked recessive lethal will, on the average, have 50 males in a brood of 100. In our sample of 10,000 females, there will be about 9,900 females who have not inherited a sex linked recessive lethal and, on the basis of simple statistical

considerations, we should expect to find among them about 7 females who have 33, or less than 33, males in a brood of 100.

If we have a sufficiently large sample of female mice available, we may determine what proportion of these mice have inherited a sex linked recessive lethal, by proceeding as follows:

We breed each female mouse until its brood totals 100. The sex of each new born mouse is determined and, with the exceptions stated below, that mouse is then killed.

In each case when we find that a female has a low proportion of males in its brood, we may suspect that an inherited sex linked recessive lethal is responsible. In order to determine whether this is, in fact, the case, we preserve 5 or 6 females out of the last litter and each of these we breed until it has a brood of 100. If any one of these 5 or 6 females has a low proportion of males in its brood, then this would confirm that an inherited sex linked recessive lethal was, in fact, responsible.

Mutation Rate and the Inherited Load of Mutations

We may study the spontaneous mutations which result in sex linked recessive lethals by applying to a natural mouse population our method of counting the number of females who have inherited a sex linked recessive lethal. A sample of 10,000 females should be sufficient for this purpose.

By counting the females who have inherited a sex linked recessive lethal we determine the inherited load of sex linked recessive lethal mutations. There exists a simple relationship between this load and the rate per generation at which an X chromosome acquires a recessive lethal through spontaneous mutation.

Let us designate with μ_m the probability that such a mutation should occur in one generation during the passage of an X chromosome through the male, and designate with μ_f the probability that it should occur in one generation during the passage of an X chromosome through the female. Since a male who had inherited an X chromosome carrying a recessive lethal would not be viable, the probability that an X chromosome in the sperm of the adult male of average age carries a recessive lethal is given by μ_m. If ε designates the probability that a female, born to adult parents of average age, inherits a sex linked recessive lethal, then the probability that an ovum of an adult female of average age contains an X chromosome which carries a recessive lethal is given by $\frac{\varepsilon + 2\mu_f}{2}$.

It follows that if we mate an adult female of average age with an adult male of average age, we may write for ε', the probability that a female offspring will inherit a sex linked recessive lethal,

$$\varepsilon' = \frac{\varepsilon + 2\mu_f}{2} + \mu_m$$

or $$\varepsilon = 2(\mu_f - \mu_m)$$

and this may also be written in the form

$$\varepsilon = 4\bar{\mu}; \text{ where } \bar{\mu} = \frac{\mu_f + \mu_m}{2}$$

Thus, in the case of sex linked recessive lethals, there exists a simple relationship between the inherited load of mutations and the rate per generation at which such mutations arise.

We have such a clear relationship between the inherited load of recessive lethal mutations and the rate per generation at which such mutations arise only for the class of the sex linked recessive lethals. Once we go outside of this class we cannot derive such a relationship because, outside of this class, it is not known how long the recessive lethal mutations persist.

Radiation Induced Mutations

We now turn to the induction of sex linked recessive lethal mutations by ionizing radiation. Let us first consider, for the sake of argument, an experiment in which we start out with a natural population of 10,000 females and an equal number of males. Let us expose the females to a certain X-ray dose d_f and designate with x_f the probability that an X chromosome contained in an ovum carries a radiation-induced recessive lethal mutation. Similarly, let us expose the males to a certain, either equal or

different, X-ray dose d_m and let us designate by x_m the probability that an X chromosome contained in a spermatozoa carries a radiation-induced recessive lethal.

Let us now mate adult females of an average age with adult males of an average age and keep one female from the first litter ~~of each female~~, while the rest of the brood is killed. We would thus end up with 10,000 female mice in the second generation. The probability that a female mouse of this second generation has inherited a sex linked recessive lethal is then given by

$$P_1 = x_m + x_f + \varepsilon$$

This number P_1 can be experimentally determined by breeding each female until it has a brood of 100 and determining the proportion of the males in the brood of each mouse in the manner described above.

If ε has been determined for a natural mouse population, then P_1 will now determine $(x_m + x_f)$. This sum is the probability that exposure of the mouse population to ionizing radiation produces a sex linked recessive lethal mutation in an X chromosome which will be passed on to the offspring.

The same result can be obtained at lesser cost if we start out in the first generation with a smaller sample of a natural population, say 1,000 females and 1,000 males. After exposure of the male and female population, we mate adult females

of average age with adult males of average age and obtain one or two litters from each female. From the litters of each female we keep 10 females and kill the rest of the brood. In this manner we end up with 10,000 female mice in the second generation. Each of these second generation females we now breed until it has a brood of 100 and, we pick out the females who have a low proportion of males in their brood. This permits us to determine, in the manner described above, which of the females of the second generation have inherited a sex linked recessive lethal.

