THE BEARING OF RADIATION EXPERIMENTS ON THE SIZE OF THE GENE

By D. E. LEA and D. G. CATCHESIDE

The Strangeways Research Laboratory, and the Botany School, Cambridge

1. Introduction

At the time when the discovery was made of the induction of mutations by radiation, the 'target theory' of the biological action of radiations was already current (Dessauer, 1923; Blau & Altenburger, 1923; Crowther, 1924). The target theory (*Treffertheorie*) supposes that, in certain cases, the biological effect of radiation is due to the dissipation of energy (in the form of ionization) in a particular region in the organism, and permits the calculation of the size of this region from determinations of the effect of measured doses of radiation. The target theory has sometimes been uncritically applied in cases where its validity is improbable, and in other cases supported on slender evidence, so that it has met with a good deal of scepticism. Recent work on the inactivation of viruses (Gowen, 1940; Wollman, Holweck & Luria, 1940; Lea, 1940*b*; Lea & Smith, 1940, 1942; Lea & Salaman, 1942; Exner & Zaytzeff-Jern, 1941; Exner & Luria, 1941; Luria & Exner, 1941) has, however, given strong support for the validity of its application to this action of radiation. In the case of the 'macromolecular' viruses, the size of the target is nearly identical with the size of the virus molecule.

It was natural, therefore, as soon as it appeared that the results of experiments on the induction of mutation by radiation were consistent with the mutation being caused by a single ionization, that these data should be used to calculate the size of the 'target' for gene mutation, and that this target should be identified with the gene. More recently, a more cautious attitude has been adopted, and some authors have gone so far as to state that the size of the target has nothing to do with the size of the gene (e.g. Timoféeff-Ressovsky & Delbrück, 1936). This attitude arises from the realization that certain unproved assumptions are involved in calculating the size of the gene from radiation data. These assumptions have been discussed by Muller (1940) and Fano (1942). No valid evidence has, however, been adduced against these assumptions, so that the matter rests as unproven rather than disproved.

Recently progress has been made in the understanding of the mechanism of biological action of radiations, particularly as a result of work on virus inactivation, and a re-examination of the position seems called for, and is given in the present paper.

The target theory makes two main assumptions:

(*a*) That ionization outside the gene does not cause gene mutation.

(*b*) That ionization inside the gene inevitably causes mutation.

Our examination leads to the conclusion that while these assumptions are doubtless simplifications of the existing state of affairs, they are not so far wrong as to lead to completely erroneous estimates of the size of the gene. It is suggested that estimates of the gene diameter based on them are probably correct to a factor of two. In view of the

paucity of information on the size of the gene, an estimate correct to a factor of two will constitute much the most precise information at present available. Existing estimates (e.g. Muller, 1935) only give upper limits, no lower limit being available.

2. Spread of the effect of an ionization

One objection raised to the calculation of gene size from radiation data is based on the idea that an ionization can exert an effect at a distance from the place where it occurs greater than the dimensions of a gene. This idea is supposed to be founded on the evidence of genetical experiments, and the argument runs as follows (Muller, 1940). Certain classes of genetically detectable chromosome aberrations are minute rearrangements involving two chromosome breaks a short distance apart. The yield of these aberrations is proportional to dose, instead of to the square or three-halves power of dose as commonly found for aberrations involving separately produced breaks. Therefore the two breaks are produced by a single ionization.

The fallacy in this argument lies in the fact that ionizations in irradiated tissue are not distributed independently and at random, but are located on the paths of ionizing particles (namely, electrons in the case of X- or γ-ray experiments, protons in the case of neutron experiments, and the α-particles themselves in the case of α-ray experiments). It is possible therefore for two breaks to be produced by the same ionizing particle. On the other hand, two breaks may be produced by separate ionizations on the tracks of separate ionizing particles. If the yield of a given type of aberration is experimentally observed to be proportional to the dose, the correct deduction is that a single ionizing particle is involved, not that a single ionization necessarily causes both breaks.

