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A RADIATION METHOD FOR DETERMINING THE NUMBER OF GENES IN THE *X*-CHROMOSOME OF *DROSOPHILA*

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(With Two Text-figures)

1. THE TARGET THEORY OF GENE MUTATION

IT is well known that gene mutations can be produced (e.g. in the sperm of *Drosophila*) by ionizing radiations. There is a considerable body of evidence suggesting that a gene mutation can be caused by a single ionization, providing it is produced in the gene or its immediate vicinity. The arguments in favour of this view have been discussed at length by others (e.g. Timoféeff-Ressovsky *et al.* 1935) and need only be summarized here. They are:

(*a*) Under a constant intensity of radiation the rate of production of mutations is constant throughout the duration of exposure, and proportional to the rate at which ionizations are produced per cubic centimetre in the irradiated germ cells. The probability that a given small dose of radiation will produce a mutation in a previously unaffected germ cell is thus independent of whether the organism has been previously irradiated (as when the given small dose occurs towards the end of the exposure) or not (as when it occurs at the beginning). This result suggests that when a mutation occurs during radiation it is not the cumulative effect of all the ionizing particles which have passed through the germ cell, but is the sudden effect of a single one of them. What distinguishes the particular ionizing particle which produces the mutation from the large number which pass through the germ cell without doing so is presumably that it happened to pass through the gene concerned, or its immediate vicinity. Evidently only ionization produced in the neighbourhood of the appropriate gene is able to produce a mutation.

(*b*) The number of mutations produced by a given dose of radiation (expressed as so many ionizations per cm.^{3} in the organism) is not affected by whether the dose is administered by prolonged irradiation at low intensity or short irradiation at high intensity. It is also independent of whether the dose is given continuously or in a series of fractions. This result strengthens the conclusion that the mutation is produced by a single ionizing particle. If it were the cumulative effect

of a number of such particles, one might expect the number of particles required to produce the effect to depend on the intervals elapsing between the arrival of the several particles.

The suggestiveness of these two arguments is perhaps increased if one contrasts the gene mutations with certain other biological actions of radiations, which are not due to the passage of a single ionizing particle through the cells (cp. Lea, 1938). The inhibition of division in tissue cells irradiated by gamma rays (Spear & Grimmett, 1933) is due to the passage of a large number of ionizing particles through the cells. The proportion of cells affected is not proportional to the dose, but there is a threshold dose below which no cells are affected. Further, the effect of a given dose is greatly reduced if it is administered slowly, giving the cell time to recover from the effect of one ionizing particle before the next arrives.

(*c*) Radiations of different sorts distribute their ionization through the tissue in different manners. Gamma-rays, for instance, ionize by means of fast electrons which pass through the material producing about 10^5 ionizations per cm. of their path. Soft X-rays ionize by means of slow electrons producing up to 10^7 ionizations per cm. of path. These ionizations are distributed along the path of the electron partly singly and partly in compact clusters of two or three, or rarely more, ionizations. In the case of ionization produced by gamma-rays, successive clusters are formed at an average distance apart of about 3×10^{-5} cm., a distance comparatively large on an intranuclear scale. Hence if several ionizations were necessary for the production of a gene mutation, so that the co-operative effect of a number of clusters was required, we should expect that gamma-rays would be less effective, per ionization, in the production of mutations than soft X-rays, the clusters produced by which are closer together. Experiment shows (Timoféeff-Ressovsky, 1937) that, per ionization, gamma-rays and hard and soft X-rays are equally efficient. The indication is that a single ion cluster (and probably a single ionization) suffices to produce a mutation, providing of course that it is produced in the right place.

Here again, it is perhaps helpful to make a contrast between the gene mutation and other actions of radiation. Fern spores (Zirkle, 1935, 1937) and bacterial spores (Lea *et al.* 1936) have been shown to require higher doses of gamma-rays and hard X-rays to kill them than of alpha-particles, showing that in these cases a densely ionizing radiation is more effective per ionization than a less dense. It is probable that here more than one ionization is necessary to produce the effect observed.

