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A THEORY OF AGEING

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It is a direct consequence of this theory that, in the author's words : "The main reason why some adults live shorter lives and others live longer is the difference in the number of faults they have inherited". This is the first consequence of the theory which is contradicted by observations on *Drosophila*. In so far as differences in adult longevity are genetically determined, by far the largest differences are those between inbred and outbred individuals[2,3]. F_1 hybrids between inbred lines live for longer than do the parental lines (sometimes for twice as long). Outbred and genetically variable wild populations have approximately the same expectation of life as do F_1 hybrids. Now inbreeding increases the proportion of loci at which individuals are homozygous. An individual which survives for an appreciable time as an adult cannot, by definition, be homozygous for a fault. Therefore inbred individuals which survive to become adults, and which do not die immediately after emergence, are not homozygous for faults at any loci, and would be expected to be heterozygous for faults at fewer loci than are members of outbred wild populations. If two inbred lines are crossed, the F_1 hybrids would be expected to carry a load of faults intermediate between the loads carried by the parental lines. Thus according to Szilard's

theory, inbred lines should have a higher expectation of life than wild populations, and F_1 hybrids between inbred lines should be intermediate between their parents. Neither of these predictions is in fact true.

Further, since males have only a single *X* chromosome, any hit on that chromosome in a male would render the cell inviable, whereas in a female not heterozygous for a sex-linked fault both *X* chromosomes must be hit before a cell becomes inviable. Therefore females should live longer than males. This again is not the case in *D. subobscura*. In some strains females do live longer than males, but in other strains, both inbred and outbred, the reverse is true. This point is particularly telling since in *Drosophila* the sex chromosomes account for about one-fifth of the total chromosome material.

The other group of facts which are inconsistent with the theory concern the rate of ageing at different temperatures[4]. Female *D. subobscura* of a particular strain have an expectation of life of about 56 days at 20° C. and of 18 days at 30·5° C. The changes responsible for death at 30·5° C. are not repaired or reversed in individuals kept for a time at 20° C. Consequently the changes responsible for death at both temperatures can properly be regarded as ageing processes. If these processes were, at each temperature, those postulated by Szilard, differing only in the rate at which hits occur, it follows that individuals kept for an appreciable time at 30·5° C. should have, when returned to 20° C., an expectation of life at that temperature lower than that of individuals of the same chronological age not previously exposed to 30·5° C. In fact, exposure to 30·5° C. for periods of the order of half the expectation of life at that temperature does not alter the further expectation of life at 20° C. of males, and significantly increases that of females.

Hence, if, despite the genetic evidence to the contrary, we assume that ageing at 20° C. is due to random hits on chromosomes, then ageing at 30·5° C. cannot be explained by the same process proceeding at a higher rate. In other words, either at 20° C. or at 30·5° C. ageing must be due to a process different from that postulated by Szilard ; it is possible, and in my view likely, that such a process is not primarily responsible for ageing at either temperature.

It is perhaps unreasonable to criticize a theory intended to explain ageing in mammals by quoting observations on insects. Unfortunately the temperature experiments cannot be repeated on a homiotherm. But there is some evidence[5] in mice, as well as in *Drosophila*, that inbred individuals do not live

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At present there is no evidence that a gene may be responsible for anything except for the production of a specific protein molecule which might be endowed with a specific enzymatic activity. In a wild population, a given gene may be present in the form of a variety of alleles and the corresponding enzymes may differ in their turnover number. For the purposes of discussion here, I shall call an allele 'weak' if the turnover number of the corresponding enzyme is small. If this turnover number is very small, the allele might be a recessive lethal. A completely homozygous strain is, of course, free of recessive lethals, but it may contain a number of 'weak' alleles.

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an individual belonging to the wild-type strain may die at an age at which f, the 'surviving' fraction of its somatic cells, has fallen to about $f^* = \frac{1}{7 \cdot 4} \approx \frac{1}{e^2}$.

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where x_r is the number of hits at death ; r is the number of the inherited faults ; $m = 23$ is the number of chromosome pairs and f^* is the surviving fraction of the somatic cells at the age of death.

The most probable age at death, t_r, is given by : $t_r = 6 \times x_r$ years.

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For the F_1 hybrid we obtain t_r, the most probable age at death, by writing : $r = 0$ and $\ln \frac{1}{f^*} \approx 2$. We thus obtain $t_r = 93 \cdot 5$ years. This is 12 years more than the value for the wild type.