The number of ionizing particles which cross each square micron of tissue depends on the wave-length of the X-rays, and is of the order of $N = 0{\cdot}01$–$0{\cdot}03$ per μ^2 per r. for wave-lengths commonly used. The number per roentgen is greater for γ-rays, less for α-rays and neutrons. For doses of a few thousand roentgen therefore many ionizing particles traverse each sperm. Two breaks chosen at random will be much more likely therefore to be produced by different ionizing particles than by a single ionizing particle. Hence the yield of chromosome interchanges increases more rapidly than the first power of the dose. However, for minute rearrangements involving two breaks close together in the chromosome, the two breaks are more likely to be caused by a single ionizing particle than by two separate particles. The argument can be made quantitative as follows. Suppose the type of minute rearrangement considered involves the production of two breaks at a separation (at the moment of production) less than or equal to a certain distance ρ. Then clearly the two breaks are likely to be produced by the same ionizing particle if ρ is so small that more than one ionizing particle is unlikely to pass through a circle of radius ρ. If however ρ is so large that with the dose D used many ionizing particles are likely to pass through a circle of radius ρ, the two breaks will usually be produced by different ionizing particles. The criterion for a minute rearrangement to be 'single-hit' is thus $ND\pi\rho^2 < 1$. Taking $N = 0{\cdot}02$ per μ^2 per r., $D = 2000$ r., we obtain $\rho < 0{\cdot}1\,\mu$. Thus any minute rearrangement involving two breaks less than $0{\cdot}1\,\mu$ apart at the time of formation will have a yield proportional to dose. In this connection it is of interest to notice that Demerec & Fano (1941) concluded, on experimental grounds, that minute deficiencies of length up to $0{\cdot}06\,\mu$ were 'single-hit' processes, while larger deficiencies were 'two-hit' processes.

The observation that the yield of minute rearrangements is proportional to the dose does not, therefore, give any information either for or against the hypothesis that a single ionization causes both the breaks participating in the rearrangement. If the yield of a given type of aberration is found to increase more rapidly than the first power of the dose, it is correct to deduce that the breaks involved are produced by separate ionizing particles, and therefore of necessity by separate ionizations. The further deduction can be made (with the values of N and D used above) that the breaks are separated by a distance exceeding $0{\cdot}1\mu$ at the moment of their production.

Having concluded that the proportionality to dose of the yield of minute rearrangements does not afford evidence for or against the spread of an ionization, we consider what other information there is bearing on this point. The production of isochromatid breaks in plant material, i.e. the simultaneous breakage at approximately the same locus of both the chromatids of a chromosome irradiated in early prophase at a time when it is already split, affords some information on this point. The yield of this type of aberration is proportional to dose (Sax, 1940), so that both chromatids are broken by the same ionizing particle, and the question is whether this means that the one ionizing particle passes through both chromatids, and produces ionization in each of them, or whether both can be broken as a result of the spreading effect of a single ionization or group of ionizations. The answer is provided by an experiment of Catcheside & Lea (1943) on the production of aberrations in *Tradescantia* chromosomes by irradiation in the prophase of the pollen tube division. X-rays of very long wave-length (8·3 A.) were used. The ionizing particles in this case are electrons of low energy having a range in tissue of only about $0{\cdot}05\mu$, so that it would hardly be possible for the same ionizing particle to produce ionization in both chromatids. No isochromatid break was found, apart from one showing signs of spontaneous origin, in experiments in which 19 chromatid breaks were produced. Radiations of shorter wave length, having ionizing particles of greater range, produced isochromatid and chromatid breaks in the ratio of one to six. We conclude therefore that to break both sister chromatids it is necessary for ionization to be produced in each of them and that a chromatid cannot be broken by one, or a group, of ionizations at a distance of the order of $0{\cdot}1\mu$ away. The evidence of this experiment is thus against the hypothesis of the spread of the effect of ionization.

