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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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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, Yg_2/yg_2, were used in all experiments. The dominant Yg_2 produces full green color, so that the heterozygote has normal green leaves. The Yg_2 locus is located near the end of the short arm of chromosome 9. Loss of the Yg_2 allele (deletion) or change in its function (mutation) in heterozygotes gives a yellowish-green phenotype in leaf 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

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or damage [1]. This was considered to be due mainly, if not exclusively, to breaks in chromosome 9 between the centromere and the $\underline{Yg}_2$ locus, with loss (following involvement in chromosomal aberrations) of the $\underline{Yg}_2$-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 $\underline{Yg}_2/\underline{yg}_2$ 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 give maximum 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 ($\underline{yg}_2$) sectors. The fifth seedling leaf (leaf 5) matured somewhat later and was then scored in the same way. The size of the $\underline{yg}_2$ 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 $\underline{yg}_2$ 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 $\underline{yg}_2$ 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 $\underline{yg_2}$ 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 $\underline{yg_2}$ sectors per leaf are presented in Table 1. 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 $\underline{yg_2}$ 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 $\underline{yg_2}$ 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.

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:

$$y = k\ (\zeta D + D^2) \qquad (1)$$

where ζ is the energy average of Z, produced by any one single charged particle in the site. The value of Z 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 k and ζ. For this purpose a least square fit of the data to the linear-quadratic model:

$$y = a\ D + b\ D^2 \quad \text{with} \quad a = k\zeta \text{ and } b = k \qquad (2)$$

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 1 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 energy deposited in one absorption event indicates that the same primary cellular phenomenon is involved in 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. 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 consequence 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 2500-3000 rad, in contrast to only about 5 rad for a sensitive wet higher plant system [6]. Doses which for most biological systems would be considered high, show a characteristic low dose response 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 2. One finds that for leaf 5 the value is considerably larger than expected. The same observation holds for leaf 4, although in this case the value is at best tentative due to considerable statistical fluctuations. It should in this context be noted that the standard deviations given in Table 1 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 4 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 apparent value for leaf 5 is approximately 182. 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 subsequent 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 independent, 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:

$$y = (a\ D + b\ D^2)\ e^{-a^1\ D\ -\ b^1\ D^2} \qquad (3)$$

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:

$$y = a_n\ D_n\ e^{-a_n^1\ D_n} \qquad (4)$$

$$y = (a_x\ D_c + b\ D_x^2)\ e^{-b^1\ D_x^2} \qquad (5)$$

The solid curves in Figs. 3 and 4 follow these relations with the values of the coefficients given in Table 2.

Although the parameters listed in Table 2 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 $\underline{Yg_2}$ locus to give $\underline{yg_2}$ sectors is considered to be due to chromosome breaks, one occurring between this locus and the centromere, followed by rearranged joining so that in subsequent somatic cell divisions the part of chromosome 9 containing dominant $\underline{Yg_2}$ 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 increased exponentially with increased dose. It was then shown [11] that after exposure to 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 $\underline{yg}_2$ sectors is consistent with those on chromosome exchanges and amenable to the same explanation. The chromosomes 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 unreplicated 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 could 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."

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, rejoin 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 $\underline{Yg}_2$ locus and eventual appearance of $\underline{yg}_2$ 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 $\underline{yg}_2$ 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 deposited within a site by a charged particle to produce the observed cytogenetic effect is computed as about 2000 rad.