2. THE EFFECT OF THE ION DENSITY OF THE RADIATION

We are interpreting the radiation-mutation experiments, then, on the view that a mutation is caused when an ionization is produced within a certain volume v which we refer to as the target volume, and which for simplicity we suppose to be a sphere of radius r. This volume we can loosely identify with the volume of the gene. Remembering the cluster distribution of ionization, we may say then that the probability of a mutation occurring in a certain gene with a dose of say 1000 r. units is equal to the probability of a cluster being produced in volume v by this dose. (For a discussion of the cluster distribution of ionization, and its importance in theories of the biological effect of radiations, see Lea, 1934; Lea *et al.* 1936, 1939; Jordan, 1938.) 1000 r. units of X- or gamma-rays produces $1{\cdot}71 \times 10^{15}$ ionizations per cm.^{3} in tissue, i.e. $5{\cdot}7 \times 10^{14}$ clusters per cm.^{3}, since on the average there are three ionizations per

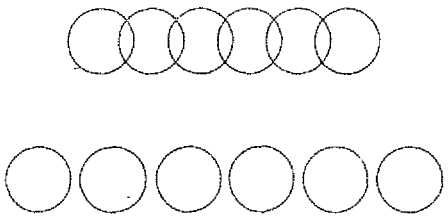

Fig. 1.

cluster. The probability of a cluster being produced in the target volume v by a dose of 1000 r. is thus

$$5{\cdot}7 \times 10^{14}\, v. \qquad \ldots\ldots(1)$$

We come now to the effect of ion density. The calculation we have just made tacitly assumed that the clusters are separated from one another by distances large compared with the target dimensions. If they are more crowded together than this, the calculation is in error, and the probability of mutation production is less than given by equation (1). This may be seen more clearly as follows. Let us imagine described round each of the $5{\cdot}7 \times 10^{14}$ clusters which are found in 1 c.c. a sphere of radius r equal to the target radius. For a mutation to be produced in a particular gene, the centre of the corresponding target volume will have to lie somewhere within the total volume enclosed by these spheres. If the spheres do not overlap, the total volume they enclose is $5{\cdot}7 \times 10^{14}\, v$, and this is the probability that the mutation will be produced. If on the other hand there is some overlapping of the spheres (see Fig. 1) the

total volume they enclose will be less than $5{\cdot}7 \times 10^{14}\, v$ by a factor F (>1), which we may term the "overlapping factor". The probability that a particular point, namely the centre of the target, will fall somewhere within the sphere complex, which occupies a volume $5{\cdot}7 \times 10^{14}\, v/F$ cm.^{3} in each c.c. is evidently $5{\cdot}7 \times 10^{14}\, v/F$.

Thus the proportion of mutations produced by a densely ionizing radiation will, per ionization, be less than the proportion produced by a less densely ionizing radiation by the factor F defined as above. The factor F obviously depends on the ratio of the radius of the target and the mean separation of clusters in the path of the electron. If the clusters have separation l and the spheres described round them have radius r, it is a matter of simple solid geometry to show that the volume enclosed by the non-overlapping spheres (see Fig. 1) is greater than the volume enclosed by the same number of overlapping spheres in the ratio

$$\tfrac{4}{3}\pi r^3 : \tfrac{4}{3}\pi r^3 - \tfrac{1}{12}\pi\,(16r^3 - 12r^2 l + l^3). \qquad \ldots\ldots(2)$$

In practice, however, the clusters are not formed at equal intervals along the path of the ionizing particle, but are distributed according to the mean free path law. If L is the mean separation of adjacent clusters, the probability of the actual separation being in the range l to $l+dl$ is $e^{-l/L}.dl/L$. The volume ratio between the non-overlapping and the overlapping spheres thus becomes, in place of the expression (2) above,

$$\tfrac{4}{3}\pi r^3 : \tfrac{4}{3}\pi r^3 - \tfrac{1}{12}\pi \int_{l=0}^{2r} e^{-l/L}.(16r^3 - 12r^2 l + l^3).dl/L, \qquad \ldots\ldots(3)$$

reducing to $$F = (2\xi/3)/\{1 - 2\,(1 - e^{-\xi})/\xi^2 + 2e^{-\xi}/\xi\}, \qquad \ldots\ldots(4)$$

where $\xi = 2r/L$.

