

Factors Limiting the Number of Radiation-Induced Chromosome Exchanges

I. Distance: Evidence from Non-Interaction of x-Ray- and Neutron-Induced Breaks

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ABSTRACT

Soaked seeds of *Vicia faba* were exposed to fractionated doses of x-rays or x-rays and fast neutrons. When the two-hit (exchange) chromosome aberrations were scored at the first mitosis of the root tip, it was observed that with short fractionation times the radiation-induced breaks from the two x-ray doses could rejoin with one another to form exchanges in proportion to the square of the total dose. If, however, one dose was x-rays and the second neutrons, then no quantitatively determinable interaction occurred between the breaks induced by each of the doses, and the aberration yield was simply the sum of that induced by each fraction.

The phenomenon of non-interaction as observed by these dose fractionation studies and also by the linear dose response curve for two-break aberrations induced by neutrons has led to calculations of the distance over which two breaks can rejoin. The distance is evidently much smaller than the previously accepted value of 1μ .

The results of experiments on x-ray-induced chromosome aberrations (see reviews by Lea, 1946, and Giles, 1954) are best explained on the assumption that break frequency is proportional to dose and that a non-linear dose relation for chromosome exchanges reflects their dependence on the simultaneous presence of breaks of independent origin. From several lines of inference (*loc. cit.*) it may be concluded that rejoining is much more likely to restore the original configuration, *i. e.*, to effect restitution, than to produce an aberration of the exchange type. As early recognized by Sax (1940), the relative probability of restitution or exchange is strongly influenced by two factors: first, the distance between broken ends; and second, the time during which they remain unjoined.

The time factor has been studied by dose fractionation and variation of the dose rate. For

example, if the exchange frequency produced by two doses separated in time is the sum of the frequencies given by each alone, it is inferred that the time interval is sufficient to allow rejoining of the first set of breaks so that they cannot interact with the second. Conversely, the time is insufficient for rejoining if the yield of exchanges is greater than additive. In the event of no rejoining before the second irradiation, the yield of two-break aberrations should be proportional to the square of the dose. According to these criteria, x-ray experiments on the seed of *Vicia faba* (Wolff and Atwood, 1954; Wolff and Luippold, 1955) indicate a class of breaks in which rejoining does not begin until at least $\frac{1}{2}$ hour after irradiation and is delayed longer by high than by low doses. The induced delay in rejoining is a process independent of chromosome breakage itself, since the protective effect of anoxia or of 2,3-dimercaptopropanol (BAL) during irradiation, expressed as dose reduction, is greater for the rejoining delay than for the break frequency. Moreover, the delay is influenced

* Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

TABLE I
Yield of Dicentric and Rings versus Neutron Dose

Dose	Dicentric and rings per 100 cells		
	Experiment 1	Experiment 2	Experiment 3
<i>r. e. p.</i>			
0	0.0	0.0	0.3
30	6.0	10.6	11.0
45	8.3	15.6	17.6
60	11.0	19.0	21.0
75	—	26.3	27.3

by postirradiation conditions that do not affect the break frequency. It is reasonable to surmise that some delay in rejoining occurs among breaks produced by ionizing radiations, regardless of quality. In other words, the time factor should provide no special hindrance to the interaction of breaks produced by different radiations.

The distance factor reveals itself in non-random rejoining. For instance, exchanges between parts of the same chromosome are more frequent than expected on the basis of random interaction of breaks throughout the entire chromosome complement (Sax, 1940). A remarkable illustration of the spatial isolation of breaks within the same nucleus is given by a comparison of haploid and diploid cells (Conger and Johnston, 1956). Since the average number of breaks in a diploid at a given dose is double that in a haploid, the exchange frequency might be expected to equal that in a haploid that had received twice the dose; that is, a fourfold increase in exchanges. In fact, however, the exchanges are only twice as frequent in the diploid. As Conger has pointed out, the exchange frequency depends not on the *number* of breaks per nucleus, but on the *concentration*.

In contrast to the results with x-rays, the exchanges induced by neutrons have a linear relation to dose and are independent of the dose rate (Giles, 1940, and Table I). This shows that the breaks involved in a neutron-induced exchange are nearly always produced by the same recoil proton. Lea (1946, p. 250) bases a rough estimate of the "rejoining distance" on this difference in kinetics: "Suppose that a given break will only take part in exchange if a second break is produced within a distance h . For a 2-hit exchange to be produced it would therefore be necessary for a second proton to pass through a sphere of radius h centered at a given break. The fact that neutron exchanges with 50 ν units (23 protons crossing the

nucleus) are not usually 2-hit means that a second proton will not usually pass through this sphere. The sphere of radius h must therefore have a cross-sectional area less than $\frac{1}{23}$ of the cross-sectional area of the nucleus of radius 6 μ . We deduce that h is less than 1.3 μ .

