ON THE MUTATION OF THE TOBACCO MOSAIC VIRUS

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The first application of the ionization theory to the calculation of a volume within which radiation must be absorbed to produce a biochemical effect was by Clark and Northrop (16) in connection with the inactivation of trypsin by x-rays. The purpose of this note is to analyze, by similar methods, the x-ray–induced mutation of the tobacco mosaic virus to the aucuba type on the basis of the physicochemical concepts of the biological effects of radiations (1). The experimental data used are those of Gowen (2, 3) on the irradiation of dry virus with 1.5 Å wave length. Applying the general methods of this field we assume that ionizations within a certain volume v of the virus are the primary cause of the induced mutation. We consider the ionizations in the irradiated sample as random events induced by the radiation. We take 32.5 e. v. as the energy required by each such event, thus assuming that the latter consists of the formation of a single pair of ions. A possible clustering of ions would affect our conclusions as to the calculated size of v, increasing the latter by a factor equal to the number of ions per cluster. Somewhat at variance with the most frequent procedure we do not make any restriction *a priori* as to the number n of ionizations which must occur in v in order to induce a detectable mutation; we require that n be obtainable from the experimental data. It is frequently assumed that n is unity, although examples are known in which this is not true (4). We indicate with p the probability of occurrence of an ionization in v if it occurs on an average of once per cubic micron of the irradiated sample. In accordance with the generally known fact in radiobiology that p is very small, we express the relation between the frequency P of the induced mutation and the x-ray dose D as a Poisson distribution. We will take this distribution as cumulative

$$P(pD, n) = \int_0^{pD} e^{-x} x^{n-1} dx/(n-1)! \qquad (1)$$

which implies the assumption that possible ionizations of v beyond the n-th ionization do not produce any detectable inactivation or further mutation of the virus. This type of assumption, frequently made, is here discussed as to its validity in connection with the experimental material analyzed. Unless otherwise stated we intend the dose measured as the number of ionizations per cubic micron; it follows then from the theory of the Poisson distribution that the average dose per mutation is n/p ionizations per cubic micron. But a mutation

requires n ionizations in the volume v, consequently $n/p = n/v$. Therefore $p = v$.

For the experiments here under discussion the observed frequency of spontaneous mutation of TMV to AMV (tobacco mosaic virus to aucuba mosaic virus) is 0.00079. Let S be the dose of x-rays which would produce the same frequency of mutation if the sample were free from spontaneous occurrences of this mutation. Then, taking into account that $p = v$, we have:

$$P(vS, n) = 0.00079 \tag{2}$$

where the function P is defined by equation (1). Let M be the total frequency of both mutations, those induced by the dose D and the spontaneous ones. Then:

$$P(vS + vD, n) = M. \tag{3}$$

M is obtained experimentally by counting the number T of tobacco mosaic lesions that a sample produces before irradiation and the number A of aucuba lesions that an equal sample produces after administration of a dose D. Since the analysis attempts to determine the probability p or the volume v within which the primary process of the mutation TMV → AMV is developed, account must be taken of those effects which influence the number of lesions without being related to the particular mutational process considered. These effects are:

1. The x-rays induce not only the mutation TMV → AMV but also mutations to other variants and inactivations of the virus. Both variants, the original TMV and the newly formed AMV, are subject to these effects. Table 2 on page 189 of Gowen's paper (3) suggests strongly that these effects are not negligible; it shows that the number of aucuba lesions increases with the dose up to a certain point only to decrease thereafter. The existence of such a maximum can be explained by more numerous inactivations of the virus at higher doses. It would be difficult to explain it without any of the effects here mentioned, since one would expect an increase of the number of mutations with the increase of the dose.

2. If the virus is used in higher concentrations the number of lesions increases less than proportionally to the increase of the number of virus particles, because at high concentrations more virus particles penetrate a cell than are necessary to produce a lesion.

