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 as long as outbred ones. In addition to this purely observational point, there is one more general reason why Szilard's work has made a theory of ageing by somatic mutation less, and not more, promising than it had previously appeared to be. It is assumed that the 'target' is a whole chromosome; a 'hit' renders ineffective all the genes carried by that chromosome. This assumption is made because, as Szilard shows, if it were assumed that the target were an individual gene, it would be necessary also to assume that each individual carried a load of faults so high as to be inconsistent with the known fertility of consanguineous marriages. There are events, particularly mitotic errors and chromosome breakages, which would deprive cells of whole chromosomes or of large segments of chromosomes, but they do not seem likely to be common enough to be the main cause of ageing. Most biologists would be happier with a theory which assumed as the unit event a hit on a gene, using the word gene here to refer to a functional unit or cistron. Perhaps the most important thing Szilard has done is to show that such a theory, at least in its simplest form, would run into difficulties.

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[1] Szilard, L., *Proc. U.S. Nat. Acad. Sci.*, **45**, 30 (1959).
[2] Clarke, J. M., and Maynard Smith, J., *J. Genet.*, **53**, 172 (1955).
[3] Maynard Smith, J., *J. Genet.*, **56**, 227 (1959).
[4] Maynard Smith, J., *J. Exp. Biol.*, **35**, 832 (1958).
[5] Mühlbock, O., *CIBA Colloquia on Ageing*, **3**, 115 (1957).

ALL the observations quoted by Mr. Smith in his interesting communication 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 strains. Further, 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 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.

Again, for the purposes of discussion here, I shall adopt a somewhat over-simplified picture, and 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, so far as their biochemical activity is concerned.

My 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, $f^* = \frac{1}{2 \cdot 72} \approx \frac{1}{e}$; whereas 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 $f^* = \frac{1}{7 \cdot 4} \approx \frac{1}{e^2}$.

We may compute for this case the most probable age at death, for man, from formula (14) given on p. 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, is given by: $t_r = 6 \times x_r$ years.

For the inbred strain we obtain t_r, the most probable age at death, by writing: $r = 0$ and $\ln \frac{1}{f^*} \approx 1$. We thus obtain $t_r = 63 \cdot 6$ 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 $t_r = 81 \cdot 5$ years. The actual value for white females in the United States is: $t_r = 80 \cdot 5$ years.

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 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 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 disproved by experiments and, sooner or later, such might be its fate. At present I am not aware, however, of any valid observations which contradict this theory. In these circumstances, I am not at present disposed to agree with the appraisal of the theory implied in the last paragraph of Mr. Smith's communication.

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

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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)