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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
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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)

Bac

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

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.

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 into quantitative relationships which can be checked against data obtained from available observations or experiments as yet to be made. The proposed work would take as its starting point preliminary theoretical studies which I carried out in the past three years.

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

I anticipate that my work will have a strong theoretical orientation. But in order to be able to function as a theoretical biologist, it is necessary to have intimate knowledge of experiments relating to a variety of biological materials and involving diverse techniques.

Biology has not quite reached the stage which was attained by physics half a century ago when enough relevant facts were established to permit a theoretical physicist to come up with significant insights. Yet in biology we might be very well on the verge of a similar situation, and a few scientists who are so inclined may now perhaps attempt to function as theoretical biologists. Accordingly, these days it might be well for a few scientists to put less emphasis on their own experiments and spend more time trying to keep in touch with the experiments of others in the hope of being able to recognize new patterns and to try to gain insight into some general biological laws. It may be that the main difference between the theoretical physicists of the past and the would-be theoretical biologist of the present is quantitative rather than qualitative. The would-be theoretical biologist would probably not be able to keep on studying the results of others and thinking about them for a very long stretch of time. Much sooner than a theoretical physicist, he will feel impelled to do further experiments (or to induce someone else to do them) because he will need to cut down the number of possible avenues along which his further thinking may be tempted to wander.

How successfully a man may be able to function as a theoretical biologist is likely to depend (apart from his inclinations and abilities) on whether he is put in the position where he can maintain close cooperations with a number of laboratories, initiate some new experiments or observations, and perhaps participate actively in experimental work carried out in laboratories where he holds no position of administrative responsibility.

On a rather limited scale I have tried, in the last few years, to function somewhat as such a theoretical biologist. Below I list a number of 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.

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

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 his co-workers and 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.

- -

When in 1946 I was faced with the task of converting myself into a biologist, I teamed up with Dr. Aaron Novick, 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 Dr. Delbrück's course in Cold Spring Harbor in bacterial viruses, and Dr. Van Niel's course in bacterial biochemistry at Pacific Grove. Dr. Novick and I worked as a team until ~~xxxxxxxxxxxx~~ the Institute of Radiobiology and Biophysics was dissolved.

A list of publications is attached, containing a short description of each paper. 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 very interesting results (18) but decided to shift after a while to the study of bacteria.

The two phenomena in which we were particularly 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, with strong emphasis on molecular biology. The paper I published most recently (#25) attempts to give a quantitative theory of the process of aging which should be applicable to mammals.

RG 6876

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 universally 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. 20

(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 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 by Fermi in the same year 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 chain reaction qualities of 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, insofar as these matters can be made public, 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 today the use of the Chemostat appears to be indispensable.

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 of longevity of individuals are attributed to the difference of the number of defective "vegetative genes" they have inherited.

August 17, 1960.

Miss Irene Fagerstrom,
Room 603,
5801 Ellis Avenue,
Chicago 37, Illinois.

Dear Miss Fagerstrom,

I am enclosing the application for continued support of my research grant RG-6876(C1). I wondered whether you could complete the forms which I am enclosing, file them with N.I.H. and notify me that this has been done. The deadline is September 30, but I am anxious that the application be filed as soon as possible. If necessary, you can call me over the telephone, collect, at extension 133, TRafalgar 9-3000 in New York City. My mail address is Room 812, The Memorial Hospital, 444 East 68th Street, New York 21, N.Y.

(1) I was not able to fill in on page 2 (Progress Report) the Statement of Accomplishment, because my Statement is too long and I had no continuation sheets available. I am therefore attaching to this letter my Statement of Accomplishment, with the request that your office copy it on page 2 of the application form. It will take one or two continuation sheets to accommodate my Statement. The rest of page 2 should be filled in also by your office.

(2) I am enclosing a letter addressed to the Division of Research Grants which I have signed. You will find enclosed two signed copies and one unsigned copy for your files. One of these signed copies is typed on a sheet sent to me by N.I.H., the other is typed on the stationery of the University of Chicago. Please forward to N.I.H. the one which you deem more appropriate.

