PROCEEDINGS SERIES [*15w.*]

BIOLOGICAL EFFECTS

OF NEUTRON IRRADIATION

[2 .]

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RELATION BETWEEN MUTATION YIELD AND CELL LETHALITY OVER A WIDE RANGE OF X-RAY AND FISSION NEUTRON DOSES IN MAIZE*

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Abstract

RELATION BETWEEN MUTATION YIELD AND CELL LETHALITY OVER A WIDE RANGE OF X-RAY
AND FISSION NEUTRON DOSES IN MAIZE.
Dry maize seeds, of a genetic stock heterozygous for the yellow-green locus ($\underline{Yg_2}$ /$\underline{yg_2}$), were irradi-
ated with fission neutron doses up to 2700 rads and with 250 kVp X-rays to 40 000 rads. The frequency of
yellow-green (yg) sectors in seedling leaves 4 and 5 is largely a measure of the frequency of breakage (or
incomplete exchange) and loss of the part of the short arm of chromosome 1X carrying $\underline{Yg}$. Plots of dose
versus $\underline{yg}$ sector frequency per leaf show a linear increase with neutrons which reaches a maximum
(leaf 4 = 5. 5, leaf 5 = 2. 5) followed by a rapid decline; and a curvilinear increase with X-rays reaching
a higher maximum (leaf 4 = 8. 5, leaf 5 = 3. 6) followed by a decrease. The observed rise and subsequent
fall of numbers of $\underline{yg}$ mutations can be accounted for by a model in which the decline of the curves is
due to cell killing which may also be due to chromosome breakage and deletion. The interpretation is
based on the principles of the dual action theory, which explains radiobiological action in terms of micro-
dosimetry. One of the observations accounted for is the higher maximum mutation rate for X-rays as
compared to neutrons.

INTRODUCTION

The aim of this paper is to explore the effects of neutron and
x radiation, over a wide range of doses, on a particular genetic effect
and on lethality in maize; and to relate the observations to microdosimetric
data for x rays and neutrons.

MATERIALS AND METHODS

Seeds of a genetic stock of maize (Zea mays L.) heterozygous at
the yellow-green locus, $\underline{Yg_2}/\underline{yg_2}$, were used in all experiments. The dominant
Yg_2 produces full green color, so that the heterozygote has normal green
leaves. The $\underline{Yg_2}$ locus is located near the end of the short arm of chromo-
some 9. Loss of the $\underline{Yg_2}$ allele (deletion) or change in its function
(mutation) in heterozygotes gives a yellowish-green phenotype in leaf

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cells and cell lineages of the altered genotypes. The frequency of yellow-green streaks or sectors in leaves of seedlings grown from irradiated seeds was used as a measure of the frequency of radiation induced genetic change or damage [1]. This was considered to be due mainly, if not exclusively, to breaks in chromosome 9 between the centromere and the Yg_2 locus, with loss (following involvement in chromosomal aberrations) of the Yg_2-containing segment.

Prior to exposure to radiation, the seeds were equilibrated over a saturated aqueous solution of CrO_3 in an atmosphere of 35% relative humidity. The water content of Yg_2/yg_2 embryos, excised from maize seeds so stored, was determined to be 6.7%.

Seeds were irradiated with x rays under the following conditions: 30 ma, 45 cm from source, 1.0 mm Al filter, 4.4 mm HVL Al, dose rate 1658-1845 rad/min. Other seeds were exposed to fission neutrons generated by thermal neutron bombardment of a ^{235}U plate in the thermal column of a Brookhaven National Laboratory reactor. The computed dose rate was from 27.5 to 198 rad/min. During the irradiation the seeds were maintained in the ambient atmosphere, i.e. with oxygen availability of about 21%. Immediately after irradiation, within 4 sec, the seeds were immersed in distilled deionized water (22° C) which was determined to have the same content of dissolved O_2 (about 21%) as the air. The oxygen availability during and after irradiation was such as to enhance radiation response with low LET irradiation.

The irradiated seeds, together with unirradiated controls, were sown immediately in moist soil, one per 4-inch pot, coded, and arranged in a completely randomized experimental design in a controlled environment growth chamber. The conditions in the chamber were 18 hr of light at 1700 to 2000 foot candles intensity in a temperature of 21° C (dark) and 24° C (light).