Because we are using here a small sample of females in the first generation, the spontaneous sex linked lethals inherited by these females would introduce a sampling error into our result. In order to avoid such a sampling error, we eliminate from the tabulation of our data any second generation female who has inherited a sex linked recessive lethal, if any of her 9 sisters have also inherited a recessive sex linked lethal. The remaining females of the second generation who have inherited a sex linked recessive lethal (and whose sisters have not) must have inherited a sex linked recessive lethal which either has spontaneously arisen in one of her parents or was induced in one of her parents by the exposure to the ionizing radiation. Therefore, the probability that a female mouse retained in the tabulation of our data has inherited a sex linked recessive lethal is given by

$$P_2 = x_m + x_f + \mu_m + \mu_f$$

for which we may also write

$$P_2 = x_m + x_f + \frac{\varepsilon}{2}$$

* * * * * *

Let us, now, assume that we expose both the male and female population to the same dose of ionizing radiation, d. We may then experimentally determine by the methods described above the dose d = D, for which we have

$$x_m + x_f = \mu_m + \mu_f = \frac{\varepsilon}{2}$$

This dose, D, would induce as many sex linked recessive lethal mutations as would spontaneously arise in one generation and we may, therefore, refer to it as the "doubling dose."

The same dose, D, would also induce as many recessive lethal mutations carried by some other chromosome than the X chromosome as would spontaneously arise in one generation on that other chromosome. It follows that if a population were exposed, generation after generation, to the doubling dose, D, in the mutational equilibrium, which would be gradually approached, the load of inherited mutations would be twice as great as the load of inherited spontaneous mutations. If we know how big the

doubling dose is, we may then assess the generic damage suffered by a population which, generation after generation, is exposed to some dose d of ionizing radiation.

Naturally, our primary interest would be to find the doubling dose, D, not for mice, but for man. The question in what manner one might attempt to deduce the doubling dose for man from experimental results obtained with mice goes beyond the scope of this presentation.

The End

The author wrote this paper while serving as Consultant to the Basic Research Program, National Institute of Mental Health, National Institutes of Health, U.S. Public Health Service, Dept. of Health, Education, and Welfare.

March 10, 1961

5-37

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THE UNIVERSITY OF CHICAGO
CHICAGO XX · ILLINOIS 60637
OFFICE OF THE VICE PRESIDENT · SPECIAL PROJECTS
5801 ELLIS AVENUE

24 September 1963

Dr. Leo Szilard
c/o Dr. Martin Kaplan
World Health Organization
Palais des Nations
Geneva, Switzerland

Dear Dr. Szilard:

Attached are your file copies of the materials which were submitted on September 23, 1963 to NIH in behalf of your research project, GM-06876-05.

If we may be of further assistance, please advise.

Sincerely,

Irene E. Fagerstrom

Irene E. Fagerstrom
Assistant Vice President
(Special Projects)

cc: Dean A. A.Albert

Prof. Szilard?

21st August, 1963

Division of Research Grants
National Institutes of Health
Bethesda 14, Maryland

Gentlemen:

Attached is an application for continued support of research grant, GM-06876-04.

The papers listed below are at present privately circulated among scientists who may be assumed to be interested, as a result of work done under this grant. Ten multigraphed copies of each paper are enclosed with this application.

Sincerely yours

Leo Szilard

Leo Szilard

Papers

"On the Occasional Dominance of the 'Perceptible Phenotype' in Man", dated July 12, 1963.

"The Aging Process and the 'Competitive Strength' of Spermatozoa", dated July 25, 1963.

... the Science Information Exchange.
... Not for publication or publication reference.

U. S. Department of
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

Submit with completed Application to: Division of Research Grants, National Institutes of Health, Bethesda 14, Md.

TITLE OF PROJECT:

QUANTITATIVE STUDIES OF GENERAL BIOLOGICAL PHENOMENA

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS or PROJECT DIRECTORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project. Include day-month-year of birth of principal investigators.

Principle investigator: Leo Szilard, Professor of Biophysics in the Enrico Fermi Institute for Nuclear Studies at the University of Chicago. Born February 11th, 1898. No other professional personnel is engaged on the project.

NAME AND ADDRESS OF APPLICANT INSTITUTION: The University of Chicago
5801 S. Ellis Avenue
Chicago 37, Illinois

SUMMARY OF PROPOSED WORK — (200 words or less — Omit Confidential data.)

In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

In a population, like for instance the population of the United States, within one cohort, the ages at death have a considerable scattering around the median value. The curve which gives for a cohort the number of deaths per year, as a function of the age, resembles a Gaussian with a standard deviation of about 10 years. Some of the scattering of the ages at death is likely to be due to the genetic differences between the individuals who make up the population and the remainder of the scattering must be attributed to non-genetic causes.