(3) I am enclosing page 1 of the application form which needs to be filled in by your office.

(4) I am further enclosing the Notice of Research Project. I have filled in the Summary of Proposed Work, but your office would have to fill in the rest.

Another point, I wonder whether you could call up Mrs. Noreen Mann in the Research Institutes at extension 3715 and ask her to furnish you with ten copies each of the three papers which I published this year. Two appeared in the Proceedings of the National Academy of Sciences and one appeared in Nature. These ought to be forwarded to the Grant Division of N.I.H. with a covering letter indicating the reference number of my grant.

With best wishes,

Yours very sincerely,

Leo Szilard

Enclosures

August 20, 1960.

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

Gentlemen:

Attached is an application for continued support of research grant, RG-6876(C1).

The papers listed below have been published during the current year as a result of work done under this grant. Ten reprints of each paper have been submitted or will be furnished as soon as they are available.

Sincerely yours,

Leo Szilard

Publications

(1) Leo Szilard "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.

(2) Leo Szilard "The Molecular Basis of Antibody Formation", Proceedings of the National Academy of Sciences, Volume 46, p.293 (March) 1960.

(3) Leo Szilard "Dependence of the Sex Ratio at Birth on the Age of the Father", Nature, Volume 186 pp.649-650, (May) 1960.

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30 August 1960

AIR MAIL

Dr. Leo Szilard
The Memorial Hospital, Room 812
444 East 68th Street
New York 12, New York

Dear Dr. Szilard:

Enclosed for your information is copy of the renewal application covering the period indicated (January 1, 1961 to December 31, 1961) for your NIH grant.

The application was signed by Mr. Harrell and mailed from here with all enclosures (including reprints) on Friday afternoon, August 26.

I have requested NIH to acknowledge receipt to this office. Duplicated copies of the application will be sent to you by NIH within the next few weeks. It will be helpful if we may be supplied with two copies. Also, notification of the grant award will be sent directly to you by NIH. Again, will you kindly see that copies are sent to me in order that we may expedite processing of the grant at this end (advice to Comptroller's Office, etc.).

Please let me know if you have any questions.

Sincerely,

Irene E. Fagerstrom

Irene E. Fagerstrom
Assistant Business Manager
(Special Projects)

Enclosure

cc: Mrs. N. Mann w/o encl

PHS-2590
Rev. 7-58

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RG-6876(C1)

☒ **APPLICATION FOR PREVIOUSLY RECOMMENDED YEAR OF RESEARCH GRANT SUPPORT**

☐ **APPLICATION FOR PREVIOUSLY RECOMMENDED YEAR OF TRAINING GRANT SUPPORT**

Date August 20, 1960

Application is hereby made for the previously recommended renewal grant in the amount of $ 26,735

for the period from January 1 1961 through December 31 1961 inclusive
Month Day Year Month Day Year

for the purpose of continuing a research project or training program on the following:
(Limit to 53 typewriter spaces)

TITLE OF RESEARCH PROJECT
OR AREA OF TRAINING Quantitative Studies of Cereal Biological Phenomena

Name, Title & Address of Principal Investigator or Program Director:

Leo Szilard
Professor of Biophysics
1155 E. 57th Street
Chicago 37, Illinois

Name & Title of any Co-principal Investigator(s) or Program Director(s):

Name of School and Department:

The University of Chicago
Enrico Fermi Institute for Nuclear Studies

Name, Title & Mailing Address of Financial Officer to Whom Check Should be Mailed:

Albert P. Cotton
Bursar
The University of Chicago
5801 S. Ellis Avenue
Chicago 37, Illinois

PROPOSED BUDGET		NO.	AMOUNT
Personnel:			$
a. Professional		1	12,748
b. Other			
Training stipends:			
a. Predoctorate			
b. Postdoctorate			
c. Other			
Permanent Equipment			
Consumable Supplies			
Travel:	Domestic		7,500
	Foreign		
Other Expenses			4,000
Sub Total			$ 24,248
Indirect Cost (Overhead)			2,487
TOTAL REQUEST (may not exceed the amount previously recommended for this period)			$ 26,735

AGREEMENT: It is understood and agreed by the applicant that the terms and conditions set forth on the face sheet of the original grant request which resulted in the activation of this project or program will apply to the awarding of any funds under this application for continued support.