"Turning now to x-ray-induced exchanges, the fact that these exchanges are usually 2-hit with 50 r means that more than one electron is usually found within a radius h of a given break. Thus the sphere of radius h must have a volume $\frac{1}{303}$ of the volume of the nucleus, since with this dose 303 electrons are found in the nucleus. It follows that h exceeds 0.9 μ ." This analysis, however, would be equally applicable over a wide range of doses with a consequent range of h values. Moreover, the assumption that "more than one electron is usually found within a radius h " is not warranted simply by the fact that the exchanges are two-hit. The exchange frequency in the example given would be of the order of 0.1 per cell, hence the required coincidence of electrons might equally well be assumed to be rather rare at 50 r. In short, the likelihood of finding two electrons within a certain volume is unrelated to the exchange kinetics, but dependent only on the dose. Nevertheless, some relation between the rejoining distance and the difference in kinetics is evident since with unrestricted rejoining, exchanges would usually involve breaks produced by separate particles, regardless of the quality of the radiation.

With this idea in mind, it is interesting to consider what will happen when breaks produced by two radiations of different quality are present simultaneously. A radiation that produces a high yield of two-break aberrations on a single particle basis would be expected to produce additional breaks separated from each other by more than the critical distance for rejoining. Ordinarily, these additional breaks would rejoin, and would therefore be undetectable. Their presence might be revealed, however, by their interaction with breaks produced by a different, less densely ionizing radiation. This interaction would manifest itself as a greater than additive yield of exchanges from the combined treatments.

In the experiments to be reported here, the seeds of *Vicia faba* were exposed to x-rays, which yielded exchanges proportional to the square of the dose (Wolff, 1954); to neutrons, which produced a linear increase in two-break aberrations; and to combined neutron and x-ray treatments. The combined irradiations gave simple additive aberration