It is proposed to develop a method that would permit us to determine how much of the observed scattering of the ages at death is due to the genetic differences between individuals, i.e. to determine what the mean deviation of the curve, giving the number of deaths per year as a function of the age for a cohort, would be if there were no scattering of the ages at death, other than that which is due to genetic causes.

SIGNATURE OF PRINCIPAL INVESTIGATOR or PROJECT DIRECTOR: Leo Szilard

Identify the Professional School (medical, dental, public health, graduate, or other) with which this project should be identified:

SCHOOL: Research Inst. for Nuclear Studies

INVESTIGATOR — DO NOT USE THIS SPACE

PHS. 166
REV. 12-61

FORM APPROVED
BUDGET BUREAU NO. 105-R002.

...SHEET FOR
...590 (REV. 2-62)

Name of Principal Investigator Szilard, Leo
Current Grant No. GM-06876-04
Current Grant Period 1/1/63 - 12/31/63

I. ESTIMATED CURRENT YEAR LEVEL OF EXPENDITURES:

The following table has been developed to help you in arriving at figures to be inserted in Column 3 of the detailed budget page. Please return this work sheet with your completed application for continuation support.

Budget Categories	Actual Obligations through 30 June 1963 (insert date)	Estimated Additional Expenditures through remainder of current grant period.	TOTAL ESTIMATE FOR ENTIRE CURRENT YEAR (Entries for Column 3 of Budget Page shown by "x")	
1. Personnel	$ 6,149.04	$ 6,944.96	$ 13,094.00	x
2. Movable Equipment	-			x
3. Consumable Supplies				x
4. Travel	1,740.16)	3,200.00	6,377.04	x
5. Other expenses on which indirect costs are allowed	1,436.88) 68.10	100.00	168.10	x
6. Sub-total	9,394.18	10,244.96	19,639.14	-
7. Indirect Cost Allowance	1,409.13	1,536.74	2,945.87	-
8. Other expenses on which indirect costs are not allowed	-0-	-0-	-0-	x
9. TOTAL ESTIMATE	$10,803.31	$11,781.70	$22,585.01	-

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II. ESTIMATED UNOBLIGATED BALANCE AT TERMINATION OF CURRENT GRANT YEAR:

1. Total funds available in current grant for the entire year (include funds that were permitted to be carried over from prior year grant, if any) ..... $ 32,670.00
2. Less total estimated expenditures under current grant (from line I.9., above) ..... - 22,585.01
3. Estimated free balance at termination of current grant (1 minus 2) $ 10,084.99

III. PLANNED USE OF ANTICIPATED FREE BALANCE DURING COMING GRANT YEAR: (Public Health Service research grant policy permits any unspent funds UP TO $5,000 OR ONE HALF OF THE CONTINUATION GRANT, whichever is smaller, to carry forward to the continuation grant account and remain available for use during the continuation grant year. Explain below your planned use of the estimated free balance shown in item II.3.)

I anticipate to use the free balance during the coming grant year for secretarial services, communications, and travel expenses.

The increased office and secretarial expenses, as well as the increased travel expenses, are anticipated because, in pursuance of work relating to population genetics and vital statistics which I am about to begin (manuscript in preparation), I expect to have to step up communications with a number of different institutions, who are engaged in similar work.

(continue on reverse if more space is needed)

Grant Number
GM-06876-05

BUDGET JUSTIFICATION

4. TRAVEL

I plan to make two trips to Europe.

On one of these trips to Europe I plan to visit one of the three institutes listed below:

(a) The World Health Organization, Geneva, Switzerland

(b) The Institute of Genetics (Cavalli Sforza) at the University of Pavia, Italy.

(c) The National Institute for Medical Research (Peter B. Medawar), Mill Hill, London, England.

Work on population genetics which I am planning to perform will make it necessary for me to visit one out of these three places in Europe, in order to assemble data in vital statistics which are difficult to obtain in the United States.

On the other trip to Europe, I plan to visit the Institute of Animal Genetics (C. H. Waddington), at the University of Edinburgh, England. The purpose of this trip is to discuss and if possible to make arrangements for the insemination of female animals with mixtures of spermatozoa derived from two different males.

During the coming grant year I also plan to make three trips from the East coast to the Salk Institute for Biological Studies at La Jolla, California.