If we enquire what are the mechanisms by which an ionization might produce an effect at a distance, one of the most promising is by the intermediary of 'activated water'. Chemical effects produced by X-rays on pure substances in dilute aqueous solution have been shown by the researches of Fricke (1934, and many other papers) on a variety of inorganic and simple organic compounds, and of Dale (1940, 1942) on enzymes, to be due not to the direct ionization of solute molecules but to ionization (or excitation) of the water molecules. This leads to the formation of an intermediate substance of short life, the exact nature of which is not at present known, but which is commonly referred to as 'activated water'. The activated water reacts with the solute molecules. In this way the number of molecules of the solute reacting is very much greater than the number of molecules of solute which are directly ionized by the radiation, being comparable to the number of molecules of water which are ionized. (In dilute solution, the number of molecules of water which are ionized greatly exceeds the number of solute molecules ionized, approximately in the ratio of the relative proportions by weight of water and solute.) If this sort of effect is the mechanism of the production of mutations by radiation

(as suggested by Fricke & Demerec, 1937), calculations of the size of the gene made on the assumption that the effect is direct will be invalid. The error will be in the direction of the calculated gene size being larger than the true size.

However, there is reason to believe that while mutation via activated water may occur, the mutation rate by this mechanism is in practice small compared with the mutation rate by direct ionization in the genes, so that the error made in neglecting the indirect effect is slight. The evidence is the fact that the presence of proteins, or of a variety of organic substances in the solution, diminishes the indirect effect, by reacting with the activated water and so protecting the solute. This has been demonstrated for enzymes by Dale (1942), and for viruses by a number of workers. In the experiments of Luria & Exner (1941) on the inactivation of bacteriophage by X-rays, a concentration of gelatin as low as 0·01 % was sufficient to make the indirect action negligible compared with the direct action. Protection against indirect action by 0·1 % of protein in solution was observed by Friedewald & Anderson (1941) studying the inactivation of the Shope rabbit papilloma virus by X-rays, and by Lea, Smith, Holmes & Markham (1944) studying the inactivation of tobacco mosaic virus by γ-rays. If it is permissible to argue from virus to gene, then in the presence of the protein concentration found in the cell the genes should be fully protected against indirect action of radiation by the activated water mechanism.

Another hypothesis which has been proposed which would permit a certain degree of spread of the effect of an ionization is the 'point-heat' theory of Dessauer (1923), which has been revived by Jordan (1938). On this view an ionization, or particularly a cluster of ionizations, produces a local rise in temperature persisting for a minute fraction of a second, and one might imagine that such a transient temperature rise might have a mutational effect in view of the fact that heat shocks are known to have this effect. Jordan calculates that a temperature rise of 100° C. would extend to a distance of about $0{\cdot}004\mu$ from a cluster of five ionizations and last about 10^{-12} sec. It is problematical whether a gene exposed to a temperature shock of this short duration would have any large probability of undergoing mutation. The range of spread given by this mechanism would not affect calculations of gene size from radiation data unless the probability of mutation by the temperature shock was nearly 100 %.

Still another mechanism which has been proposed is the transference of energy through a molecule (Timoféeff-Ressovsky, Zimmer & Delbrück, 1935). It is known that when a molecule is dissociated by ultra-violet light, the particular chemical bond which is dissociated is not necessarily in the part of the molecule which absorbs the quantum of energy, so that transference of energy between atoms in a molecule can occur. How far such transference could occur along a chromosome is not known; it is a question on which one might hope for some help from the theoretical chemist. It is to be noted that such transference of energy, resulting in the effect being exhibited not at the point at which the ionization occurs, but at some other point, would not lead to major error in the calculation of gene size from mutation rate, unless we imagined that by some means the energy of ionizations produced outside the gene was conducted to the gene without there being an equal probability of transference in the reverse direction.

Spread of the effect of ionization, whatever the mechanism, would tend to make the efficiency per ionization of radiations of different ion-density equal. On the other hand, if there is no appreciable spread of the effect of an ionization, then densely ionizing radiations, i.e. radiations in which consecutive ionizations along the path of the ionizing

particle are separated by a distance of less than a gene diameter, will be less efficient per ionization than less densely ionizing radiations (Timoféeff-Ressovsky & Zimmer, 1938) on account of the wastage of ionization when several ionizations are produced in a gene.