If effects 1 and 2 were absent M would be equal to A/T. To correct this ratio for these effects one can proceed in the following manner.

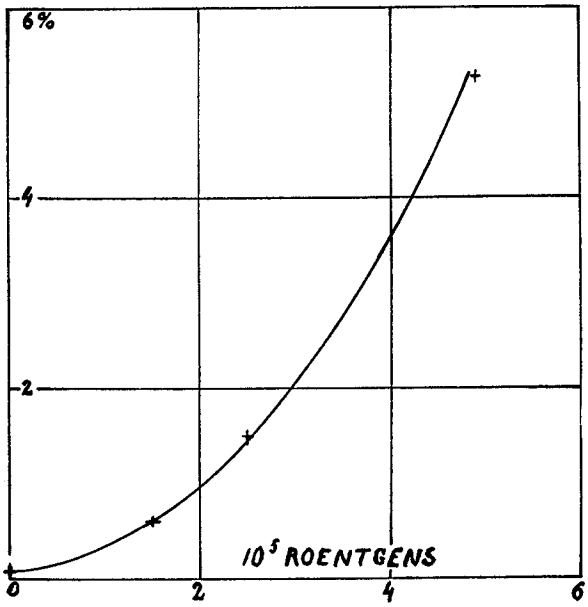
The inactivation of the TMV and its mutation to other variants can be taken into account, since the probability Q of a TMV to survive as such a dose D roentgen of 1.5 Å x-rays is known:

$$Q(D) = \exp(-415 \times 10^{-8} D). \tag{4}$$

This expression is a good fit of the data of Gowen (2) within the range of his mutation experiments. In this way

$$MQ(D) = A/T. \tag{5}$$

As to the remaining effects, *viz.* (*a*) inactivation or further mutation of the AMV formed during the irradiation from the TMV, and (*b*) the concentration effect, it may be easily seen that to a certain degree at least they counterbalance each other. In fact, (*a*) decreases the number A of aucuba lesions; (*b*) decreases the



TEXT-FIG. 1. Frequency of mutation M against the administered dose D. Theoretical curve and points calculated from experimental data. Experimental range: $D = 0 — 490{,}000$ roentgens, $M = 0.079 — 5.275$ per cent.

number T of tobacco mosaic lesions and leaves unaffected the number A, because the number of the original TMV is extremely high with respect to the AMV obtained by mutation. In this way the effects (*a*) and (*b*) reduce the numerator and the denominator of the ratio A/T, so that we may assume, as a first approximation, that they leave this ratio unchanged. This is not an exact argument, but there are no data for a more accurate estimate of M.

The crosses in Text-fig. 1 represent the values of M calculated by means of equations (4), (5) from Gowen's data for A and T. Using these values of M, the quantities v, n, and S are obtainable from equations (2), (3), in which the dose D is expressed as number of ionizations per cubic micron. The transforma-

tion of roentgen into this unit can be made on the basis of the following data calculated according to the method outlined by Lea (1).

Number of Ionizations per Cubic Micron per Roentgen of 1.5 Å X-Rays

Virus protein (1)	1.45
Nucleic acid of the TMV, calculations on the basis of the average chemical composition as obtained by Loring (5)	3.74
Phosphorus, calculations on the basis of an assumed density of 2.2	28.1

Stanley, Knight, and Lauffer (6) found that mutations of the TMV are accompanied by changes in its protein component. On the contrary Pfankuch *et al.* (7) believe that these changes are in the nucleic acid. Phosphorus, because of its high absorption coefficient, cannot be disregarded in an analysis of a radiobiological experiment.