Fig. 2 shows this function F plotted against $\xi = 2r/L$. As of course is to be expected, F tends to the value unity for $L \gg r$, i.e. well separated clusters. For $L \ll r$, i.e. densely ionizing radiations in which the clusters overlap to form a column of ionization, F tends to the value $4r/3L$.

3. Interpretation of the neutron experiments on *Drosophila*

Recently Zimmer & Timoféeff-Ressovsky (1938) have shown that neutrons produce mutations in *Drosophila melanogaster*, the proportion of sex-linked lethal mutations obtained being 1·6 times less than obtained with X-rays and gamma-rays, referred to equal numbers of ionizations per c.c. of tissue. Neutrons ionize by the intermediary of protons, which in these experiments had a mean energy of $1{\cdot}8 \times 10^6$ V. These protons will produce on the average $1{\cdot}1 \times 10^7$ ionizations per cm. in tissue, or $3{\cdot}7 \times 10^6$ clusters per cm. The mean separation of clusters

is thus $L = 2{\cdot}7 \times 10^{-7}$ cm. Now the factor of 1·6 by which the neutrons were less effective than X-rays is clearly to be equated to the factor F of our theory. But Fig. 2 shows that $F = 1{\cdot}6$, when $2r/L = 1{\cdot}4$. Hence the target radius must be given by $2r = 1{\cdot}4 \times 2{\cdot}7 \times 10^{-7}$, i.e.

$$\text{radius } r = 1{\cdot}89 \times 10^{-7} \text{ cm.},$$
$$\text{volume } v = 2{\cdot}83 \times 10^{-20} \text{ cm.}^3, \qquad \ldots\ldots(5)$$

since $v = \frac{4}{3}\pi r^3$.

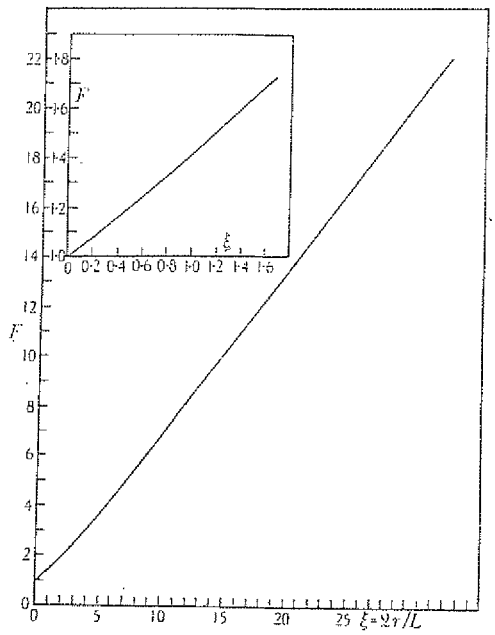

Fig. 2. The overlapping factor. (The inset shows the early part of the graph on an enlarged scale.)

What is observed in these experiments is a sex-linked lethal mutation. There are a large number of genes in the X-chromosome of *Drosophila* capable of exhibiting such a mutation; the target sizes for the individual genes will of course differ to some extent, and the size we have calculated is an average for all the genes of the X-chromosome capable of giving lethal mutations. We can proceed further by noting that equation (1) permits us to calculate the total target volume of all these genes. Equation (1) as it stands gives the mutation rate for a single gene of target volume v. In experiments in which sex-linked lethal mutations are studied, the mutation rate observed is the sum of the frequencies

of mutations in all the genes of the X-chromosome capable of exhibiting lethal mutation, i.e. is

$$5{\cdot}7 \times 10^{14} \times \Sigma v,$$

where Σv is the sum of the target volumes.