Whether or not densely ionizing radiations are less efficient per ionization than less densely ionizing radiations can thus be made a test of whether the effect of an ionization can spread over distances great enough to invalidate the calculation of gene size from radiation experiments.

The importance of making neutron experiments was pointed out by Timoféeff-Ressovsky (1937), and experiments by Zimmer & Timoféeff-Ressovsky (1938), Dempster (1941), Demerec, Kaufmann & Sutton (1942), Fano (1943), and Giles (1943) have all agreed in showing that, per ionization in the tissue, neutrons are less effective than X-rays. We conclude therefore that there is no evidence in genetical experiments suggesting that spread of the effect of an ionization occurs, and that it appears improbable that sufficient spread can occur to invalidate on this account calculations of the gene size from radiation data.

3. The evidence of virus and enzyme inactivation

Postulate (*b*) (p. 41), made in relating the rate of radiation-induced mutation to gene size, is that an ionization in the gene always causes change in it. The energy involved in the process of ionizing an atom exceeds the energy needed to break the chemical bonds uniting the atom to its neighbours in the molecule. It is plausible therefore that ionization of an atom should lead to chemical change in the molecule of which it is a part. The typical result of experiments on the chemical action of radiation (Lind, 1928) is a yield of about one molecule reacting per ionization produced. There are some instances of yields much greater or much smaller than this being obtained, but chain reactions enhancing the yield, or recombination of the products of dissociation diminishing the yield, can usually be recognized in these cases.

Most experiments on the chemical action of radiations have been made on simple inorganic substances in the gas phase, and it is clearly of importance to see whether the same result holds for more complicated molecules, in particular proteins, if we are going to assume its truth for genes. In Table 1 we have collected the results of some recent experiments on enzymes and viruses. The experiments were performed under conditions in which only the direct effect of the radiation was concerned, and the criterion of effect used was loss of enzyme activity, or loss of virus infectivity, which may reasonably be considered analogous to lethal mutation in the case of the gene. In the table, α is the probability of a particular enzyme molecule or virus particle being inactivated by a dose of 1 roentgen, and is analogous to the mutation constant employed by Timoféeff-Ressovsky & Delbrück (1936). In virus work it is more usual to express the result by stating the reciprocal of α, which is referred to as the inactivation dose.

It is seen that the target weight approximates fairly closely to the molecular weight. The maximum deviation, a factor of 4·5 in weight, corresponds to a factor of 1·7 in diameter. (By diameter we mean the diameter of a sphere of equal volume. The inactivation rate determines the weight but not the shape of the molecule. Extreme departure from the spherical shape may be recognizable if data are available for radiations of different ion-densities.) Using the radiation method to determine the diameters of these enzymes and viruses, we should in no instance have been in error by a factor of more

than 1·7. Exner & Zaytzeff-Jern (1941) and Luria & Exner (1941) have presented tables comparing the target diameters of bacteriophages with the diameters of the phage particles themselves, and have obtained a similar measure of agreement.

In working out the target sizes from the inactivation doses, it was assumed that spread of the effect of an ionization did not occur, so that allowance had to be made for the reduced efficiency, per ionization, resulting from the fact that ionizations are not produced individually and at random, but in small clusters along the tracks of ionizing particles. The method of calculation allowing for this effect has been described previously (Lea, 1940*a*; Lea & Smith, 1942).