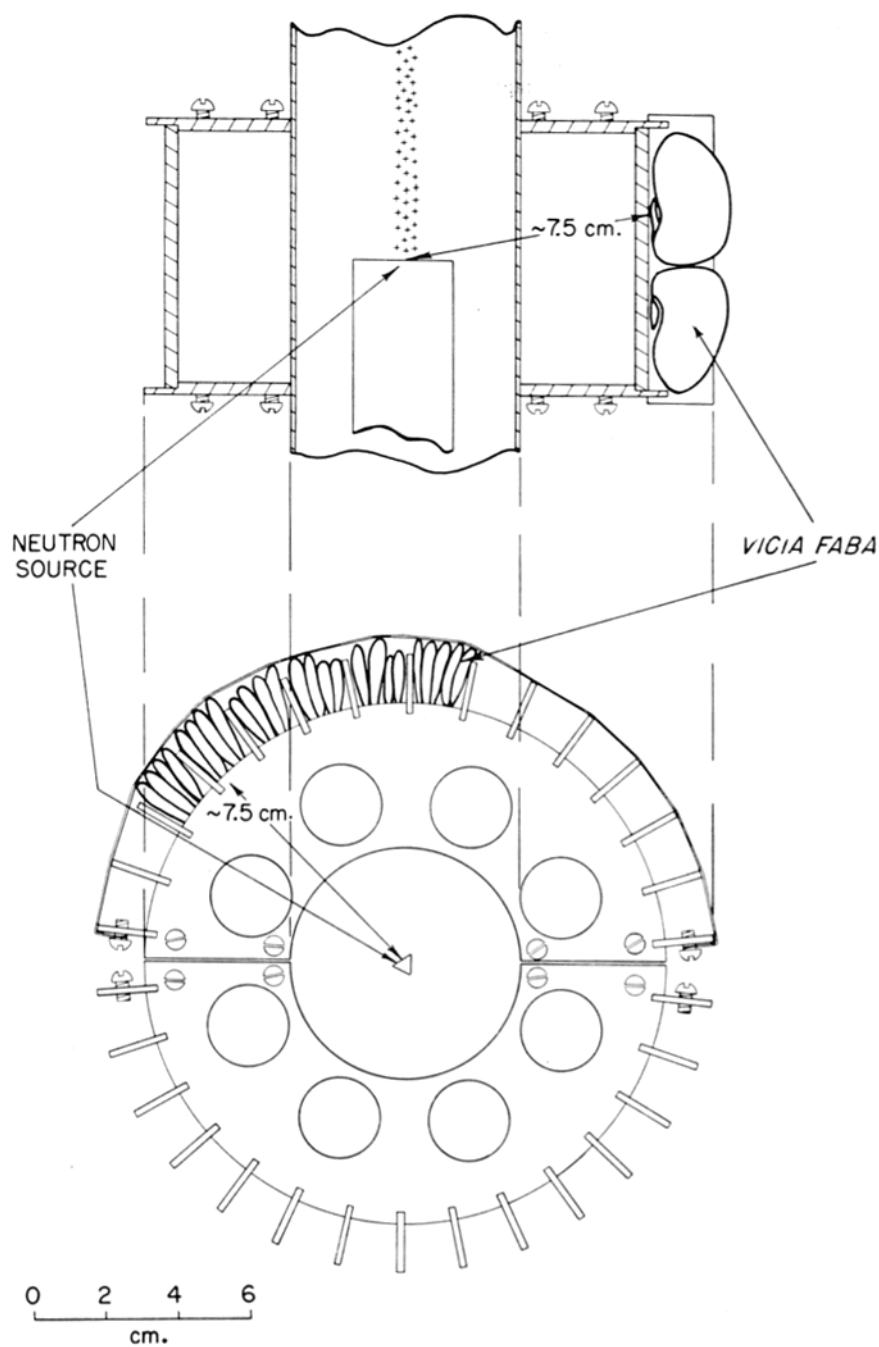


FIG. 1. Details of bean holder around neutron-producing target.

frequencies; that is, no interaction could be detected between breaks produced by neutrons and x-rays. It will be shown that in order to explain this result, the generally accepted maximum distance of 1μ within which broken ends may exchange must be revised downward.

Methods

The seed coats of *Vicia faba* seeds were chipped and the seeds soaked in distilled water for 5 hours at 22°C ., then peeled and irradiated. Subsequently, they were allowed to germinate according to the method of Gray and Scholes (1951). Three days later, when the first

mitotic root tip divisions were occurring, the roots were cut off and placed in a 0.2 per cent colchicine solution for 2 hours for accumulation of cells in metaphase. The root tips were then fixed in C. E. Ford's modification (unpublished) of Flemming fluid. Feulgen squashes were made, and at each dose 300 cells from six slides were scored for chromosome dicentrics and rings. For all x-irradiations, the seeds were arranged with their embryos up in open culture dishes and irradiated with a G. E. Maxitron tube operated at 250 kvp with 3 mm. of Al filtration at 200 r per minute. For neutron irradiations, the seeds were placed in a specially designed lucite holder (Fig. 1) 7.5 cm. from the target of a Cockcroft-Walton linear accelerator. The monoenergetic 14.1-Mev. neutrons were generated by the D, T reaction.

For the combined dose experiments, the first dose was 600 r administered either to seeds soaked in water or soaked for the last $\frac{1}{2}$ hour in a 2×10^{-3} M BAL solution. The breaks produced remain open for 2 hours after irradiation in water, but only for $\frac{1}{2}$ hour in seeds irradiated in BAL (Wolff, 1954). The second dose of radiation was administered at such a time that, except in the case of the BAL-treated seeds, the breaks from the first dose would be open at the time of the second dose.

OBSERVATIONS

In all experiments, whether or not the x-ray-induced breaks were still open at the time of the neutron dose, the two-hit aberration yield was only the sum of the two-hit aberrations induced by each dose alone (Table II, experiments 1, 2, and 3E). This is contrary to the results obtained when the second dose is also x-rays (experiment 3D and Wolff and Atwood, 1954). In experiment 3, a group of seeds soaked in water were irradiated with 600 r of x-rays. Within 5 minutes, some of the seeds were exposed to a second dose consisting of 45 r.e.p. of neutrons. As soon as the neutron exposure was completed, other seeds from the original group which had been exposed to x-rays were given a second dose consisting of 300 r of x-rays. As may be seen from experiment 3E, the aberration yield for the combined x-ray-neutron experiment was only the sum of the individual yields. When the second dose was x-radiation (experiment 3D), however, the aberration yield was proportional to the square of the total dose administered. This indicates that the breaks induced by the first dose were open and capable of rejoining with other x-ray-induced breaks, even at a time after the neutron irradiation had been completed.