When fully mature, the fourth seedling leaf (leaf 4) was harvested and scored, under coded number, for frequency of yellow-green (yg_2) sectors. The fifth seedling leaf (leaf 5) matured somewhat later and was then scored in the same way. The size of the yg_2 sectors is a function of the number of cell divisions and the amount of cell expansion that takes place subsequent to irradiation. Consequently, the sectors are larger in leaf 5. The relative frequency of yg_2 sectors in the two leaves is a function of the number of primordial leaf cells in the seed embryo at the time of irradiation and the target size [2]. In these stocks the leaf 5 primordium appeared in the dormant seed as a four-layered structure with slightly more than 500 cells. However, since the epidermal layers of the leaf are devoid of chloroplasts and therefore not involved in yg_2 expression, leaf 5 at the time of irradiation presented a relevant target of about 250 cells. The number of cells in embryonic leaf 4 was more difficult to establish but was estimated by different techniques to be about 2900. It was concluded that the potential number of relevant leaf 4 target cells was about 1500. This estimate is probably too high, however, since many (possibly about half) of the cells in leaf 4 are differentiated and may not be capable of contributing yg_2 sectors.

To determine the volume of the nucleus at the time of irradiation, primordia were dissected from the seed embryo, placed in a 0.1% solution of the surface-active compound Tween 20 for 5 min, then fixed and stained with acetocarmine. The diameter of the nucleus (or length and width if cylindrical) was measured, and the volume was calculated in cubic microns. The mean nuclear volumes were 159 μ^3 for leaf 4 and 122 μ^3 for leaf 5.

RESULTS AND COMPUTATIONS

The dose-response data for frequency of yg₂ sectors per leaf are
presented in Table I. The range in doses for x rays is from 0.50 to 40.0
krad and for neutrons from 0.03 to 2.69 krad. These results are shown
plotted arithmetically in Fig. 1 for leaf 4 and in Fig. 2 for leaf 5.
The neutron and x-ray curves have in common that after an initial rise
they reach a peak, saturate, then decline [3].

In Fig. 3 the experimental results for leaf 4 are shown in a
logarithmic plot of the frequency of yg₂ sectors per leaf as a function
of absorbed dose. In the logarithmic plot straight lines of slope 1
correspond to proportionality between observed yield and absorbed dose;
lines of slope 2 correspond to a quadratic dependence between yield and
absorbed dose. In these experiments no spontaneous incidence of yg₂
sectors was observed. The curves differ insofar as: 1) neutrons are
much more effective per unit of absorbed dose; 2) the neutron-induced
yield of mutations peaks at a somewhat lower frequency; 3) the slope of
the rising part of the neutron curve is approximately 1 which corresponds
to proportionality between effect and absorbed dose. The x-ray curve,
on the other hand, is consistent with a linear increase at smallest doses
which turns into a quadratic dependence with increasing dose.