As discussed in § 2, evidence for the absence of spread is afforded by the observation that the efficiency per ionization diminishes with increase of ion-density of the radiation, i.e. in the order γ-rays, X-rays, neutrons, α-rays. Table 2 shows that this is true for a virus, and moreover demonstrates that consistent estimates of the target size are obtained with different radiations, which is evidence of the correctness of the assumption that spread of the effect of an ionization sufficient to upset calculations of this sort does not

Table 1. *Inactivation of Viruses and Enzymes by X- or γ-rays*

Enzyme or virus	Molecular weight	α	Target weight	Ratio	Reference
Ribonuclease	$1{\cdot}5 \times 10^4$	$2{\cdot}9 \times 10^{-3}$	$3{\cdot}0 \times 10^4$	2·0	1
Myosin	10^5–10^6	$1{\cdot}8 \times 10^{-7}$	$2{\cdot}3 \times 10^5$	0·2–2	1
Phage S-13	$1{\cdot}7 \times 10^6$	$1{\cdot}7 \times 10^{-6}$	$1{\cdot}5 \times 10^6$	0·9	2
Tobacco ringspot virus	$3{\cdot}4 \times 10^6$	$2{\cdot}1 \times 10^{-6}$	$2{\cdot}3 \times 10^6$	0·7	3
Tobacco necrosis virus	$7{\cdot}2 \times 10^6$	$1{\cdot}5 \times 10^{-6}$	$1{\cdot}6 \times 10^6$	0·22	3
Bushy stunt virus	$10{\cdot}6 \times 10^6$	$2{\cdot}2 \times 10^{-6}$	$2{\cdot}3 \times 10^6$	0·22	3

(1) Lea, Smith, Holmes & Markham (1944).
(2) Lea & Salaman (unpublished).
(3) Lea & Smith (1942).

Table 2. *Inactivation of phage* S-13 *by different radiations (Lea & Salaman, unpublished)*

Radiation	γ-rays	X-rays (1·5 A.)	α-rays (5 eMV.)
Inactivation dose ($1/\alpha$) in roentgen	$0{\cdot}58 \times 10^6$	$0{\cdot}90 \times 10^6$	$3{\cdot}5 \times 10^6$
Inferred target diameter in mμ	15·5	15·5	16·0

occur. Additional evidence of the same sort has been provided by experiments of Lea & Smith (1942) on plant viruses. The differences between the efficiencies per ionization of different radiations are more marked in virus experiments than in mutation experiments because the virus is larger than the gene.

To conclude the discussion of viruses and enzymes: the obvious test of the proposed method of determining the size of a gene from radiation data is to apply it to something as nearly as possible resembling a gene but of known size. Viruses and enzymes would seem to be the most appropriate test objects, and we have seen that applied to viruses and enzymes the estimate of size given by the radiation method is correct to within a factor of two in diameter. It is a reasonable presumption that this will be the case also when the method is applied to genes.

4. Visible mutations

The arguments we have given have been directed to showing that the assumptions that ionization inside the gene causes change in the gene, and that ionization outside does not cause change in the gene, will enable us to make an estimate of the size of the gene which will probably be reliable to a factor of two. We have tacitly assumed that change in the

gene is something which is observable and the rate of which can be measured, and we must now examine how far this is the case. Visible mutation, or a lethal mutation not due to chromosome deletion, may safely be taken to indicate that chemical change has occurred in the gene concerned,* but the converse, that absence of visible mutation shows that no change has occurred in the gene, is less certain.

For example, in the white eye alleles in *Drosophila* we have a large number of different states of a gene which are distinguishable because eye-colour is a character in which many quantitatively slight differences can be recognized. The different alleles also affect the colour of the Malpighian tube in the larva, but if we had to rely only on the colour of the Malpighian tube we should recognize fewer alleles, and hence in radiation experiments often fail to recognize the occurrence of a mutation when mutation had in fact occurred. Even using the more sensitive eye-colour as a means of detecting mutation, changes in the gene can occur without being detected, since it is known that alleles exist having the same eye-colour but differing in other properties (e.g. viability and fertility, Timoféeff-Ressovsky, 1933*b*). It is evident therefore that the experimentally determined frequency of mutation of a given locus will usually underestimate the frequency with which permanent and viable changes are produced in the gene. (This point has been emphasized by Timoféeff-Ressovsky, 1937.) This consideration probably applies especially forcibly to the experiments on the induction of mutations in a plant virus described by Gowen (1941). Here a mutation was recognized by the production of a local lesion on the leaf inoculated with virus, in place of the usual mottle. It would clearly be unreasonable to assume conversely that if after irradiation the virus still produced a mottle on the leaf there was therefore no permanent change induced in the virus molecule by the radiation. The fact that an ionization in the virus molecule had rather a small chance (order of 10^{-3}) of producing this particular mutation does not mean that an ionization in the virus molecule has only this small probability of producing a viable inherited change.