NAME OF INSTITUTION THE UNIVERSITY OF CHICAGO

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION

W. D. Harvell
Vice President - Business Affairs

(Leave blank) NIH record of additional years of support	
1st $	
2nd	
3rd	
4th	

Form Approved
Budget Bureau No. 68-R 249.7

☆ U. S. GOVERNMENT PRINTING OFFICE: 1958-475983

PHS-2590
Rev. 7-58

PROGRESS REPORT

Grant No. H-6876(C1)
(Repeat number shown on Page 1.)

Principal Investigator or Program Director Leo Szilard Institution THE UNIVERSITY OF CHICAGO

Title of project or area of training: Quantitative Studies of General Biological Phenomena
(Repeat title shown on page 1)

STATEMENT OF ACCOMPLISHMENT COVERING PERIOD 1 Jan 1960 thru 30 Sept 1960

A model for the control of the rate of production of repressible enzyme 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. 689-690 (May) 1960, which is based on a theory of ageing, previously presented by the author, (Proc. Nat. Acad. Sc. 45, 30. 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 Y-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.

Form Approved
Budget Bureau No. 68-R 249.7

☆ U. S. GOVERNMENT PRINTING OFFICE: 1958-475983

THE UNIVERSITY OF CHICAGO
CHICAGO 37 · ILLINOIS

BUSINESS MANAGER · SPECIAL PROJECTS
5801 ELLIS AVENUE

7 September 1960

Dr. Leo Szilard
The Memorial Hospital, Room 812
444 East 68th Street
New York 12, New York

Dear Dr. Szilard:

Your application for renewal of your NIH grant was acknowledged by the Division of Research Grants on 30 August 1960.

As stated in my letter of 30 August 1960, duplicated copies should reach you in a few weeks. We shall appreciate receiving two copies of it and of the grant award as they are received.

Sincerely,

Irene E. Fagerstrom

Irene E. Fagerstrom
Assistant Business Manager
(Special Projects)

enclosure

THE UNIVERSITY OF CHICAGO
CHICAGO 37 · ILLINOIS

BUSINESS MANAGER · SPECIAL PROJECTS
5801 ELLIS AVENUE

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The Memorial Hospital, Room 812
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Irene E. Fagerstrom
Assistant Business Manager
(Special Projects)

enclosure

THE UNIVERSITY OF CHICAGO
CHICAGO 37 · ILLINOIS

BUSINESS MANAGER · SPECIAL PROJECTS
5801 ELLIS AVENUE

August 14, 1961

Re: RG-6876-C2

Dr. Leo Szilard
Hotel Dupont-Plaza
DuPont Circle
Washington 6, D. C.

Dear Dr. Szilard:

We have received via Mrs. Mann the materials sent by Public Health Service pertaining to the renewal of your grant. You will recall that last year you prepared the renewal proposal (with exception of budget estimate) and forwarded the material to us for completion and transmittal to Public Health.

We shall be pleased to follow the same procedure this year and accordingly are forwarding to you the forms.

Please return all copies to this office. At the time we transmit them to PHS we will send you copies for your file.