ACKNOWLEDGEMENT

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Table 1

Dose-Response Data for Number of *yg*$_2$ Sectors Per Leaf Following Irradiation of Seeds with X Rays or Neutrons

X rays						Neutrons					
		yg$_2$ sector/leaf						*yg*$_2$ sector/leaf			
Dose	No.	Leaf 4		Leaf 5		Dose	No.	Leaf 4		Leaf 5	
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						

Table 2

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

$$y_n = a_n D_n e^{-a_n^1 D}$$

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

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

$$y_x = (a_x D_x + b D_x^2) e^{-b^1 D_x^2}$$

	Leaf 4	Leaf 5
a_x (rad^{-1})	9.5×10^{-5}	3.3×10^{-5}
b (rad^{-2})	4.5×10^{-5}	1.7×10^{-6}
b^1 (rad^{-2})	5.0×10^{-9}	4.5×10^{-9}

FIGURE LEGENDS

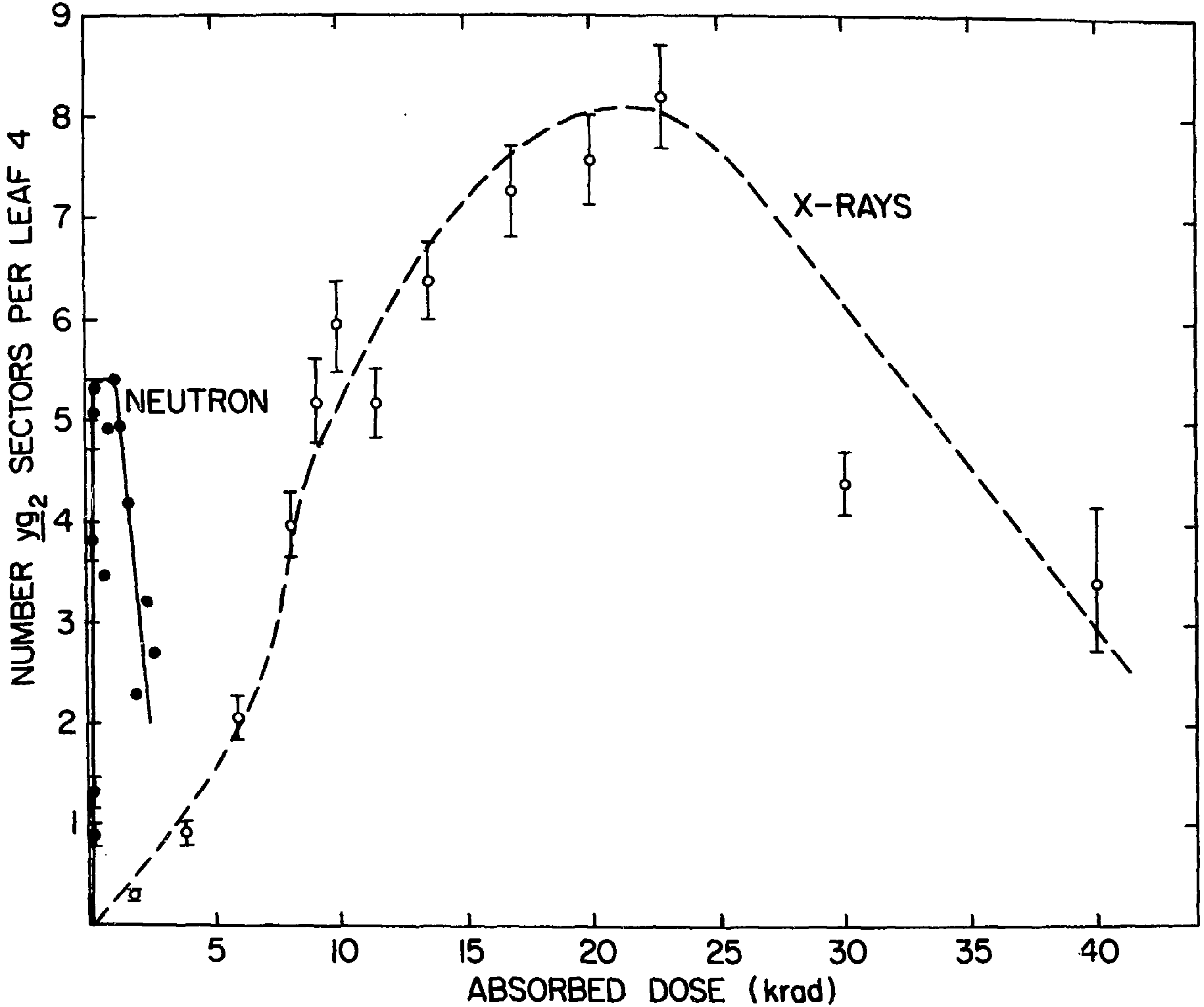
Fig. 1. Frequency of *yg*$_2$ sectors induced in maize leaf 4 by a wide range of neutron and x-ray doses; arithmetic plot.