The complete deficiency of a gene, when homozygous, is usually lethal in *Drosophila*. If as a result of ionization a gene suffers a change which causes the loss of the power of reproduction or alternatively the loss of all its characteristic activity, a recessive lethal will usually be recorded. Lethal mutation at a given locus (excluding deficiency caused by deletion of a part of the chromosome containing the locus) appears not to be much more frequent than visible mutation (data bearing on this question are given by Patterson (1932), Demerec (1937), Muller (1940)), which is perhaps a little surprising.

In Table 3 we quote a number of mutation rates in *Drosophila* (from Timoféeff-Ressovsky's experiments) which are seen to range from 1 to 16×10^{-8} per locus per roentgen. Target diameters can be calculated as if these mutation rates represented the total probabilities of obtaining change in the gene concerned. The target diameters obtained in this way range from 2 to 7 mμ. Since, as just explained, changes in the gene can occur without producing visible mutation, these estimates will all be smaller than the sizes of the genes concerned. Since, however, it is for the more frequently occurring mutational steps that numerical data are available, these estimates may not be underestimates of the *mean* size of a gene.

* We are not accepting the extreme view that all mutations are position effects, and are regarding the typical point mutation as due to chemical change in a gene.

5. Lethal mutation

If we are prepared to draw analogies between the action of radiations on viruses and enzymes on the one hand, and on genes on the other, lethal mutation (excluding chromosome deletion) should be analogous to virus or enzyme inactivation. Target diameters calculated from the rates of lethal mutation may be expected to agree within a factor of two with the sizes of genes, in view of the results obtained in the study of enzyme and virus inactivation.

In discussing lethal mutations we have the advantage that some information is available of the relative efficiency of radiations of different ion-density. This information is set out in Table 4 and shows that the yield of lethals per 1000 r. diminishes progressively as the ion-density is increased in the order γ-rays or hard X-rays, soft X-rays, neutrons, α-rays. In no case can one be certain that the difference obtained between the given radiation and hard X-rays is established with complete certainty. In the soft X-ray experiments correction was necessary for the absorption of the X-rays in the tissues, and

Table 3. *Rates of some (visible) mutation steps*

All in units of 10^{-5} per locus per roentgen. (Timoféeff-Ressovsky, 1933*a*; Timoféeff-Ressovsky & Delbrück, 1936.)

+ to *m*	2·4	*f* to +	2·4
m to +	1·0	+ to any allele of *w*	15·9
+ to *f*	6·6	*w* to any other allele	1·2

Table 4. *Relative efficiency of different radiations in inducing sex-linked lethals in* Drosophila

Radiation	% yield for 1000 r.	Relative dose for given yield	Inferred gene diameter	Ref.
X-rays or γ-rays	2·89	1·00	—	1
Soft X-rays (2–3 A.)	2·23	1·30	4·4	2
Neutrons	1·99	1·45	9·0	3
α-rays	0·84	3·44	6·6	4

(1) Timoféeff-Ressovsky (1939).
(2) Wilhelmy, Timoféeff-Ressovsky & Zimmer (1936).
(3) Zimmer and Timoféeff-Ressovsky (1938), Dempster (1941), Demerec, Kaufmann & Sutton (1942), Fano (1943), Giles (1943).
(4) Ward (1935), recalculated by Lea (1940*b*).

in view of the uncertainty this correction introduced the authors did not consider their experiment was inconsistent with equal efficiency of hard and soft X-rays. Several workers are agreed that neutrons are less effective, per ionization in the tissue, than X-rays, but neutron dosimetry is not yet so well established as X-ray dosimetry and some systematic error in this respect cannot be regarded as impossible. The α-ray experiments were made by irradiating fertilized eggs in the polar cap stage and are not therefore really comparable with the X-ray experiments made by irradiating mature sperm.