(15)

GRANT NUMBER SHOWN ON PAGE 1 →

SUMMARY PROGRESS REPORT

GRANT NUMBER: GM-09876-03 2d 04

PRINCIPAL INVESTIGATOR (Name): Leo Szilard

INSTITUTION: The University of Chicago

SUMMARY OF ACCOMPLISHMENTS COVERING PERIOD

FROM: 15 Sept 1962 THROUGH: 21 Aug. 1963

TITLE OF PROJECT (Repeat title shown in Item 1 on first page): Quantitative Studies of General Biological Phenomena

1.) It has been shown that the observed frequent occurrence of a striking resemblance between a child and one of its parents might be explained in one of two ways:

a) The perceptible phenotype might be determined by a number of different genetic loci, all of which are located on one pair of homologous autosomes. Such an autosome might possess a certain "strength" and a "strong" autosome might suppress the homologous autosome if it is substantially less "strong". (For details see the enclosed paper "On the occasional dominance of the 'perceptible phenotype' in Man", dated July 12, 1963.)

b) The genes which determine the perceptible phenotype might all be located within the same operon and different operons might be more strongly or less strongly repressed. This could then account for the observed resemblances, if one assumes that the perceptible phenotype is determined by the ratio of the quantities of the products of these genes in the diploid cell.

2.) An experimental method has been devised for determining whether the competition which exists between spermatozoa for fertilising an ovum might serve the purpose of protecting the ova against being fertilised by a spermatozoon which might contain genetic material that has deteriorated as a result of the aging process. The method devised consists in inseminating females with a mixture of spermatozoa, derived from two donors, and in determining how the fraction of the offspring which is derived from the older donor, decreases with increasing age differences of the two donors. (For details see the enclosed paper "The aging process and the 'competitive strength' of spermatozoa", dated July 25, 1963.) Arrangements for carrying out experiments of this type are at present under discussion.

GM-06876-04

July 12, 1963

ON THE OCCASIONAL DOMINANCE OF THE "PERCEPTIBLE PHENOTYPE" IN MAN*

by

Leo Szilard
The University of Chicago

People generally believe, on the basis of everyday experience, that sons and daughters frequently resemble one, or the other, of the parents. Frequently, people remark that a boy or a girl is the ~~split image~~ "spitting image" of the father or the mother. It is by no means self-evident, however, how such striking resemblances might come about as frequently as people, rightly or wrongly, believe that they do.

It is, of course, conceivable that people might be wrong and such striking resemblances might be much less frequent than they believe them to be. In the circumstances, a valid determination of just how often a boy or a girl shows a marked resemblance to one of the parents would appear to be very desirable at this point and I intend to describe elsewhere a method that would permit this to be accomplished.

In the meantime I propose to assume the validity of the generally held belief that a marked resemblance of a child to one of the parents is rather frequent. I shall also assume that a really striking resemblance between unrelated individuals is extremely rare; in my whole life I have come across only one or two cases where a boy or a girl appeared to be the ~~split image~~ "spitting image" of someone whom I knew and to whom they were not related.

The way a person looks, moves, and the quality of his voice, make up most of what may be called the "perceptible phenotype" of the individual. Because of the very great variety of perceptible phenotypes within a population, it seems reasonable to assume that the perceptible phenotype is determined by a fairly large number of genes. In order to be able to explain a high frequency for a girl or boy to appear to be the split image of the father or the mother we shall

* This work was performed under a research grant of the National Institutes of Health

first of all assume that all the relevant genes form a single linkage group which we shall designate as the "PP (perceptible phenotype) linkage group". This assumption would appear to be a necessary condition for a high frequency of marked resemblances between a child and one of the parents. It cannot by itself explain, however, why such resemblances should occur with a high frequency, because each individual carries two homologous copies of the PP genes, one copy inherited from the father and one copy inherited from the mother and one would expect the presence of two different PP groups to blur any resemblance of an individual to either of its parents.

In order to explain a striking resemblance of an individual to one of its parents, one would have to postulate that the PP group of genes, derived from that parent, has somehow acquired dominance over the PP group inherited from the other parent. Thus the question arises whether it may be possible to think of a plausible mechanism which could account for the occasional dominance of the perceptible phenotype.

We are led to postulate a particular mechanism which could account for this phenomenon on the basis of the following considerations:

It appears to be well established that in many of the somatic cells of the female only one of the two X-chromosomes is functional and the other X-chromosome undergoes heterochromatinisation, becomes heteropycnotic, and forms the so-called sex-chromatin body. It appears that this heterochromatinisation is induced in a large part of one of the X-chromosomes of the somatic cells at an early embryonic stage. Generally speaking, the two X-chromosomes of a female have an equal probability to undergo heterochromatinisation in her somatic cells, but once one of the X-chromosomes has undergone heterochromatinisation in a somatic cell, then thereafter that chromosome will form the sex-chromatin body in all of the descendants of that somatic cell.[1,2,3]

Recently, however, there have been found exceptions to this general rule.[4] In the cases of two female patients, each of whom carried an X-chromosome which was structurally abnormal, it was found that in all the somatic cells of the patient this abnormal X-chromosome was consistently heteropycnotic and late

replicating. In these two cases one might choose to say that the X-chromosome which had the abnormal structure happened to be "weaker" than the other, normal, X-chromosome, and that the normal X-chromosome established its dominance by suppressing the abnormal X-chromosome.