Fig. 2. Frequency of *yg*$_2$ sectors induced in maize leaf 5 by a wide range of neutron and x-ray doses; arithmetic plot.

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 2.

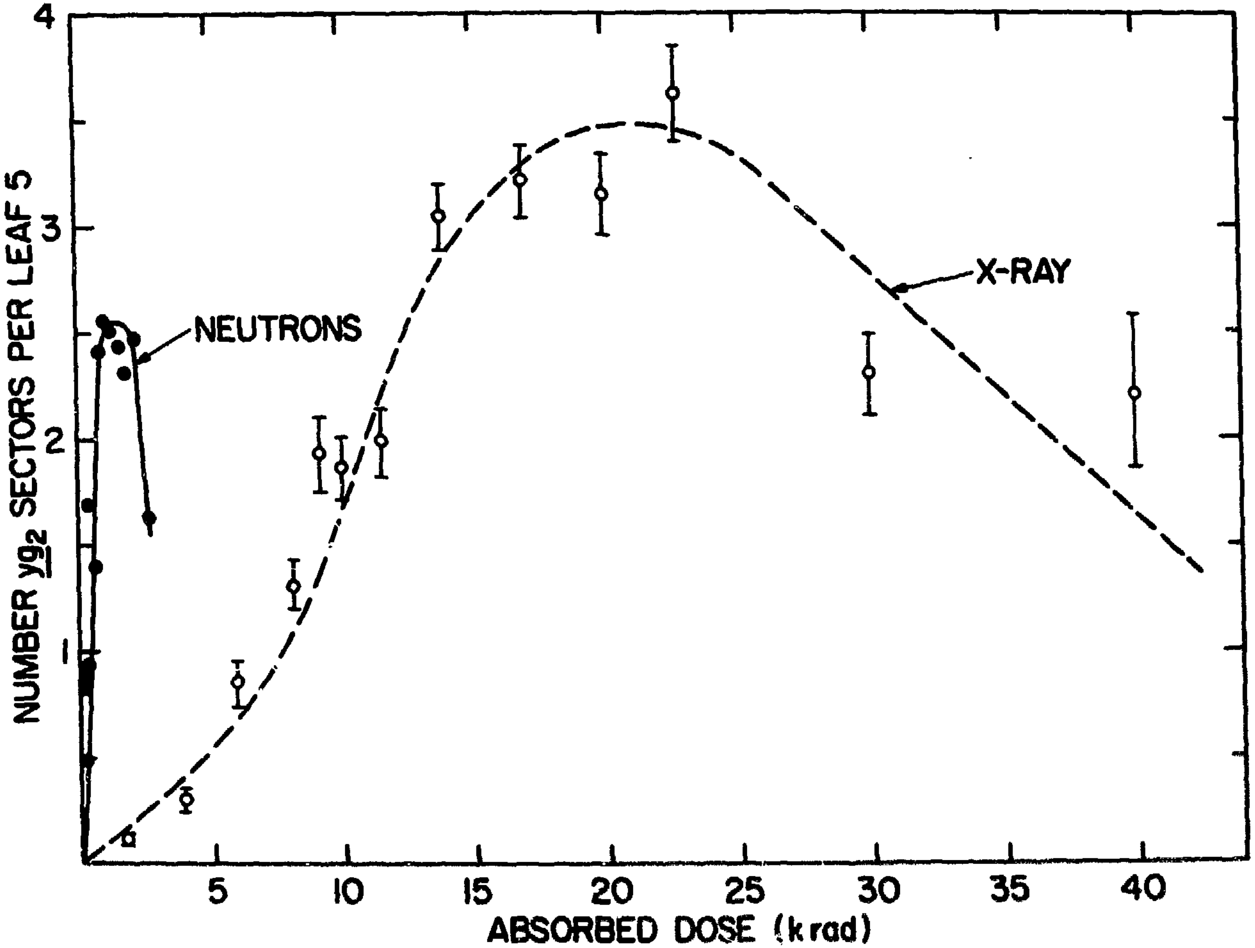
Fig. 4. Mean numbers of *yg*$_2$ sectors in leaf 5 as a function of x-ray and neutron dose. Same remarks as for Fig. 3.