However, the combined evidence of Table 4 is strongly suggestive that the efficiency, per ionization, of the different radiations does diminish with increase of ion-density.

From the relative efficiency of radiations of different ion-density it is possible to calculate the size of the gene on the assumptions that spread of the effect of ionization does not occur, and that the probability that an ionization in the gene, or strictly an ion-cluster, produces change in the gene is unity. The method of calculation has been described (Lea, 1940*a*): the details of the calculation have subsequently been improved by taking more exact account of the spatial distribution of ionization in the irradiated tissue, which

accounts for the estimates of gene size given in the fourth column of Table 4 being a little different from those previously given on the basis of essentially the same experimental data. The estimates of gene diameter range from 4 to 9 mμ, the differences being due to errors in the experiments and uncertainties in the calculation. It is satisfying that these estimates are consistent with the estimates of 2–7 mμ obtained in § 4 by an independent method.

As explained in the earlier paper (Lea, 1940*a*), by combining the *total* yield of sex-linked lethal mutations with the estimate of gene size based on the *ratio* of the yields with different radiations, we can deduce the number of genes contributing to the sex-linked lethals, i.e. the number of genes in the *X*-chromosome. In view of the sensitivity of this calculation to errors in the experimental determination of the ratio of efficiencies as well as to any inadequacy in the calculation, it is hardly justified to regard this as an accurate method of determining the number of genes, as first proposed. However, the fact that the method gives a reasonable estimate of this number provides a further argument in support of the validity of the general assumptions involved in the theory, and therefore of the estimates of gene size they lead to.

6. Discussion

The estimate obtained for the mean diameter of a gene in *Drosophila* is 4–8 mμ. If there are about 1000 genes in the *X*-chromosome, this means that a length of 4–8μ of the chromosome thread is taken up by the genes themselves.

According to an interpretation of recessive lethals put forward by Lea & Catcheside (1945) the probability that an ionizing particle which breaks a chromosome in the euchromatic region shall cause a lethal mutation is 0·38, and this leads to the inference that a fraction 0·38 of the length of the euchromatic region of the chromosome is occupied by genes. This would make the total length of the euchromatin of the *X*-chromosome 10–20μ.

This estimate is at least ten times smaller than the length of the salivary gland chromosome, and if it is accepted one must presume that the salivary gland chromosomes have multiplied in length as well as in breadth as compared with ordinary chromosomes. The 10–20 μ represents the length of the chromosome which is breakable, and one might suppose that only a tenth of the length of the chromosome thread was in fact breakable, which would bring the total length more into agreement with the length of the salivary gland chromosome. However, such an assumption would imply that an ionizing particle had only a small chance of causing a break when it traversed a chromosome at random, and would make the occurrence of pairs of breaks (caused by the ionizing particle traversing the chromosome thread twice, by intersecting two consecutive turns of the spiral) very infrequent compared with single breaks. Breaks in pairs, however, occur rather frequently (roughly one pair to two single breaks according to the analysis of Lea & Catcheside, 1945), which seems to exclude this possibility.

If the genes were elongated, and not spherical, some increase of length above the figure of 4–8 mμ could be allowed, but a highly elongated gene, nearly 100 mμ long, which would be required to make the length of the chromosome thread 200 μ, cannot be reconciled with the radiation data as we have interpreted them.

Summary

At various times it has been proposed that the size of the gene can be estimated from experiments on the yield of mutations obtained with known doses of X-rays. The assump-

tions involved in calculations of this sort are discussed, and some of the objections which have been raised against them answered. It is pointed out that similar assumptions, applied to experiments on the inactivation of enzymes and viruses, lead to estimates of the sizes of these bodies correct to a factor of two in diameter. The estimate arrived at for the size of the gene in *Drosophila* is 4–8 mμ diameter. The length of the euchromatic region of the *X*-chromosome thread in the sperm is deduced to be 10–20 μ.

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