These two examples show that, at least in the case of the X-chromosome, there is known to exist a mechanism which may occasionally permit one of two homologous chromosomes to establish its dominance by rendering the other chromosome non-functional.

The PP linkage group of genes cannot be assumed to be located on the X-chromosome, because if it were so located, then boys would always show a striking resemblance to their mother and never to their father. Therefore we must assume that the PP group is located on one of the autosomes which we may designate as the PP-autosome. In order to explain the occasional dominance of the perceptible phenotype of the father or of the mother, we propose to postulate that each PP linkage group of genes possesses a certain "strength". We further postulate that if the PP linkage group of genes which an individual inherits from, say, the father has a much greater "strength" than the homologous group of genes which that individual inherits from its mother, then during early embryonal development the stronger PP linkage group suppresses the weaker one (in the same manner as the normal X-chromosome appears to have suppressed the abnormal X-chromosome in the case of the two patients mentioned above) and, accordingly, this individual would then be the ~~split-image~~ "splitting image" of its father.

The mechanism described above might have evolved because of the evolutionary advantage that would be possessed by a population in which the dominance of the perceptible phenotype is a frequent occurrence, over a population in which it is not.

In Man there appears to be a strong "aesthetic selection" at work in the choice of the mate which is guided by the perceptible phenotype. If there were no correlation, or only very weak correlation, between the perceptible phenotype and the genotype of the individual, then such an aesthetic selection could not make any constructive contribution, either to the development of new evolutionary

traits, or even to the prevention of deterioration of the genotype which may result from spontaneous mutations.

If the dominance of the perceptible phenotype is sufficiently frequent, then there is a strong correlation between the perceptible phenotype and the genotype of the individual and because the aesthetic selection may be assumed to be a very powerful one, PP-autosomes, which may have undergone major changes through spontaneous mutations, could spread rapidly through the whole interbreeding population when this autosome undergoes a further mutation resulting in an aesthetically attractive perceptible phenotype.

There is one type of conspicuous "exception" which gives the appearance of contradicting the general scheme of things, described above, and which represents a paradox that needs to be resolved.

This exception is exemplified by the colour of the eye. In contemporary populations we find both dark eyed and blue eyed individuals, "blue eyes" being recessive and "dark eyes" being dominant. The eye colour is determined by a single gene locus which has a conspicuous effect on the perceptible phenotype of the individual and which must consequently also affect the "aesthetic selection" in the choice of the mate. Yet, this gene locus cannot be part of the PP linkage of genes, otherwise "dark eyes" could hardly be always dominant.

This paradox may be resolved as follows: In early prehistoric times different populations, living in geographically adjacent areas, lived in comparative isolation from each other. Let us now assume that such a population was homozygous for "blue eyes" while another population, living in an adjacent area, was homozygous for "dark eyes". Let us further assume, that at some point in time cross-breeding between these two, previously isolated, populations has occurred and has led to a mixed population which was heterozygous for eye colour. Because aesthetic selection for "blue eyes" or "dark eyes" may be assumed to be exceedingly rapid, the period of time which it would have taken for the mixed population to become homozygous either for "blue eyes" or for "dark eyes" may be assumed to have been so short as to be negligible, from an evolutionary point of view. After a short transitional period of time, the mixed population would have

become homozygous for eye colour and thereafter aesthetic selection would have again been solely guided by the PP linkage group of genes.

The contemporary populations, which include both blue eyed and dark eyed individuals have resulted from cross-breedings which have taken place comparatively recently - as the consequence of the high mobility of populations, newly acquired in historic, and late prehistoric, times.

Until I saw a way of resolving the above described paradox it has formed for me a mental block, which kept me from finding any plausible explanation for the occasional dominance of the perceptible phenotype.

References

1. Melvin M.Grumbach and Akira Morishima. Acta Cytol. 6, p. 46; 1962

2. Jacobs, P.A. et al. Lancet, 1, p. 1183; 1961

3. Jacobs, P.A. et al. Lancet, 1, p. 1212; 1962

4. Melvin M. Grumbach, Akira Morishima and J. Herbert Taylor. Proc. Nat. Acad. Sc. 49, pp. 581-592 (May) 1963

Preprint 18 May, 1964

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by

Leo Szilard
The Salk Institute for Biological Studies
La Jolla, California

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*This work was performed under a research grant, administered by The University of Chicago, of the General Medical Sciences Division of the National Institutes of Health.