TABLE I. DOSE-RESPONSE DATA FOR NUMBER OF yg_2 SECTORS PER
LEAF FOLLOWING IRRADIATION OF SEEDS WITH X-RAYS OR NEUTRONS

X rays						Neutrons					
		yg₂ sector/leaf						yg₂ sector/leaf			
Absorbed		Leaf 4		Leaf 5		Absorbed		Leaf 4		Leaf 5	
Dose	No.					Dose	No.				
krad	leaves	Mean	SE	Mean	SE	krad	leaves	Mean	SE	Mean	SE
0.50	343	0.08	.014	.002	.007	0.03	60	0.92	.131	0.50	.090
0.75	477	0.09	.014	0.04	.009	0.07	60	1.33	.150	0.83	.106
1.00	165			0.05	.017	0.15	148	3.80	.194	0.87	.077
1.80	87	0.30	.052	0.12	.034	0.27	119	5.08	.340	0.98	.089
3.95	87	0.92	.117	0.30	.064	0.39	118	5.30	.332	1.69	.115
5.92	60	2.08	.216	0.85	.111	0.65	116	3.46	.221	1.40	.101
8.10	146	3.97	.321	1.30	.121	0.95	131	4.91	.253	2.41	.118
9.17	87	5.21	.425	1.93	.177	1.16	83	5.43	.259	2.54	.123
10.00	85	5.93	.453	1.86	.151	1.36	102	4.98	.193	2.52	.098
11.52	108	5.17	.338	1.98	.164	1.63	68	4.21	.167	2.44	.112
13.68	81	6.37	.379	3.04	.166	1.98	20	2.30	.231	2.30	.231
16.96	83	7.25	.454	3.21	.168	2.37	109	3.23	.117	2.47	.102
20.00	55	7.56	.436	3.15	.190	2.69	25	2.73	.219	1.62	.198
22.79	60	8.18	.495	3.62	.230						
30.00	30	4.39	.310	2.30	.187						
40.00	9	3.44	.709	2.22	.364						
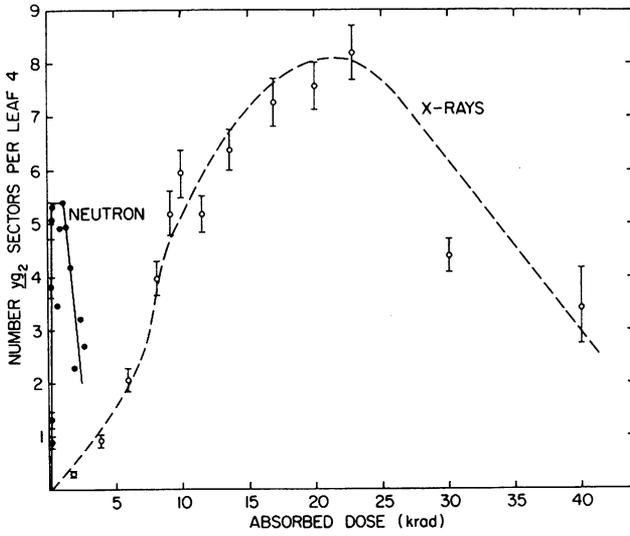
FIG. 1. Frequency of yg$_2$ sectors induced in maize leaf 4 by a wide range of neutron and X-ray doses; linear representation.
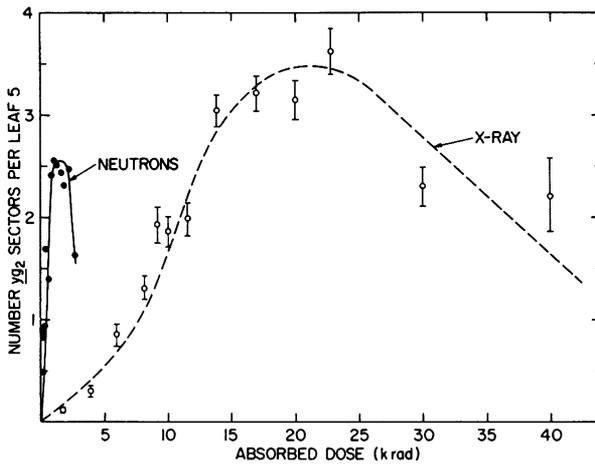
FIG. 2. Frequency of yg$_2$ sectors induced in maize leaf 5 by a wide range of neutron and X-ray doses; linear representation.
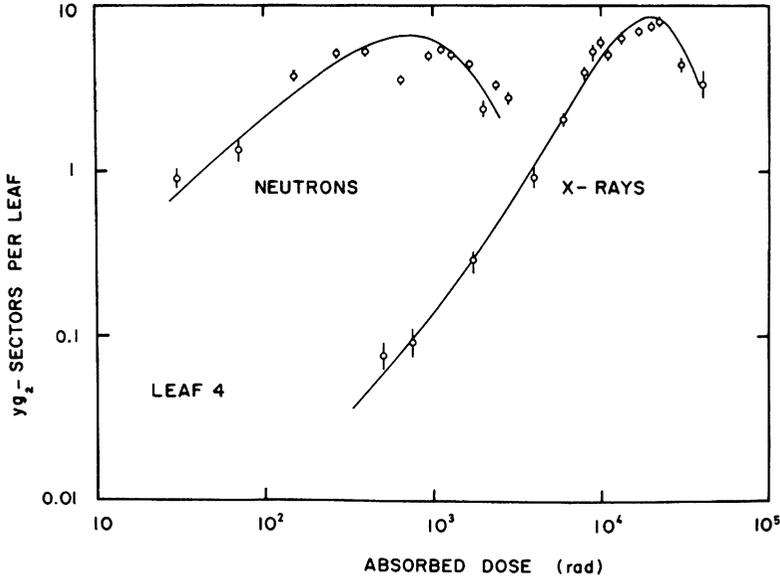
FIG.3.   Mean numbers of yg_2 sectors in leaf 4 as a function of X-ray and neutron dose (log-log plot).   The vertical bars indicate standard deviations due to the Poissonian fluctuations of the numbers of observed events.   The curves represent the least squares fit according to Eqs (4) and (5) with the parameters given in Table II.