It may thus be seen that a substantially shortened life expectancy of the homozygous, inbred strain, as compared with the wild type, need not be inconsistent with the theory. However, an increased life expectancy of the F_1 hybrid as compared with the wild type strain is a necessary consequence of the theory.

This consequence of the theory could be tested by experiments on short-lived mammals, say mice. In order to render the experiment more sensitive, one may first expose to ionizing radiation a population of wild type mice over several generations and may thereby increase the number of faults in the population. Starting with such a 'wild' population, enriched in faults, one would then select two unrelated families and derive from them two inbred homozygous strains. The theory demands that the F_1 hybrid of these two inbred strains should live appreciably longer than the population from which the two families were selected. Given a suitable opportunity, I propose to arrange for experiments of this sort. A

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Laboratory. Contact with the universities was maintained, but no details of any extra-mural contracts with universities are given in the report. Six members of university staffs were appointed as vacation consultants during the summer months, and nineteen vacation students worked at the Laboratory.

Two open days were again held, and of the 7,900 people invited, 3,250 attended. Two international symposia were held during the year. The ninth in the series, which took place during June 4–6, dealt with the physical chemistry of metallic solutions and intermetallic compounds, and the tenth, on "The Mechanization of Thought Processes", during November 24–27, evoked such considerable interest that the attendance had to be limited to the two hundred who could be accommodated. The proceedings of the symposium on visual problems of colour, held in September 1957; No. 19 in the series of Notes on Applied Science entitled "Signal Generators, Attenuators, Voltmeters and Ammeters at Radio-Fre-

inspected the Laboratory and appeared generally satisfied with the work being done, but expressed concern at the possibility of overlap of work and facilities between the Ship Division of the Laboratory and the Admiralty in relation to research on hull and propeller design. There is formal liaison between the two establishments through the Froude Ship-Research Sub-committee, and informal liaison between the superintendent of the Division and senior officials in the Admiralty.

A building plan for the long-term development of the Laboratory site is being considered in consultation with the Ministry of Works, and a centre, with lecture, conference and restaurant facilities, has at long last been authorized. A new physics building and a building properly equipped for the mechanical working of difficult materials have been included in the 1959–64 proposals. Reference is also made to the Ship Hydrodynamics Laboratory at Feltham [which was opened by the Duke of Edinburgh on October 19, see p. 926 of this issue of *Nature*].

YELLOWSTONE PARK EARTHQUAKE

ON August 18 an earthquake occurred at 06h. 37m. 13s. G.M.T. from an epicentre near the western boundaries of Yellowstone National Park (epicentre lat. 44½° N., long. 111° W.). The earthquake had a magnitude of 7·1 or rather greater on the Richter Scale. The shock appears to have had a normal depth of focus. Most damage appears to have been caused by the earthquake near the Hebgen dam in south-western Montana. The dam is built at an altitude of 6,000 ft. and holds up a lake some 37 miles long in a narrow canyon, through which flows the Madison River. The dam is 87 ft. high and 718 ft. long. In addition to cracking parts of the base of the floor of the lake and producing minor cracks in the earth and rock section of the dam, the earthquake started a huge wave in the lake, which threatened further damage. Fortunately, the wall held fast. The surge of water in the lake and canyon is reported to have caused an air blast which stripped the clothing from one person.

Some seven miles below the dam, landslides from an 8,000 ft. high mountain blocked the road and the river. The road and the surrounding countryside were severely fissured. Roads leading into the western side of Yellowstone Park were closed. Perhaps two hundred people, including ranchers, campers, fishermen and tourists in about fifty cars, were trapped between the landslide and the lake. Ten people are reported to have been killed and sixty injured. Telephones and electric power installations were out of action. Buildings shifted on their foundations, chimneys fell and fuel pumps toppled over.

The elastic waves of the earthquake were recorded by seismographs at observatories throughout the world. At Kew Observatory the provisional readings are:

eP	06 hr.	48 min.	11 sec.	G.M.T.
iS	06 hr.	57 min.	13 sec.	G.M.T.
MH	07 hr.	18 min.	00 sec.	G.M.T. amplitude 570μ
F	12 hr.	00 min.	00 sec.	G.M.T.

In the past the regions most affected seismically in this part of the United States have been east of Helena, Montana (lat. 46° N., long. 111·2° W.), and north-east of the centre of Helena (46·6° N., 112° W.).