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References

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4. Melvin M. Grumbach, Akira Morishima and J. Herbert Taylor. Proc. Nat. Acad. Sc. 49, pp. 581-592 (May) 1963

GM-06876-04

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Preprint

25 July 1963

THE AGING PROCESS AND THE "COMPETITIVE STRENGTH" OF SPERMATOZOA*

by Leo Szilard
The University of Chicago, Chicago, Illinois

In a paper, printed in 1959, I presented a theory of the nature of the aging process in mammals, that attributes aging to changes which take place within the chromosomes of the somatic cells.[1] I postulated in particular that in spontaneous random events, designated as "aging hits", a whole chromosome, or a large fraction of a whole chromosome, is rendered non-functional. In the case of Man I estimated an average of one aging hit over a period of six years for a somatic cell, containing 46 chromosomes (23 chromosome pairs). This theory makes quantitative predictions which, if correct, ought to be capable of experimental verification.

If one assumes that the chromosomes contained in the spermatogonia of the male do not escape such aging hits, then one may be led to entertain speculations of the following kind:

Let us assume that each spermatozoon, contained in the ejaculated semen of a male, possesses a certain "competitive strength" and that in the mating process spermatozoa which have a "competitive strength" that is markedly below normal lose out - in the competition for reaching and fertilizing a mature ovum - to spermatozoa which possess full competitive strength. Let us further assume that a spermatozoon derived from a spermatogonium which, because of the advancing age of the individual, no longer contains an intact diploid set of chromosomes, has a markedly reduced competitive strength and, accordingly, a markedly reduced chance of fertilizing an ovum in the natural mating process.

* This work was performed under a Research Grant of the National Institutes of Health

If such a selection takes place in the natural mating process, then the ova are safeguarded against being fertilized by a spermatozoon that carries a chromosome which has become non-functional as a result of an "aging hit".

It would seem of interest to test the validity of these assumptions by experiments of the following general type:

Let us assume, for the sake of argument, that we have available two strains of a mammalian species, a strain A and an isogenic strain B. Let us further assume that these two strains differ from each other with respect to one gene locus, which controls an easily visible phenotypic character and that strain A is homozygous at this locus for a dominant allele, while strain B is homozygous at this locus for an allele which is recessive.

Let us now select a young male A and a number of males B_1, B_2, B_3, who differ in age, their ages being t_1, t_2, t_3, ... One set of experiments consists in inseminating young females of strain B, who are all of the same age, with semen which is composed of a mixture of spermatozoa of two individuals, a male A and of one, or another, of the males B. Within the offspring of each female B, that has been inseminated with a mixture of spermatozoa of two individuals, those derived from the male A and those derived from one of the males B can be distinguished from each other; those derived from A show the phenotypic character of the dominant allele, while those derived from a male B (homozygous for the recessive allele) do not show the dominant phenotypic character.

What is of interest to us here is the ratio of the number of offspring of male A and number of offspring of a male B, derived from inseminations of

B females with mixtures of spermatozoa of A and B_1, of spermatozoa A and B_2, of spermatozoa A and B_3 etc. We shall designate these ratios as

$$\left[\frac{A}{B_1}\right] = \beta_1 \; ; \; \left[\frac{A}{B_2}\right] = \beta_2 \; ; \; \left[\frac{A}{B_3}\right] = \beta_3 \; ; \; etc.$$

In order to simplify the interpretation of these ratios β it is assumed that in all experiments the sperm mixtures used for insemination contain the same number of spermatozoa A and spermatozoa B.

If the males A and B_1 are of equal age and if the genetic difference between them is "irrelevant", then we ought to have $\beta_1 \approx 1$. This is what we would expect to find if the strain A is genetically identical with the isogenic strain B - except for one "irrelevant" gene locus which controls the visible phenotypic character, used for distinguishing the offspring of the male A from the offspring of a male B.

If experiments of the sort described above are carried out, we would then expect that if we use males B_1, B_2, B_3 of increasing ages t_1, t_2, t_3 etc., the ratios β will increase, and more particularly we would expect that, for equal increments of age, these ratios will increase by the same factor.

Further, we would expect that if we inseminate a number of females B with the mixed semen of the same pair of males A and B at different points in time, distributed over a time interval of a few years, the ratios β will remain the same.

On the basis of my theory of aging, one may venture to predict how the ratios β might increase for increasing ages t_1, t_2, t_3 ... t_i...t_k... of the males B. According to the theory we might expect:

$$\frac{\beta_k}{\beta_i} = e^{\gamma(t_k - t_i)}$$

For Man in particular the theory predicts for γ the value of

$$\gamma = 1/6 \text{ years}$$

For other species of mammals the theory predicts as a rough approximation for the value of γ

$$\gamma \approx \frac{100}{6 t_\infty} \sqrt{m/23}$$

where t_∞ is the lifespan of the species, expressed in years, and m is the number of chromosome pairs of the species (the number of the chromosomes in the haploid set).