       The corresponding relations for leaf 5 are shown in Fig. 4. One finds the same characteristics, although the initial shape of the neutron curve is doubtful and seems to be subject to considerable statistical uncertainties.

       The results are in agreement with the assumption that the effect probability is proportional to the square of the energy concentration in sensitive sites of the cell nucleus.   It has been shown [4] that the dependence of the effect on the square of the specific energy, z, leads to a linear-quadratic dependence of the observed yield on dose:

$$y = k \, (\zeta D + D^2) \hspace{3cm} (1)$$

where ζ is the energy average of z, produced by any one single charged particle in the site.   The value of ζ for neutrons is generally so high that the quadratic component can be neglected.   For x rays, on the other hand, ζ is so small that the quadratic component dominates except at smallest doses.   k is a proportionality constant and can be understood in terms of the different number of cells in the leaf primordia.

       Since, in the present experiments with x rays, both the initial linear component and the subsequent quadratic increase of the yield can be observed, one is able to determine the values of k and ζ.   For this purpose a least square fit of the data to the linear-quadratic model:

$$y = a \, D + b \, D^2 \hspace{1cm} \text{with} \hspace{1cm} a = k\zeta \text{ and } b = k \hspace{1cm} (2)$$

TABLE II.   ESTIMATES OF THE
PARAMETERS IN RELATION TO
THE YIELD OF $\underline{yg}_2$ SECTORS

A.   Estimates of the Parameters in Relation
     to the Yield of $\underline{yg}_2$ Sectors Produced by
     Neutrons

$$y_n = a_n\, D_n\, e^{-a'_n\ D}$$

	Leaf 4	Leaf 5
a_n (rad^{-1})	.024	.006
a'_n (rad^{-1})	.0013	.0012

B.   Estimates of the Parameters in Relation
     to the Yield of $\underline{yg}_2$ Sectors Produced by
     X Rays

$$y_x = (a_x\, D_x + b\, D_x^2)\, e^{-b'\ D_x^2}$$

	Leaf 4	Leaf 5
a_x (rad^{-1})	9.5×10^{-5}	3.3×10^{-5}
b (rad^{-2})	4.5×10^{-8}	1.7×10^{-8}
b' (rad^{-2})	5.0×10^{-9}	4.5×10^{-9}

has been performed. Only data up to 10^4 rad have been included in this
analysis in order to avoid complexities due to cell killing and to the
corresponding decline of the observed yield of mutations at higher doses.