TABLE II

Effect of Fractionated Doses of x-Rays and Neutrons on Vicia faba

Experiment	First dose	Time breaks remain open	Interval between doses	Second dose	Dicentrics and rings per 100 cells	
					Observed	Expected if two doses are additive
1. With BAL						
A	600 r	30			10.7	
B	45 r.e.p.	?			8.7	
C	600 r	30	60	45 r.e.p.	19.3	19.4
2. With Water						
A	600 r	120			16.7	
B	45 r.e.p.	?			10.7	
C	600 r	120	60	45 r.e.p.	28.7	27.4
3. With Water						
A	600 r	120			13.3	
B	300 r	?			6.0	
C	45 r.e.p.	?			8.7	
D	600 r	120	10	300 r	36.7*	19.3
E	600 r	120	5	45 r.e.p.	20.7	22.0
F	Control				0.0	

* Expected value if aberrations increased as the square of the total dose = 37.2.

DISCUSSION

The additive effect of combined irradiation indicates that broken ends produced by neutrons do not join often enough with those produced by x-rays to increase noticeably the aberration frequency. The explanations that come readily to mind fall into two classes: those that depend mainly on the spatial distribution of the breaks, and those that require some qualitative difference between breaks produced by the two types of radiation. Little support can be found for a qualitative difference of the type required. Thoday's analysis (1942), for example, gives no indication of a difference in rejoining pattern. With the very densely ionizing α radiation, however, aberrations that result from failure to rejoin are relatively more frequent (Kotval and Gray, 1947). This is often attributed to a qualitative difference between breaks. A difference of this nature cannot, however, explain the present case in which breaks produced by each type of radiation rejoin perfectly well among themselves, but apparently not with those of the other type. The possibility of qualitatively different breaks in which both types rejoin has been considered by Wolff and Luippold (1956). Two

sets of breaks induced simultaneously by x-rays were distinguished by different times of onset of rejoining: in the first type, rejoining began immediately and was complete within about 1 minute, whereas in the second type, rejoining did not begin until from 30 minutes to more than 2 hours after irradiation, depending on conditions. Although a qualitative difference between breaks is a plausible explanation for the two waves of rejoining sharply separated in time, it provides no solution to the problem at hand, unless it is assumed that the neutrons produce only breaks of the first type. This preferential effect seems wholly inconsistent with the nature of the radiations. Despite the difference in the initial elementary processes, the mechanisms of energy transfer thereafter are too similar to account for the high order of specificity implied by the qualitative difference hypothesis.

Without specificity of the breaks, it is clear that the failure of interaction must have a geometrical basis. Since such an explanation involves detailed assumptions that cannot be evaluated at present, we must be satisfied with an examination of certain limiting cases. To provide the greatest contrast between the radiations of different quality, we may imagine the paths of densely ionizing particles as uninterrupted lines that produce breaks at all intersections with chromosomes, while the sparsely ionizing radiation transfers its energy at random points. Obviously, the difference in spatial distribution of breaks under these contrasting regimes depends on the form and arrangement of the chromosomes; that is, almost any experimental result could be explained with *ad hoc* assumptions of bizarre intranuclear geometry that would be difficult to disprove in detail. We shall confine the argument, however, to chromosomes of cylindrical shape and unspecified arrangement. The explanation to be sought must suffice for both the absence of interaction and the linear dose-effect curve for neutrons; *i.e.*, the additivity of a neutron and x-ray dose or of two neutron doses will be treated as having a common cause.

Since the distance within which exchange can occur is restricted, the nucleus will contain an unknown, but finite number of sites where different chromosomal regions are close enough together to permit exchange should both strands be broken. Although the site concept is necessary, a more detailed description of sites is not yet possible. It seems most reasonable to assume variation in site shape dependent on how the strands happen to

cross or approach each other for a portion of their length. Let kD be the probability of breaking at least one of the strands within a site with the dose, D . Let g represent the relative frequency with which both strands are broken as a single event; that is, in which the breakage of one strand implies the breakage of the other by the same ionizing particle. Then the probability, P , of double breaks per site is given by

$$P = gkD + [(1-g)kD]^2. \quad (1)$$

in which the linear term represents breakage of both strands as a single event. Given two breaks within a site, the probability of exchange is something less than unity; hence P may be regarded as the maximum exchange yield per site. To obtain a linear dose-effect curve, the squared term must remain negligible in comparison to the linear term. We therefore seek conditions that maximize the probability, g , of simultaneous traversal. A solution for g as a function of the distance between strands is given by Lea (1946, p. 260). In Lea's formula, which he derived with isochromatid breaks in mind, the cylinders are assumed to lie parallel. Since g would be smaller for crossed cylinders, the value $g = 0.363$, given by Lea's formula, with the strands in contact is maximal for any reasonable assumptions about the geometry of the sites. Even with this value, the increasing contribution with dose of the squared term in equation (1) would be quite evident in the range of P between 0.1 and 0.2, for instance. Hence, even if the strands are required to be in contact for exchange to occur, a linear dose effect curve for exchanges per site cannot be obtained.