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CROSS-LINKING OF DEOXYRIBONUCLEIC ACID IN SPERM HEADS BY IONIZING RADIATIONS

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Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, S.W.3

IRRADIATION with X-rays of deoxyribonucleic acid in dilute aqueous solution leads to a reduction in the size of the molecule due to attack by hydroxyl radicals[1]. Irradiation of the solid acid as the sodium salt was claimed by us to reduce the molecular weight[2] and we wrongly concluded (see below) that ionizing radiations, whether acting directly or indirectly via free radicals from water, produce breaks in the main chain. Since *in vivo* deoxyribonucleic acid is conjugated with protein, nucleoprotein obtained from the sperm of fish was irradiated and attempts were made to isolate the deoxyribonucleic acid so as to measure its molecular weight and to see if its radiosensitivity was affected by the presence of proteins. Sperm heads were chosen for these experiments since they contain essentially only deoxyribonucleic acid and protamine. They can be prepared without denaturation as they take up only a few per cent of water and no break up of the native configuration occurs due to swelling. After the nucleoprotein complex has been dissociated in 2 *M* sodium chloride, deoxyribonucleic acid can be isolated in a very pure form (less than 0·1 per cent of protein contamination) by precipitating the protamine by the usual procedure[3] with an anionic soap, sodium dodecyl sulphate. The detergent–protamine complex is removed by centrifugation at 20,000*g* for 30 min. If the sperm heads are obtained from viable sperm by cytolysis at temperatures below 4° C., the recovery of deoxyribonucleic acid is quantitative (better than 95 per cent).

Following irradiation by 20,000–1,000,000 rads with 1-MeV. electrons from a Van de Graaff machine, the sperm heads dissolved apparently completely in 2 *M* sodium chloride, but after the removal of the protamine complex it was found that a substantial fraction of the deoxyribonucleic acid had been lost. In this dose range, no deoxyribonucleic acid was lost if the solution in 2 *M* sodium chloride was centrifuged at 20,000*g* for 2 hr. It was found that the loss of deoxyribonucleic acid was related to the dose as shown in Fig. 1. No significant difference was found between sperm-heads from salmon, trout and herring; and moreover, the same effect was obtained if viable whole sperm were irradiated in their seminal fluid and the nucleoprotein isolated after irradiation.

Evidence for Cross-linking

A possible reason for the loss of deoxyribonucleic acid on the addition of the detergent is that some of the protamine is chemically linked by the radiation to the deoxyribonucleic acid so that it, too, is involved in the detergent-complex[4]. But all attempts to demonstrate such a combination have failed. Thus the deoxyribonucleic acid was precipitated quantitatively from the dispersion of sperm heads in 2 *M* sodium chloride by the addition of a polyvalent cation, lanthanum chloride, and the precipitate analysed for protein by paper chromatography. No differences could be detected between the control and irradiated samples, though the latter 'lost' 30–50 per cent of their deoxyribonucleic acid on the addition of the detergent and neither contained more than 0·5 per cent protein. The best evidence that there was no combination with protein was obtained by isolating the deoxyribonucleic acid by ultracentrifugation. In a preparative 'Spinco' the deoxyribonucleic acid from a solution of sperm heads in 2 *M* salt (concentration of deoxyribonucleic acid 0·03 per cent)

Barr.

A Note on Szilard's Theory of Ageing

by

Leo Szilard

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In an interesting letter to the Editor appearing in this issue of "Nature" under the above title, J. Maynard Smith refers to the theory of the ageing processes in mammals which I recently proposed ("On the Nature of the Ageing Process", Proc. Nat. Acad. of Science, U.S.A., 45: pp. 30-45, 1959), and he cites observations which may appear to contradict this theory.

All of the observations quoted by Smith relate to fruit flies and they fall into two classes: observations which we may expect to be able to duplicate in the case of mammals and those which we may not. Since I do not propose to discuss here whether the theory might or might not be extended to insects, I am primarily concerned with the former of the two classes.

Smith states that a genetically variable, wild, population of fruit flies has a substantially higher life expectancy than inbred, fairly or wholly homozygous, strains derived from it. He also states that the F_1 hybrid obtained by crossing two different inbred strains has a substantially higher life expectancy than the two inbred strains themselves. Smith holds that these findings are not compatible with the theory of ageing that I proposed.