Together with the estimate of the two coefficients in Eq. (2)
the joint 95%-confidence region of these parameters has been derived.
As pointed out in a separate discussion of the statistical analysis [5]
this confidence region is an ellipse. Figure 5 depicts the results for
leaf 4 and 5. From the estimates of the parameters a and b listed in
Table II one obtains values, $\zeta = a/b$, for x rays of 2100 rad for leaf 4
and 1900 rad for leaf 5. The close similarity in these two essentially
independently computed values of the average specific energy produced by
one absorption event implies that the same primary biophysical phenomena
are involved in cells producing $\underline{yg}_2$ sectors in each leaf. These values
belong to diameters of the sensitive sites of approximately 0.2 to 0.3 μm.
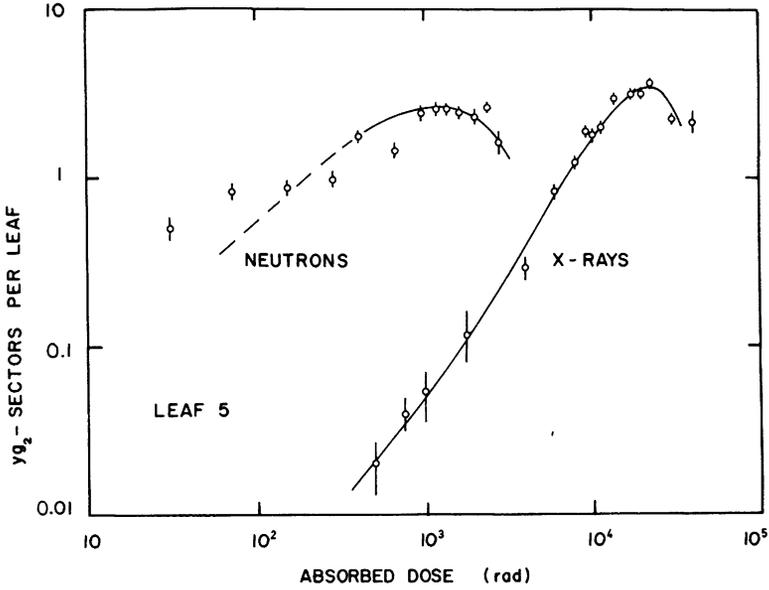
FIG. 4.   Mean numbers of $\underline{yg}_2$ sectors in leaf 5 as a function of X-ray and neutron dose.   Same remarks as for Fig. 3.
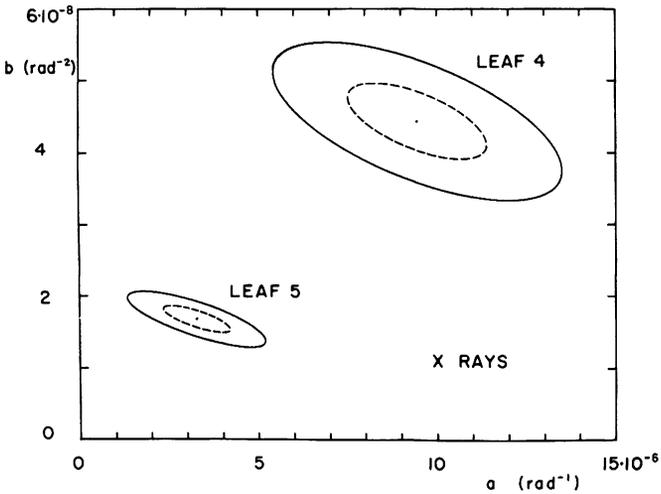
FIG. 5.   Least squares estimates and 95% confidence regions for the parameters a and b in Eq. (2) for X-rays. Only dose values up to 10^4 rads are included in the analysis.   The joint regions of standard deviation are represented by broken lines.

These distances are smaller than those observed in the effects on most
other eukaryotic cells [4]. The difference is, however, not unexpected
since in the present case one is dealing with a dry system. As a conse-
quence of the large values for ζ and small site diameters, the dry seed
system shows a linear dose-effect relation for x rays up to about 2500 rad,
in contrast to only about 5 rad for a sensitive wet higher plant system
[6]. For doses which in most biological systems would be considered high,
a characteristic low dose response is obtained in this dry system.

Since, as yet, no precise microdosimetric data are available for
sites as small as 0.2 μm, one cannot predict the value of ζ for neutrons.
The values would be expected, however, to be roughly 50 times larger than
the values for x rays. Accordingly, the observed linear component for
neutrons should exceed the linear component for x rays by about the same
factor of 50. The actually observed values of the linear coefficient for
neutrons are also given in Table II. One finds that for leaf 4 the value is
considerably larger than expected. The same observation holds for leaf 5,
although in this case the value is at best tentative due to considerable
statistical fluctuations in the initial part of the dose-effect relation.
It should in this context be noted that the standard deviations given in
Table I and Figs. 3 and 4 represent only the Poissonian fluctuations in the
number of observed $\underline{yg_2}$ sectors; other systematic variations may well occur
and are not represented by these standard deviations. The data for leaf 5
in the range of low doses indicate proportionality to a power of dose less
than 1. If substantiated this would be a finding of considerable interest.
The question must, however, remain open as long as no systematic analysis of
the experimental uncertainties has been performed. For the present discussion
only a linear relation at small doses will be considered although this may
lead to conservative estimates of the neutron RBE.