The experimentally observable value, however, is the exchange yield per cell, which depends not only on P , but also on the number of sites per cell. Thus, where N is the average number of sites per cell, the maximum exchange yield per cell is given by

$$Y = NP = N g k D + N [(1-g)kD]^2. \quad (2)$$

Consider, for example, an experiment in which a deviation from linearity would be noticed if the squared term contributes at least 5 per cent of the exchanges at a yield of 1 per cell. If g is maximized at 0.363, and $0.05 P = [(1-g)kD]^2$, then a value of $P = 0.018$ is obtained from equation (1). Since (from equation 2) $N = Y/P$, at least 55 sites would be required to maintain apparent linearity up to a yield of one exchange per cell in this hypo-

tical case. The linearity might also be explained by the assumption of a value of g larger than the theoretical maximum of 0.363. Such larger values would result if breakage occurs not only when a chromosome is traversed by a recoil proton, but also when one passes nearby. Thus it is always possible to explain the apparent linearity by the assumption of either a sufficiently large number of sites per cell, or a sufficiently large radius of influence perpendicular to the paths of the ionizing particles. By either means, the two-hit component can be arbitrarily reduced.

It can now be shown that the same factors, N or g , when arbitrarily increased, will lead to the apparent absence of interaction in the present experiments. Since the x-ray dose effect curve for exchanges in *Vicia* shows no linear component, the yield with x-rays is

$$Y_1 = N(k_1 D_1)^2. \quad (3)$$

The results of the combined irradiation is given by

$$Y_{(1+2)} = N\{gk_2 D_2 + [(1-g)k_2 D_2 + k_1 D_1]^2\}, \quad (4)$$

in which all parameters in the x-ray case are designated by the subscript 1, and in the neutron case by the subscript 2. Equation (4) may be written

$$Y_{(1+2)} = Y_1 + Y_2 + 2(1-g)Nk_1 D_1 k_2 D_2. \quad (5)$$

Since the neutron curve is linear within experimental error, the interaction term in equation (5) may be further simplified by assuming

$$Y_2 = N g k_2 D_2. \quad (6)$$

The combined yield can then be expressed in terms of the separate yields:

$$Y_{(1+2)} = Y_1 + Y_2 + \frac{2(1-g)}{g} Y_2 \left(\frac{Y_1}{N}\right)^{\frac{1}{2}}. \quad (7)$$

It is apparent that as either N or g increases in equation (7), the interaction becomes less significant and additivity of the separate yields is approached.

In the computation of the geometrical factor, g , the distance between strands is expressed in strand diameters; hence the actual distance of separation corresponding to a given g value depends on the diameter of the interphase chromosome. Although this diameter is not known in *Vicia*, Muller (1935) has estimated a value of 20μ in *Drosophila*, and it must certainly be less than

Sax's estimate (1938) of 0.1μ for the pachytene chromosomes of *Tradescantia*. This means that if rejoining occurs over a distance as great as 1μ , a very large number of sites would be needed to render negligible the interaction term in equation (7). To fix ideas on this point, let us consider an experiment typical of those presented here. Three hundred cells are scored and the separate yields with neutrons and x-rays are each 0.1 per cell, whereas the combined irradiation gives a yield not significantly different from the additive 0.2 per cell. We note that if the combined irradiation had given 0.25 per cell, the departure from additivity would have been just significant at the 5 per cent level by χ^2 test. If we assume a rejoining distance of 1μ , then strands separated by ten diameters are close enough to rejoin, and the g factor (from Lea's formula) would be only 0.028. With this value of g , the interaction term in equation (7) may be set equal to 0.05 and solved for N , with the result that at least 1930 sites would be necessary to prevent a significant departure from additivity. For mutual independence of sites to be maintained, the distance between them must exceed the rejoining distance. Since the nuclear volume in *Vicia* is only about $320\mu^3$, the required number of sites cannot be contained within the nucleus, and we must reject the initial hypothesis that the rejoining distance is of the order of 1μ . It may be concluded that a proximity much less than 1μ is prerequisite to rejoining.