For example we may expect for any species of mammals for which the chromosome number is about the same as for Man ($m \approx 23$) if the age difference, $t_k - t_i$, amounts to about one-fifth of the life-span, t_∞.

A qualitatively similar, but quantitatively different, result must be expected if a spermatozoon may possess full competitive strength, even if the spermatogonium from which it is derived does not have an intact diploid set of chromosomes, as long as the spermatozoon itself carries an intact haploid set of chromosomes. If this were the case, the values of γ would be half of the values given above.

It may be assumed that the general considerations discussed above would also hold for birds, and it is conceivable that they would also hold for reptiles.

In the case of cold-blooded vertebrates, like reptiles, it would be of interest to carry out an experiment which is not feasible in the case of either mammals or birds:

In the case of such cold-blooded animals, a male A and a male B could be maintained at temperatures which differ by, say, 10° C. If the male B, for instance, were maintained at the higher temperature, then we would expect that the ratios β would increase with time, if females were inseminated with mixtures of the sperm of the same pair of males A and B at different points in time, distributed over a time interval of a few years. For equal intervals of time the ratios β would increase by the same factor.

It must be pointed out for the sake of conceptual clarity that the concept of the competitive strength of spermatozoa must not be equated with the concept of the viability of spermatozoa. We designate a spermatozoon as viable if in the natural mating process, or in artificial inseminations which simulate the conditions of the natural mating process, the spermatozoon is capable of reaching an ovum and of giving a viable zygote by fertilizing that ovum, provided that the ovum has not already been fertilized by another spermatozoon which was faster in reaching it. One may obtain a measure of the number of viable spermatozoa, contained in a given ejaculate of a male, by inseminating females with samples of a fixed volume of highly diluted semen and determining the probability of obtaining a pregnancy as a function of the dilution factor. (See the Appendix).

It is reasonable to expect that the number of viable spermatozoa contained in the ejaculates of males may also decrease with age. The theory does not permit, however, to make quantitative predictions with the same assurance in this regard, as in regard to the decrease of the number of spermatozoa which possess full competitive strength.

For this reason, priority ought to be given to experiments with mixtures of spermatozoa of the kind described above, and arrangements for experiments

along these lines are now under discussion.

If these experiments should bear out our predictions, they would throw light on the nature of the aging process, and at the same time they would furnish an explanation as to why the male ejaculates millions of spermatozoa in the mating process when it takes only one single spermatozoon to fertilize an ovum.

.....

References:

1. Leo Szilard, Proc. Nat. Acad. Sc. (USA) Vol. 45, pp. 30-45, 1959

APPENDIX

One can obtain a measure of the number of viable spermatozoa contained per cc in an ejaculate of a given male in the following manner: One inseminates a number of females each, say, with 1 cc of the undiluted semen of that male and one determines the probability of obtaining pregnancies. This probability is then compared with the probability of obtaining pregnancies when a number of other females are inseminated with 1 cc of semen, diluted by a factor D, of the same male.

We may write for the probability, P_{conc}, for a pregnancy to result from an insemination with 1 cc of the undiluted semen of a given male

$$P_{conc} = \alpha\left(1 - e^{-n}\right) \quad \text{where } \alpha < 1 \text{ and}$$

where n denotes the number of viable spermatozoa thatreach a mature ovum, and would give a viable zygote by fertilizing that ovum, provided that no other spermatozoa have reached and fertilized that ovum earlier.

Similarly we may write for the probability, P_{dil}, of obtaining a pregnancy if 1 cc of the semen, diluted by a factor D, of the same individual is inseminated

$$P_{dil} = \alpha\left(1 - e^{-n/D}\right)$$

Because we have

$$e^{-n} \ll 1$$

we may write

$$n = D \ln \frac{1}{1 - P_{dil}/P_{conc}}$$

and for $\dfrac{P_{dil}}{P_{conc}} \ll 1$ we may write

$$n \approx D \times P_{dil}/P_{conc}$$

It should be noted that the number n is not the number of viable spermatozoa contained in 1 cc of the undiluted semen, rather it is a very much smaller number. However, n is proportional to the number of viable spermatozoa contained in 1 cc of the undiluted semen, and the factor of proportionality will be the same in all the experiments, here envisaged, provided the inseminated females are isogenic, are of the same age, and have the same physiological, as well as anatomical, vaginal, cervical, uterine, tubular and ovarian conditions.