The essential observation is that the limiting RBE of neutrons
vs. x rays at lowest doses appears to exceed the expected values of roughly
50 by a wide margin. The value for leaf 4 appears to exceed 200. Although
there is at present no explanation for this result, it should be pointed out
that values of neutron RBE of as high as 135 have been reported in this
material [1,2] and RBE values exceeding 100 have also been found in studies
of the opacification of the murine lens [7], in experiments with the induction
of mammary tumors in the Sprague-Dawley rat by neutrons [8], and in results
with somatic aberrations in Tradescantia [9]. There are some indications
that these very high values of the neutron RBE at low doses are due to an
increased production of sublesions in the densely ionizing tracks of heavy
charged particles. Such an increase could be represented in Eq. (1) by a
larger value of the coefficient k for neutrons than for x rays. Since there
have been no systematic microdosimetric studies with the necessary spatial
resolution of several nanometer one cannot, at present, attempt a quantitative
evaluation of the effect.

It remains to study the plateau of the curves and their subse-
quent decline. The simplest explanation of the observed curves is that
induction of mutations and cell killing go on simultaneously. In a first
approximation one may consider the case that these processes are inde-
pendent, although this is probably an over simplification. The simplest
assumption is then that cell survival declines exponentially with cellular
damage that follows the same linear-quadratic kinetics that are observed
for other effects. The resulting relation for the observed yield over
the full dose range is then the product of the survival probability and the
probability for mutation induction:

$$y = (a\ D + b\ D^2)\ e^{-a'\ D\ -\ b'\ D^2} \tag{3}$$

For neutrons, the quadratic terms can be neglected in both parts of the
equation. For x rays, on the other hand, the linear component plays no
role in the term for survival since that term is significant only in the
range of high doses. One, therefore, deals with the two different equations
for neutrons and x rays:

$$y = a_n \, D_n \, e^{-a_n' \, D_n} \tag{4}$$

$$y = (a_x \, D_x + b \, D_x^2) \, e^{-b' \, D_x^2} \tag{5}$$

The solid curves in Figs. 3 and 4 follow these relations with the values of
the coefficients given in Table II.

Although the parameters listed in Table II result in a satisfactory
fit we consider it premature to relate them to microdosimetric quantities
which would necessarily have to pertain to the different sensitive volumes
involved in mutation and killing. However, it may be noted that due to
the single-hit kinetics of neutrons one must expect events in which mutation
and killing occur simultaneously resulting in a smaller maximum observable
mutation frequency, which is consistent with our results.

DISCUSSION

Loss of the Yg_2 locus to give yg_2 sectors is considered to be
due to chromosome breaks, one occurring between this locus and the centro-
mere, followed by rearranged joining so that in subsequent somatic cell
divisions the part of chromosome 9 containing dominant Yg_2 is eliminated.
In the early cytogenetic literature [10] it was shown that: 1) simple
deletions are rare; 2) x-ray induced aberrations involving two chromosomes
are dependent on two independent breaks limited in both time and space;
and 3) the frequency of these two-break aberrations increases more than
linearly with increasing dose. It was then shown [11] that after neutron
irradiation (7.5 and 15 MeV) there was a linear relation of two-break
aberrations to dose. This was attributed to both breaks being produced
by single proton ionization paths, in contrast to their production with
x rays by two independent electron ionization paths. Further extensions
of these considerations, relating a dependence of the number of chromosome
exchanges on the square of the dose for x rays and a linear relation for
neutrons, were developed by, e.g. Lea [12], Wolff et al. [13] and
Neary [14].

The data on production of yg_2 sectors are consistent with those
on chromosome exchanges and amenable to the same explanation. The chromo-
somes in leaf primordia of the dormant seed embryo in maize would be
expected to be mainly in G1 of interphase [15].

Recently insight into the ultrastructure of interphase chromosomes
has been gained through electron microscopy. From observations on certain
animal chromosomes, which may apply generally, it appears that each un-
replicated interphase chromosome consists of a continuous folded fiber [16].
This fiber is comprised of a single DNA double helix tightly packed by
supercoiling and coated by protein. DuPraw states, "For example, a length
of Watson-Crick double helix 20 Å in diameter and 56 μ long would be wound
into a supercoiled fiber 80 to 100 Å in diameter and 7 or 8 μ long; the
latter could then be supercoiled again into its fully packed form, 230 Å
in diameter and only 1 μ long" [16].