The same conclusion is reached through a line of reasoning suggested by Lea's analysis of the proportion of breaks that recombine (1946, pp. 256, 259). In general, the total number of breaks produced for a given exchange yield (Y) is dependent on the distance, h , over which rejoining can occur. For radiations showing linear exchange kinetics, this relation is approximated by

$$Y_2 = \frac{hn(n-1)}{d}, \quad (8)$$

in which n is the total number of breaks per nucleus and d the total intranuclear path length of ionizing particles. Equation (8) is derived on the assumption that each break has a probability $1/d$ of being within a given μ of path, hence a probability $2h/d$ of being within $h\mu$ of a given break. Finally, each of the $n(n-1)/2$ pairwise combinations of the n breaks has the probability $2h/d$ of being a potential exchange. It may be noted that

TABLE III
Relation Between the Rejoining Distance, h , and the Aberration Yield, Y , with Combined x-Ray Neutron Irradiation

h	n x-ray (from equation 9)	n Neutron (from equation 8)	Combined Y (see text)
μ			
1	4.46	3.62	0.45
0.5	11.6	4.89	0.31
0.4	16.0	5.40	0.28
0.3	24.4	6.15	0.258
0.2	44.4	7.40	0.237
0.1	125.0	10.3	0.218

n is a minimal estimate of the number of breaks, since not all potential exchanges are realized.

For radiations giving exchange frequencies proportional to the square of the dose, the corresponding relation is

$$Y_1 = \frac{h^3 n(n-1)}{2R^3}, \quad (9)$$

in which R is the radius of the nucleus. Here it is assumed that the probability of a second break within a sphere of radius h centered at a given break is h^3/R^3 , and $n(n-1)/2$ combinations have this probability of being potential exchanges. With equal exchange yields, the total number of breaks per cell, n , in equations (8) and (9) will ordinarily differ, the nature of the discrepancy being strongly influenced by the distance, h . Table III shows the total breaks computed from equations (8) and (9) when the exchange yields of both are 0.1, and the rejoining distance varies between 1.0 and 0.1 μ .

The average intranuclear path length of ionizing particles for neutron doses (50 r.e.p.) producing exchange yields of 0.1 is $d = (\text{energy absorbed in nucleus per gram}) (\text{volume of nucleus}) (\text{density of nucleus}) \div (\text{track average LET}^1) = 78 \mu$. The nuclear radius was taken as 4.25 μ , and the track average LET as 12 kev/ μ . The significance and the methods used in calculation of the track average LET have been discussed in more detail elsewhere (Edington and Randolph, 1958). The value of d is almost independent of the density of the nucleus, since the LET increases almost linearly with density. The conclusions reached in this paper are relatively insensitive to errors in the calculation of d .

It is evident that the smaller the rejoining distance, the larger the number of breaks with x-rays

¹ Linear Energy Transfer.

in comparison to neutrons. When the irradiations are combined, the total number of breaks produced is the sum of the numbers produced by the separate irradiations; from this the combined yield of exchanges can be computed. We first note that $2Y_2$ breaks per cell are actually involved in neutron-induced exchanges. The remainder of the neutron-induced breaks are assumed to be distributed randomly; that is, they are assigned the same probabilities of interaction as x-ray-induced breaks. Thus $2Y_2$ breaks are subtracted from the sum of the neutron and x-ray breaks, and the remaining number substituted for n in equation (9). The yield owing to neutrons alone (0.1 in this case) is added to that computed from equation (9) to obtain the yield from the combined irradiation, shown in the last column of Table III. Since 0.25 exchanges per cell is a significant departure from additivity in representative experiments, h must be less than 0.3 μ to explain the result. It may be reemphasized that h obtained in this way is a maximum value, and might be further reduced by more refined experiments. Since not the slightest indication of a departure from additivity is seen in the present experiments, and, more generally, no departure from linearity has been noted with densely ionizing radiations in this and similar systems, the true value of the rejoining distance is likely to be much smaller than 0.3 μ .

SUMMARY

1. Although the frequency of chromosome exchanges induced by x-rays is proportional to the square of the dose, the frequency induced by a combined x-ray and neutron dose is only the sum of the exchanges produced by the separate irradiations.

2. The apparent absence of interaction between neutron- and x-ray-induced breaks—and also the linear dose effect curve with neutrons—can be explained on the basis that the distance h over which rejoining can occur is much less than the previously accepted value of 1 μ .

3. These experiments indicate that h in *Vicia* cannot exceed 0.3 μ .

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