Sincerely yours,

Irene E. Fagerstrom

Irene E. Fagerstrom
Assistant Business Manager
(Special Projects)

Enclosures
1. Instructions for Preparing...
2. Notice of Research Project
3. Progress Report
4. Application
5. Transmittal Letter
6. Acknowledgement Card

cc: Mrs. Norene Mann

September 6, 1961

Miss Irene E. Fagerstrom
Room 603
5801 Ellis Avenue
Chicago 37, Illinois

Dear Miss Fagerstrom:

Enclosed I am returning to you the grant application in which I filled out the scientific parts. I should greatly appreciate your putting in all the rest, including the title of the project, its number, etc. I am going to mail you, within a few days, ten copies of a paper to which there is reference made in one of the documents which are enclosed. These ten copies should be forwarded to Washington, together with the application. I should greatly appreciate your advising me, through Mrs. Norene Mann, when the application has been completed by you and sent off to Washington.

When I am in Washington I am always staying at the Hotel Dupont Plaza and you can reach me there over the telephone. However, I expect to go to a meeting in Vermont in a few days and I might be away from Washington for two or three weeks. I shall telephone you, however, from wherever I may be just to find out whether everything is in order.

With best wishes.

Yours sincerely,

Leo Szilard

Enclosures

THE UNIVERSITY OF CHICAGO
CHICAGO 37 • ILLINOIS
THE ENRICO FERMI INSTITUTE
FOR NUCLEAR STUDIES

File: Grants

August 3, 1962

Dear Dr. Szilard,

As you will see from the enclosed form letter, the Public Health Service wishes to know whether the proposed indexing terms, listed on the sheet accompanying the letter, meet with your approval. Would you be interested to see the RESEARCH GRANTS INDEX itself, referred to in the letter?

The enclosed copy of a letter I sent to Miss Fagerstrom is self-explanatory, and I assume that you will receive from her in due course these various forms for your signature.

With kindest regards, (also to Mrs. Szilard),

Very sincerely yours,

Noreene Mann

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE BETHESDA 14, MD.

NATIONAL INSTITUTES OF HEALTH
OLiver 6-4000

AUG 1 1962

TO: Recipients of National Institutes of Health Research Grants

FROM: Chief, Division of Research Grants Dale L. Lindsay

In May 1962, the Division of Research Grants, National Institutes of Health, instituted a new annual publication, the RESEARCH GRANTS INDEX, covering Public Health Service research grants for fiscal year 1961. If you have not yet received your copy of the INDEX (PHS Publ. No. 925), we will be glad to send one on request.

The format of the 1962 RESEARCH GRANTS INDEX, now in preparation, has been changed so that it is similar to that of the enclosed SAMPLE INDEX (PHS Publ. No. 876). In the 1962 volume your current research grant will appear under the subject headings shown on the attached list, which you may retain for your files. After you have reviewed these proposed indexing terms, please complete the spaces below and return this form in the enclosed envelope. Should you indicate changes, we will incorporate your suggestion verbatim wherever feasible; otherwise, we will represent the concepts as closely as possible.

☐ The indexing terms submitted for my review satisfactorily describe my current PHS project No. GM-06876-3, and may be used for the 1962 Research Grants Index.

☐ I suggest the following changes in the indexing terms:

ADD:

DELETE:

Signature of Principal Investigator

Please list below names of Co-Investigators:

yes ☐ no ☐ I would like to receive a copy of the next edition of the Research Grants Index

GM-06876-3 FEW August 1, 1962

transfer ribonucleic acid..enzymes feedback..immunity..proteins..enzymes inhibitors..~~xxxxxxxxx~~
biosynthesis

proteins..transfer ribonucleic acid..enzymes inhibitors..enzymes feedback

enzymes inhibitors..transfer ribonucleic acid..proteins

enzymes feedback..transfer ribonucleic acid..proteins

immunity..enzymes feedback..transfer ribonucleic acid

biosynthesis

mjs

August 3, 1962

Miss Irene E. Fagerstrom
Assistant Business Manager -
Special Projects
Administration 603
Faculty Exchange

Dear Miss Fagerstrom:

Below are listed various enclosures pertaining to continuation of Dr. Szilard's research project, just received from the National Institutes of Health. I am enclosing these forms herewith, assuming you will want to prepare the first six, at least, for Dr. Szilard's signature. Although the last three (i.e. 7), 8), and 9)), should go to Dr. Szilard for completion, I thought it best not to forward them separately just now, but to keep everything together, so that all the forms can be returned to the Public Health Service at the same time.