It is probably true that the observations quoted above could be duplicated with mammals and I am quite prepared to accept

this thesis for the sake of argument. As I shall presently show, however, my theory does not preclude that the homozygous inbred strains may have a substantially smaller life expectancy than the wild type strain. Furthermore, the theory demands, that the life expectancy of the F_1 hybrid be appreciably higher than that of the wild type strain, if the wild type strain carries a substantial number of faults. In order to see this, we may consider the following:

At present there is no evidence that a gene may be responsible for anything except for the production of a specific protein molecule which might be endowed with a specific enzymatic activity. In a wild population, a given gene may be present in the form of a variety of alleles and the corresponding enzymes may differ in their turnover number. For the purposes of our discussion here, we shall call an allele "weak" if the turnover number of the corresponding enzyme is small. If this turnover number is very small, the allele might be a recessive lethal. A completely homozygous strain is, of course, free of recessive lethals, but it may contain a number of "weak" alleles.

For the purposes of our discussion here, we shall adopt a somewhat over-simplified picture and we shall disregard the possibility that the enzyme levels in the somatic cells may be determined to some extent by the regulatory mechanisms of the cell, through enzyme induction or otherwise. On this over-simplified basis, we may then say, that the somatic cells of an inbred strain, which is homozygous for a number of "weak" alleles, are impoverished in the corresponding enzymes as far as their bio-chemical activity is concerned.

Our theory assumes that only a small fraction of the enzymes, less than one-fifth perhaps, is important for the functioning of the somatic cells of the adult, while practically all of the enzymes may be important for differentiation and morphogenesis during the embryonic life of the individual. Accordingly, we may then expect that an individual of the inbred strain (which is homozygous for a number of "weak" alleles) may be maldeveloped, in the sense that it may have a much smaller reserve at birth than the wild type individual, with respect to a number of physiological functions. Thus it is conceivable that an individual belonging to an inbred strain may die at an age at which, f*, the "surviving" fraction of its somatic cells has fallen to, say, $f^* = \frac{1}{2.72} \approx \frac{1}{e}$; whereas and individual belonging to the wild type strain may die at an age at which, f*, the "surviving" fraction of its somatic cells has fallen to about $f^* = \frac{1}{7.4} \approx \frac{1}{e^2}$

We may compute for this case the most probable age at death, for Man, from formula (14) given on page 33 of my paper (loc.cit.) which reads:

$$x_r + r = \sqrt{4m \ln \frac{1}{f^*}} + \ln \frac{1}{f^*}$$

where x_r is the number of hits at death; r is the number of the inherited faults; m = 23 is the number of chromosome pairs and f* is the surviving fraction of the somatic cells at the age of death.

The most probable age at death, t_r, in years is given by: $t_r = 6 \cdot x_r$ years

For the inbred strain we obtain the most probable age at death by writing: r = o and $\ln \frac{1}{f^*} \approx 1$. We thus obtain $t_r = 63.6$ years.

For the wild type we obtain t_r, the most probable age at death, by writing: $r = 2$ and $\ln \frac{1}{f^*} \approx 2$. We thus obtain $t_r = 81.5$ years. The actual value for white females in the United States is: $t_r = 80.5$ years.

For the F_1 hybrid we obtain t_r, the most probable age at death by writing: $r = o$ and $\ln \frac{1}{f^*} \approx 2$. We thus obtain $t_r = 93.5$ years. This is 12 years more than the value for the wild type.

It may thus be seen that a substantially shortened life expectancy of the homozygous, inbred, strain, as compared with the wild type, need not be inconsistent with the theory. However, an increased life expectancy of the F_1 hybrid ~~in comparison~~ as compared with the wild type strain is a necessary consequence of the theory.

This consequence of the theory could be tested by experiments on short-lived mammals, say mice. In order to render the experiment more sensitive, one may first expose to ionizing radiation a population of wild type mice over several generations and may thereby increase the number of faults in the population. Starting with such a "wild" population, enriched in faults, one would then select two unrelated ~~females~~ families and derive from them two inbred homozygous strains. The theory demands that the F_1 hybrid of these two inbred strains should live appreciably longer than the population from which the two families were selected. Given a suitable opportunity, I propose to arrange for experiments of this sort. A negative result might well prove fatal for the theory.

I should perhaps add at this point that the observed differences in the life expectancy of the male and the female do not provide a usable criterion for the validity of the theory because, f*, the "surviving" fraction of the somatic cells at death, might

differ appreciably for the male and the female.

Smith cites in his note a rather peculiar effect of the temperature on the life expectancy of the male and the female in D.subobscura. It seems to me that any future theory of ageing that may be generally applicable to insects would be put to an unduly severe test, were one to demand that it account for this particular effect.