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 rad are included in the analysis. The joint regions of standard deviation are represented by broken lines.



NUMBER $y\underline{g}_2$ SECTORS PER LEAF 4
NEUTRON
X-RAYS
ABSORBED DOSE (krad)
9
8
7
6
5
4
3
2
1
5
10
15
20
25
30
35
40

FIGURE 1



NUMBER yg2 SECTORS PER LEAF 5
NEUTRONS
X-RAY
ABSORBED DOSE (krad)
4
3
2
1
5
10
15
20
25
30
35
40

FIGURE 2

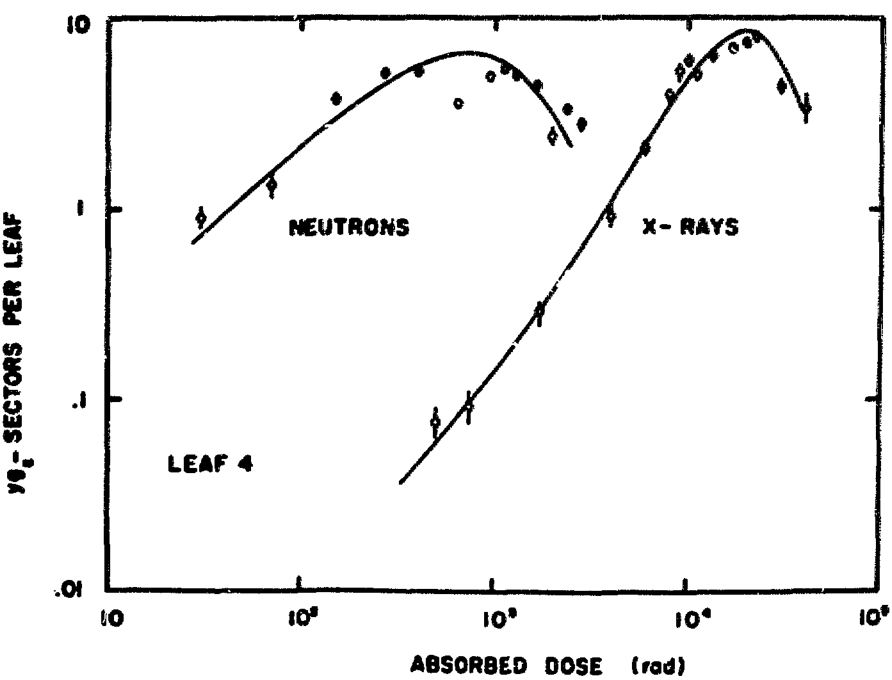

10
1
.1
.01
10
10^2
10^3
10^4
10^5
NEUTRONS
X- RAYS
LEAF 4
$y\theta_c$- SECTORS PER LEAF
ABSORBED DOSE (rad)