1) Instructions for preparing application for previously recommended year of research project grant support on Form PHS-2590.

2) Form PHS-2590; i.e. Application for previously recommended year of research grant support, page 1. (10 copies)

3) Form PHS-2590; page 2 (10 copies)

4) Form PHS-2590 Summary Progress Report (10 copies)

5) Covering letter for above application for continued support (1 copy)

6) Budget Work Sheet for PHS-2590 (1 copy)

7) Form PHS-3945: Definition of Terms used in annual invention statement (1 copy)

8) Form PHS-3945: Procedure for submission of annual invention statement (1 copy

a) Annual invention statement on public Health Service grant or award Form 3945 (3 copies)

9) Form PHS-166: Notice of Research Project (Summary of Proposed Work).

Sincerely yours,

Secretary to
Dr. Leo Szilard

Encl.

THE UNIVERSITY OF CHICAGO

DATE August 7, 1962

TO Miss Shirley Cherin DEPARTMENT Galef & Jacobs, New York 16, N.Y.

FROM Howard L. Zarse DEPARTMENT Comptroller, Govt. Accounts

IN RE: USPHS Medical Research #629
Principal Investigator, Dr. Leo Szilard
Account Code: 3-5420-00-3469

Dear Miss Cherin;

Enclosed is original of final report covering the above referenced account for funds received from the United States Public Health Service under Grant No. RG 6876-C1.

Will you please have Dr. Szilard sign the report in the space provided in the lower left hand corner of page one and return the report to this office for transmittal to the United States Public Health Service.

Your prompt attention to the above request will be greatly appreciated.

Yours truly,

H. L. Zarse

Supervisor, Government Accounts

HLZ:ay
Enclosure

September 17, 1962

Miss Irene E. Fagerstrom
Business Manager's Office
University of Chicago
5801 Ellis Avenue
Chicago 37, Illinois

Dear Miss Fagerstrom:

Enclosed you will find the letter of transmittal for my grant application and eleven copies of a preprint (of which ten go with it). Please hold this material until you receive the rest of the grant application.

With best wishes,

Sincerely yours,

Leo Szilard

Encls.

September 17, 1962

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

Gentlemen:

Attached is an application for continued support of research grant, RG-6876-C2.

The enclosure listed below has been completed during the current year as a result of work done under this grant. Ten preprints of this manuscript are enclosed.

Sincerely yours,

Leo Szilard

Enclosure:

Leo Szilard "The Sex Chromatin in Mammalian Cells, 'Dosage Compensation' in the Fruit Fly, and Enzyme Repression in Bacteria" (September) 1962.

S-38

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
		GB-2878-04

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 3-5601

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

$ 27,673

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 major 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

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

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER CA-08076-04			
	(1)		(2)	(3)
DETAILED BUDGET FOR PERIOD SHOWN ON PAGE 1	Time Spent on This Project		AMOUNT REQUESTED (OMIT CENTS)	ESTIMATED TOTAL CURRENT YEAR EXPENDITURES
	Percent of Time	Hours Per Week		
1. PERSONNEL: (List all positions and indicate time spent on project.)				
PRINCIPAL INVESTIGATOR Leo Gallard	100%		$ 12,000	
				$ 12,265
Employee Benefits, academic (11.8% of $8,250 and 3.2% of $3,750			1,084	
2. MOVABLE EQUIPMENT: (ITEMIZE) (Identify any proposed item of equipment requested in an earlier application but not yet purchased and any item of equipment which duplicates equipment already available. Provide justification for present need.)				$
3. CONSUMABLE SUPPLIES: (Itemize)				$
4. TRAVEL (Itemize)				
Travel to major research centers in The United States and Institute Pasteur			7,500	$ 7,672
5. OTHER EXPENSES ON WHICH INDIRECT COSTS ARE ALLOWED				
			3,067	$ 2,850
6. SUB-TOTAL ITEMS 1 THRU 5			$ 24,001	
7. INDIRECT COST ALLOWANCE ON ABOVE ITEMS (SEE INSTRUCTIONS)			$ 3,669	
8. EXPENSES ON WHICH INDIRECT COSTS ARE NOT ALLOWED OR MUST BE NEGOTIATED. (Note that funds may not be used for alterations and renovations without prior PHS approval. See instructions regarding indirect costs.)				$
9. TOTAL BUDGET (Same as amount shown on Page 1 of application)			$ 27,670	