Now with hard X-rays (for which the complication of the overlapping factor may be neglected) the rate of production of sex-linked lethal mutations is 3 % per 1000 r. units. Thus

$$0{\cdot}03 = 5{\cdot}7 \times 10^{14} \times \Sigma v, \qquad \ldots\ldots(6)$$

i.e.
$$\Sigma v = 5{\cdot}26 \times 10^{-17} \text{ cm.}^3.$$

Comparing equations (5) and (6) giving respectively the mean volume per gene and the total target volume of all the genes in the X-chromosome, we obtain an estimate of the number of such genes, viz.

$$N = 5{\cdot}26 \times 10^{-17} \div 2{\cdot}83 \times 10^{-20} = 1860.$$

This method thus provides a means of estimating the mean size of a gene (or strictly its target volume), and the number of genes in the

TABLE I

Mutation	Probability of mutation per 1000 r.	Target radius
$+ \rightarrow w^c$	$2{\cdot}6 \times 10^{-5}$	$2{\cdot}22 \times 10^{-7}$ cm.
$w \rightarrow w^c$	0·3	1·08
$w^c \rightarrow +$	0·8	1·50
$+ \rightarrow m$	2·4	2·15
$m \rightarrow +$	1·0	1·61
$+ \rightarrow f$	6·6	3·06
$f \rightarrow +$	2·4	2·15
	Average radius	$1{\cdot}97 \times 10^{-7}$ cm.

X-chromosome, by observations made only on sex-linked lethal mutations in which mutations of different genes are not separately distinguished. It should be possible to check this method by observations on the non-lethal mutations of individual genes. Neutron measurements are as yet not available; there is, however, a limited amount of published data giving individual mutation rates due to X-rays. The following table embodies data of seven individual mutations listed by Timoféeff-Ressovsky (1937). From the mutation rates experimentally found we have calculated the target volumes by equation (1) and so deduced the target radii.

The target sizes are seen to differ for different mutations, but it is evident that they are on the whole very much of the same order as the average target size $1{\cdot}89 \times 10^{-7}$ cm. deduced by the ion density variation method. The agreement between two methods completely different in principle is encouraging.

4. DISCUSSION

The estimate of 1860 for the number of genes in the *X*-chromosome of *Drosophila* is of the right order of magnitude. Its accuracy, of course, depends on the accuracy of the experimental determination of the relative efficiency of X-rays and neutrons in producing mutations, and it is in fact very susceptible to any error in this ratio. Owing to the difficulties of neutron dosimetry the figure of 1·6 given by Zimmer & Timoféeff-Ressovsky for the relative efficiency of X-rays and neutrons is quite likely to be in error by as much as 10 %, and an error of this amount would be sufficient to change the calculated value of the number of genes in the *X*-chromosome by a factor of 2. Our estimate of the number of genes in the *X*-chromosome is therefore provisional.

In addition to making the experimental precision of the neutron experiments as high as possible, it would be desirable also to have experiments with other radiations of high ion density particularly alpha-particles. Alpha-particles give an ionization density still higher than that produced by neutrons, and according to our theory, should be 3·5 times less effective per ionization than X-rays.

A further deduction from the present theory, which could with advantage be subjected to experimental test is the following. Considering the individual mutations listed in Table I, the mutation rates of the most frequent ($+ \rightarrow f$) and the least frequent ($w \rightarrow w^e$) are (to X-rays) in the ratio 22 : 1. The most frequent has, of course, a larger target volume than the least frequent, and should therefore show a greater difference between X-rays and neutrons. Carrying through the calculation, one finds that the mutation rates to neutrons should be in the ratio of 14 : 1, which is sufficiently different from 22 : 1 to give some hope of the difference being experimentally detectable.

5. SUMMARY

It is known experimentally that the efficiency of production of sex-linked lethal mutations in *Drosophila melanogaster* by radiation is, per ionization, less for densely ionizing radiations (neutrons) than for less densely ionizing radiations (X-rays). A theory is given correlating this effect with the target volume of the individual genes. The estimate of the target volume obtained by this method agrees with the estimate obtained by methods already known. It is further shown that on this theory the experimentally determined ratio of the mutation rates for X-rays and neutrons may be used to give an estimate of the number of genes in the *X*-chromosome of *Drosophila*. Using the experimental

figure of 1·6 for the ratio of the mutation rates, the number of genes in the *X*-chromosome is deduced to be 1860.

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