The following tentative interpretation of the elementary processes
leading to the radiation response described in this paper is consistent

with the information available at this time. Radiation-induced lesions in
two of the continuous folded chromatin fibers, on the same or different
chromosomes, join in rearranged position so that two-break exchange
aberrations (dicentrics, centric rings, interstitial deletions), as
commonly seen in the light microscope, are formed. Certain of these,
involving the short arm of chromosome 9, can lead to subsequent loss of
a chromosome segment containing the Yg_2 locus and eventual appearance of
yg_2 sectors in the leaf.

The diameters computed from microdosimetric considerations of
the maize data, namely 2000 to 3000 Å, can be formally interpreted as the
diameter of sensitive sites which are subject to dual damage, or as
the effective diameter of the sphere of potential interaction around a
break produced by ionization. The interaction, scored eventually as a
yg_2 sector, is due primarily to the illegitimate joining of two breaks
produced by two independent charged particles in the site (as with x
rays) or the illegitimate joining of two breaks produced by the same
charged particle in a site (as with neutrons and low doses of x rays).
The amount of energy produced within a site by a charged particle to
yield the observed cytogenetic effect is computed as about 2000 rad.

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DISCUSSION

B. V. CONGER: The RBE values you obtain are very high, but since the
conditions under which the seeds were treated were such that some oxygen-
enhancement would be expected, they are probably not as high as they could
be. By controlling seed water content and oxygen availability so as to pro-
vide maximum radioresistance, you could possibly obtain RBE values 2 to 3
times higher, i. e. in the range of 600 to 700. If you lowered the water content
of your seeds still further and soaked them in oxygenated water after irradi-
ation, I suspect that you would have even greater oxygen enhancement, which
would lower the RBE values.

H. H. SMITH: Yes, I agree, we could have modified the conditions to
obtain still higher RBE values.

B. V. CONGER: What were the approximate times after sowing when
counts were taken of the frequency of yellow-green sectors in leaves
4 and 5?

H. H. SMITH: This is done as soon as the leaves reach maturity, which
is approximately 4 weeks after sowing in the case of leaf and about 5 weeks
in that of leaf 5 under our growing conditions.

K. H. CHADWICK: As I have already mentioned that the peak heights for
mutations should be the same regardless of radiation type, I should like to comment
on the apparent contradiction between your results and our proposals. I do not
believe that there is in fact a contradiction. I would be inclined to draw a
double peak curve through the neutron data in your Fig. 3. It will then be
found that the first peak is matched by a small peak at the edge of the X-ray
curve at the same height. I would say that you have two different sensitivities
in your cell population. The first peaks agree quite well; the second peak
for the neutrons and the main peak for the X-rays are different because of
the additivity of the mutations from the more sensitive cells. At the second
neutron peak I suspect that the more sensitive cells are no longer contribu-
ting, whereas in the case of the X-rays some sensitive cells are still pro-
ducing mutations and pushing up the main X-ray peak.

Your unineme proposals for the chromosome are most welcome; in this
connection we would refer you to the paper cited as Ref.[6] in paper IAEA-
SM-179/18, where we discuss the association between DNA double-strand
breaks, the unineme concept and chromosome aberration production. A more
detailed paper on this subject will be appearing in the journal " Theoretical
and Applied Genetics" shortly.

H. H. SMITH: I look forward to reading your papers. With regard to the suggestion that the peak areas of our X-ray and neutron curves may consist of two peaks each, the confidence limits around these points are not sufficiently narrow at present, in my opinion, to warrant such an interpretation.

P. D. HOLT: You have assumed in your paper that mutation and cell killing are independent, but have indicated that this assuption is probably only an approximation. Have you any idea what error is involved?

H. H. SMITH: We have made some computations on the basis of the assumption that in the production of a Yg_2 sector the target is primarily the distance of the Yg_2 locus from the centromere in chromosome IX, that in the case of cell killing the target is the whole length of the genome, and that both phenomena involve two chromosome breaks and illegitimate rejoining with a loss of chromosomal material — cell killing involving multiple events of this same type. Our computations indicated tentatively that two such dual events would on average cause cell death, but we do not believe that our neutron data are sufficiently accurate at present to confirm an interpretation of this kind.