FIGURE 3

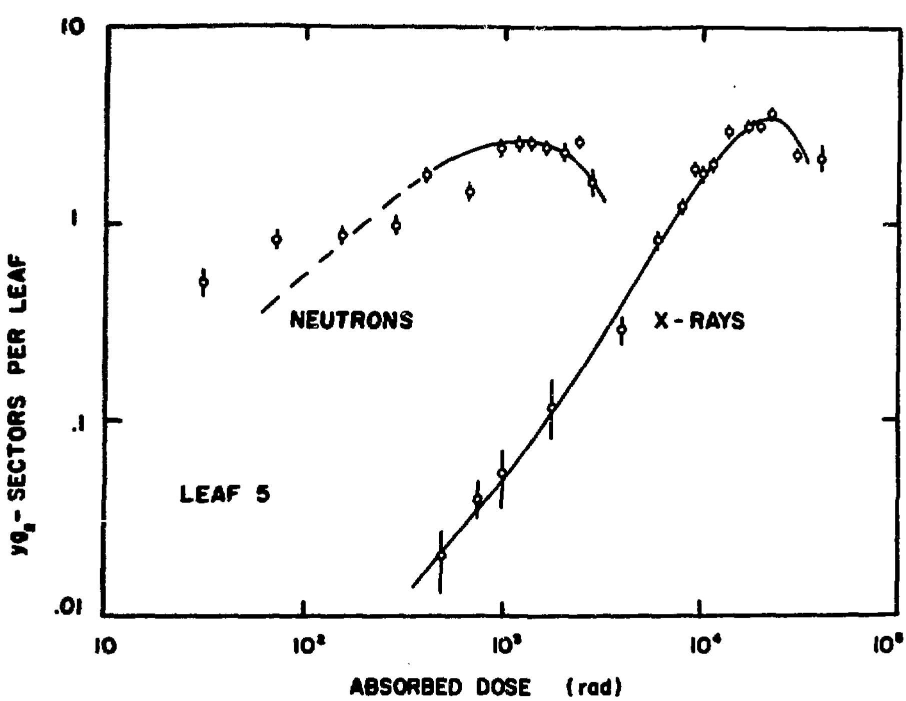

10
1
.1
.01
10
10^2
10^3
10^4
10^5
yg_2 - SECTORS PER LEAF
ABSORBED DOSE (rad)
NEUTRONS
X - RAYS
LEAF 5

FIGURE 4

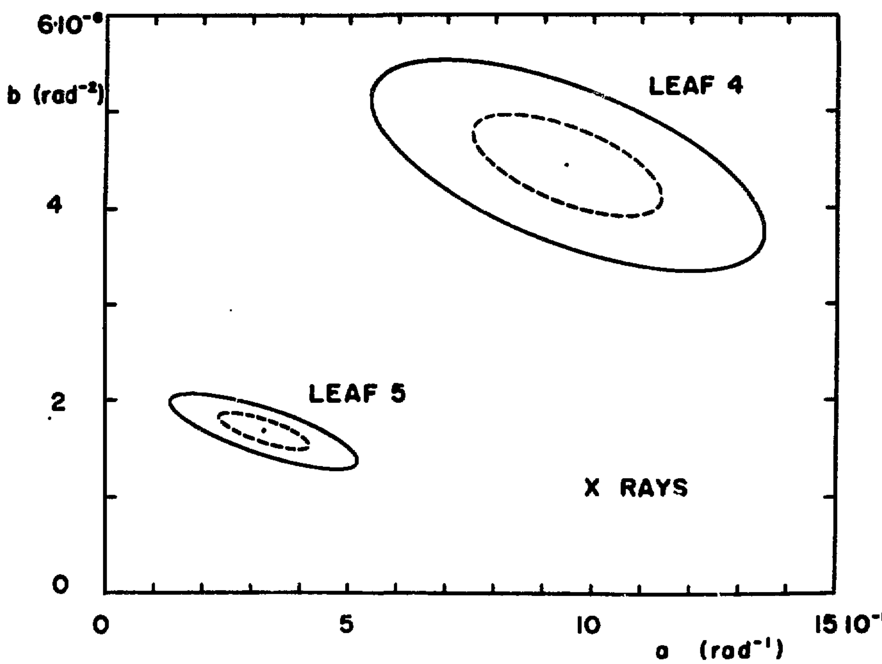


FIGURE 5