Because the theory of ageing that I proposed makes quantitative predictions, it is capable of being disproven by experiments and, sooner or later, ~~this~~ such might be its fate. At present I am not aware, however, of any valid observations, which contradicts this theory. In these circumstances, I am not at present disposed to go along with the appraisal of the theory, implied in the last paragraph of Mr. Smith's Note.

J.B.

29th August, 1958.

Szilard Aging Theory--Notes

1. General.

The probability of r chromosome pairs of which one and only one have suffered one or more "aging hits" and jointly of d pairs which have suffered at least one "aging hit" in each of the pair, we call P(r,d,t). In any period of time dt, short enough so that double events can be neglected, there are the following possibilities:

I. No hits.

II. A hit on a viable chromosome, whose homologue is also viable.

III. A hit on a chromosome which is viable but whose partner has previously been hit.

IV. A hit on a chromosome which has itself previously been hit.

We choose the time unit such that the probability is unity per unit time, that a hit occurs: $t = \xi$ in Szilard's paper. $\underline{m}$ is the number of homologous pairs.

The above process may be represented by a differential equation, as follows:

(1) $P(r, d, t + dt) = (1 - 2mdt)P(r,d,t) + 2(m - r - d + 1)P(r-1, d, t) + (r-1)$

$(r+1)\, P\,(r+1, d-1, t) + (r + 2d)P(r,d,t)$

The r.h. terms express the probabilities connected with the previous four possibilities.

The above equation may be transformed by using the Laplace generating function

(2) $F(x, y, t) = \sum_{r=0}^{\infty} \sum_{d=0}^{\infty} P(r, d, t)x^r y^d$

by simply multiplying both sides by $x^r y^d$ and summing. The result is

(3) $\partial F/\partial t = 2m(x-1)F + (x - 2x^2 + y)\partial F/\partial x - 2y(x - 1)\partial F/\partial y$

On the other hand we may independently derive F(x, y, t) by an argument like that leading to Szilard's (2), and it is

(4) $F(x, y, t) = (p^2 + 2pqx + q^2y)^{m-r_o - d_o}\,(px + qy)^{r_o} \cdot y^{d_o}$

with the initial conditions $F(x, y, 0) = x^{r_o}y^{d_o}$, and in which $p = e^{-t}$.

Deductions from (3) or from (4) may be used, whichever is easier. For example the function $R(t) = \partial F/\partial x \Big|_{\substack{x=1 \\ y=0}}$

may be derived from (4), in terms of $\phi(t) = \partial F / \partial x \Big|_{\substack{x=1 \\ y=1}}$

and $f(t) = F\Big|_{\substack{x=1 \\ y=0}}$. This is

(5) $R = f(\phi - r_o q)/1-q^2$

On the other hand from (3),

(6) $df/dt = -R$

So we have the equation

(7) $-df/f = (\phi - r_o q)\, dt\, (1-q^2)$

The right hand expression has the significance of the conditional expectation of the number of faults, conditional upon the non-appearance of any "dead" pairs. The integral of (7) in its series expansion gives various approximations for t in terms of f, involving Szilard's (21) for $r_o = 0$.

2. Special--Physiological Age.

Instead of using (7), we may proceed as follows: Suppose, starting from $r = 0$ there occurred r aging hits. The expected time required is given by

(8) $r = 2me^{-t} - 2me^{-2t}$

$e^{-t} = \frac{1}{2} + \frac{1}{2}(1 - 2\rho_0 - 2\rho_0..)$

(9) $t = \rho_0 + \rho_0^2 + ..$

On the other hand if there are exactly r aging hits, the probability of no "death" is, from (4),

(10) $f_{|r = r_0} = \left(\frac{p^2}{1-2pq} \right)^{m-r_0}$

$= p^2 + ap^3q + ...$

and at time $t \approx \rho$ this amounts to

(11) $f_{|r=r_0} \approx e^{-\rho^2}$

Now this process, from $r = r_0$ at time 0, takes a time

(12) $t = \rho_0 \sqrt{\frac{1}{m}} + \frac{\rho_0^2}{2m}$

This means, then, that the physiological age lost by n_0 initial faults is less than $\rho + \rho^2$ and greater than $\rho_0 + \rho_0^2 - \rho_0 \sqrt{\frac{1}{m}} - \frac{\rho_0^2}{2m}$.

This method permits of iteration.