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER
SUMMARY PROGRESS REPORT	GM-05376-04
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 these 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.

FORM APPROVED; BUDGET BUREAU 68-R773

PHS-3945
1-62

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

ANNUAL INVENTION STATEMENT ON PUBLIC HEALTH SERVICE GRANT OR AWARD

I hereby certify that, to the best of my knowledge and belief, all inventions are listed below which might possibly be construed in any manner to be Public Health Service grant or award supported or related and which were conceived and/or reduced to practice, or made the subject of patent application by persons engaged in the performance of work under Public Health Service grant or award

No. RG-6876, for the period 1 Sept 1961 through 31 August 1962

(For General Research Support grants the Annual Invention Statement would include only those inventions related to a specific research project aided by such funds. If no inventions have been made under any Public Health Service grant or award, insert the word "None" under Title of Invention.)

NAME OF INVENTOR	TITLE OF INVENTION	DATE REPORTED TO PHS
	None	
	—	
	—	

Use Continuation Sheet if Necessary

Signature, in ink, is required in the space provided below, appropriate to the type of grant or award being supported: SIGNATURE OF INSTITUTIONAL OFFICIAL REQUIRED IN ALL INSTANCES.

TYPE OF GRANT OR AWARD	SIGNATURES
1. FOR A RESEARCH GRANT X	Leo Rifkind (PRINCIPAL INVESTIGATOR OR PROJECT DIRECTOR)
2. FOR A TRAINING GRANT	(PROGRAM DIRECTOR)
3. FOR THE RESEARCH CAREER AWARD PROGRAM	(AWARDEE)
4. FOR A FELLOWSHIP AWARD	(a) (FELLOW)
	(b) (SPONSOR)
5. FOR A GENERAL SUPPORT GRANT (SEPARATE INVENTION STATEMENT FOR EACH IDENTIFIABLE RESEARCH PROJECT)	(PRINCIPAL INVESTIGATOR)

APPROVED:

SIGNATURE (INSTITUTION OFFICIAL RESPONSIBLE FOR PATENT MATTERS): Irene E. Fagerstrom — DATE: 9/28/62

TITLE: Irene E. Fagerstrom
Assistant Vice President
(Special Projects)

MAILING ADDRESS: The University of Chicago
5801 South Ellis Avenue
Chicago 37, Illinois

file grant

May 21, 1964

Mr. W.R. Rossman
Office of the Comptroller
The University of Chicago
Chicago 37, Illinois

Dear Mr. Rossman:

I am returning you enclosed the document which you have sent me for signature - with my signature.

Yours sincerely,

Leo Szilard

LS:jm

Enclosure

September 9, 1962

(Preprint.)

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

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

---

*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.

~~Leo Szilard~~ September 9, 1962

(Preprint.)

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

by Leo Szilard
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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 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 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 ~~are~~ is the same. 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.

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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.

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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

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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 ~~responsible for~~ the repressor of a sex linked enzyme, which shows ~~showing~~ "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:

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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 chromosomes of the female becomes suppressed in these cells.

References:

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In mammals, as 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 ~~normal~~ male and the ~~normal~~ 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 ~~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 these 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 problem.

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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.

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(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.

To be inserted on page 2 of Progress Report

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

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