We may directly compare, $m(E)$ and $m(F)$, measures of the number of viable spermatozoa contained in 1 cc of the semen of two different males E and F, by diluting their semen with a suitable factor D, inseminating a number of females with 1 cc samples of the diluted semen of E and inseminating a number of other females with 1 cc samples of the diluted semen of F. We may then write for the ratio of the probability $P(E)$ for obtaining a pregnancy in an insemination of the diluted sperm of E and the probability $P(F)$ of obtaining a pregnancy in an insemination of the diluted sperm of F

$$\frac{P(E)}{P(F)} = \frac{1 - e^{-m(E)/D}}{1 - e^{-m(F)/D}}$$

where $m(E)$ is a measure at the number of viable spermatozoa per cc in the undiluted semen of E, and $m(F)$ is a measure of the number of viable spermatozoa per cc in the undiluted semen of F.

In order to obtain reasonably accurate information concerning the relative numbers of viable sperm of the two males E and F from $P(E)$ and $P(F)$ it is necessary to choose the diluting factor D sufficiently high.

For sufficiently high dilutions we have

$$\frac{m(E)}{m(F)} \approx \frac{P(E)}{P(F)}$$

In order to illustrate the conceptual difference between the "competitive strength" of a spermatozoon and the "viability" of the spermatozoon, we may consider a spermatozoon that is derived from a spermatogonium in that one or several chromosomes have suffered aging hits, but carries a haploid set of chromosomes which are unaffected by aging hits. On the basis of the assumptions described above, this spermatozoon might have a markedly reduced "competitive strength" and might therefore lose out in the competition with spermatozoa which are in possession of full competitive strength, and yet conceivably this spermatozoon might be fully viable, in terms of the definition given above.

The End.

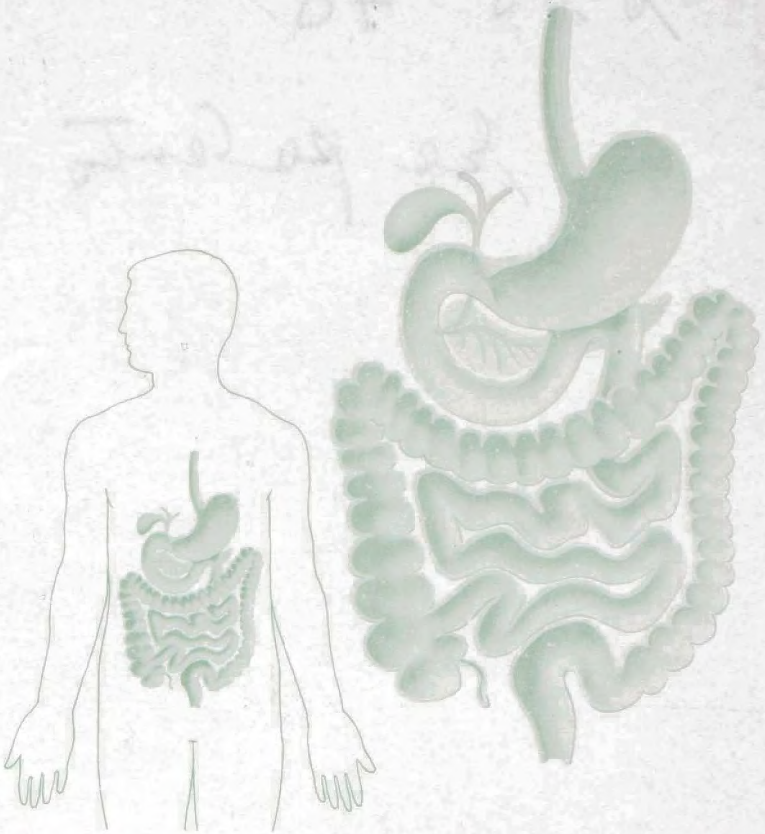
S-38 draft (14)

(3)

It is proposed to develop further a theory on enzyme repression, presented in ~~Proc. Nat. Acad. Sci. 46, 277, 1960~~ and a related theory on antibody formation, presented in Proc. Nat. Acad. ~~Sci. 46, 293, 1960~~. In their present form both of these theories are based on the "premise" that the repressor controls the rate at which the protein forming site forms a specific protein, rather than the rate at which this site itself is formed. The theory on antibody formation is based on the postulate that the repressor is capable of combining with the specific antibody and it explains on the basis of this postulate - which is independent of the above-mentioned "premise" - the production of antibody in the primary response. The theory leans, however, on this "premise" for the explanation of immune tolerance in the new-born rabbit. It is proposed to evaluate experiments which are in progress (in a number of different laboratories) in order to determine if the above-mentioned "premise" may have to be abandoned. With this possibility in mind, conceivable alternative explanations of immune tolerance will be explored. (1961)

bbs 48

Les parents



for the spasm reactors in your practice