The calculations, whose results are given in the figure and in the table below, have been carried out in the following manner by means of the tables of Molina (8). First of all from equation (2) the dependence of vS on n has been numerically tabulated. These values of vS have been then substituted into equation (3) and from the equations thus obtained a numerical relation between v and n has been found for each pair of experimental values of M and D. For each n the standard deviation σ of all the calculated values of v from their arithmetical mean $\bar{v}$ has been computed. The result is:

n	1	2	3	4	5	6	7	100
$\sigma/\bar{v}$ in per cent	45.7	14.3	4.5	2.0	2.7	5.3	7.2	12.0
$\bar{v}_n/\bar{v}_4$	0.03	0.27	0.63	1	1.32	1.71	2.07	15.49

with $n = 4$ giving the smallest standard deviation of 2 per cent. If this value of n is accepted as the correct one, the size of the volume v in which the primary absorption of the radiation energy must take place in order to induce a mutation comes out to:

(*a*) 1.42×10^{-6} cubic micron if that volume consists of protein; it is about 3 per cent of the total virus protein. Pfankuch (9) suggested that possible protein differences between TMV and a variant of it can amount to at most 10 per cent. The above figure for v is practically identical with the 1.34×10^{-6} cubic micron which Bernal and Fankuchen (14) found by x-ray diffraction as the volume of a hexagonal unit cell (87 Å side length and 68 Å height) making up the virus protein. Clark and Northrop (16) found a volume of the same order of magnitude, 2.47×10^{-6} cubic micron, within which the absorption of x-rays takes place in causing inactivation of trypsin.

(*b*) $v = 5.5 \times 10^{-7}$ cubic micron if this volume consists of nucleic acid; it is about 25 per cent of the total nucleic acid content. Since there are 8 molecules of the nucleic acid in a virus (10), at least two of these molecules would be affected by the mutational process.

(*c*) $v = 7.31 \times 10^{-8}$ cubic micron if the volume consists of phosphorus. It

amounts to about 50 per cent of the whole nucleic acid phosphorus, so that phosphorus of at least every second nucleic acid molecule would have to be active in absorbing the mutating x-rays.

The above figures are calculated on the basis of the following data: volume of the virus 5×10^{-5} cubic micron (11), nucleic acid content 5.5 per cent of the virus (10, 12), phosphorus content 9.13 per cent of the nucleic acid (5), density 1.3 for the virus protein and 1.6 for its nucleic acid (13).

In the last line of the table $\bar{v}_n$ is the size that $\bar{v}$ would have for the corresponding value of n and $\bar{v}_4$ is the accepted size of $\bar{v}$. It is seen that our conclusions would not differ by very much if n were chosen as 5 instead of 4.

The question how 4 ionizations in any place within a volume of the size of v, which is quite large on the atomic scale, can produce such a fine effect as a mutation from one variant to another, is answered either by the fact that the volume v may consist of many identical units, or by the facility of transfer of energy within v. Examples of transfer of energy over volumes much larger than v are known (4).

The calculations give $S = 282{,}000$ ionizations per cubic micron if v is protein, and 729,000 if v is nucleic acid. Since cosmic radiations and γ-rays from rocks give about 0.2 roentgen per year (15) or in a round figure one ionization per cubic micron every two years, it would take more than half a million years for these natural radiations to produce the mutations that have been observed to occur spontaneously in the course of the experiment. This comparison shows once more how insignificant the natural radiation is for the process of spontaneous mutation.

SUMMARY

Experiments on x-ray–induced mutations of the tobacco mosaic virus (Gowen) are analyzed on the basis of the ionization theory. The size of the volume within which the primary process of mutation develops is calculated on the basis of three alternative assumptions; *viz.*, (1) that this volume consists of protein, (2) that it is nucleic acid, (3) that it is phosphorus. It is found that the volume calculated under assumption (1) is identical with the hexagonal cell unit which Bernal and Fankuchen found in the virus protein by x-ray diffraction. Assumptions 2 and 3 lead respectively to the conclusions that one-fourth of the total nucleic acid content or one-half of the total phosphorus content is involved in the mutational process. The relation between the induced and the spontaneous mutation is examined and it is found that natural ionizing radiations are completely insignificant for the spontaneous mutation of the virus.

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