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MASTER

NEUTRON-INDUCED MUTATION EXPERIMENTS

Progress Report

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Abstract

Experiments have been carried out at the University of Wisconsin and Brookhaven National Laboratory to study the relative mutagenic effectiveness of neutrons of different energies employing X-linked recessive lethal and specific locus mutation tests. The energies and doses employed to date are .43 MeV (500, 1000, and 1500 R, in progress), .68 MeV (250, 500, 1000, and 1500 R), 2 and 6 MeV (250 and 500 R), and 15 MeV (250, 500, 1000, 1500 and 3000 R). .68 MeV neutrons appear to have an RBE between 3.3 - 4.5, 15 MeV neutrons an RBE between 1.9-2.2, and 2 and 6 MeV neutrons RBE's of intermediate values. The data for both .68 and 15 MeV neutrons do not yet differentiate between a linear and quadratic dose/frequency response curve for the doses studied. The specific locus mutation data also indicate the highest RBE for .68 MeV, followed by 2 and 6 MeV respectively.

Introduction

Mutation-induction experiments with neutrons have been started at the University of Wisconsin, with irradiations being carried out using the RARAF facility at Brookhaven National Laboratory. These experiments are being undertaken in collaboration with Professor Harold Rossi and his group. The two main purposes of these experiments have been (1) to determine the RBE for several energies of neutrons in the range of .34-15 MeV in gonial cells of Drosophila, and (2) to obtain information from specific locus mutations induced by neutrons in gonidia for the purposes of interspecies comparisons (Abrahamson et al., 1973). A third area of inquiry which has arisen is that of determining whether the dose/frequency response curve for any particular neutron energy best fits a linear or a quadratic equation.

With regard to the first and third aims, it becomes necessary to have a thorough knowledge of the dose response curve with respect to some standard radiation quality in a well defined germ cell stage. In eucaryotes, surprisingly this has only rarely been accomplished. Indeed the Webber and DeSerres (1965) studies were the first demonstration that a non-linear mutation relationship for metabolically active cells existed through the dose range studied of acutely delivered X-rays, a relationship that was best expressed by the quadratic equation $Y = C + \alpha D + \beta D^2$, where Y is the yield of mutation, C is the control rate and α and β are the coefficients of the dose D. This equation has been

known to describe the induced frequency of chromosome aberrations for decades (Lea, 1955) but clearly was not considered to play an important role in mutation induction. One probable reason for disinterest in this relationship was that most of the Drosophila studies on mutations were and are carried out on mature sperm, a metabolically inert stage, which appears to show a linear mutation induction relationship throughout its dose range. The exceptional observation was that of Edington (1956) in which a quadratic relationship was demonstrated.

Within the past several years however, the importance of the quadratic mutation induction relationship for X-rays independently reemerged from two separate studies. Firstly Abrahamson and Wolff (1975) have attempted a complete analysis of the mouse mutation studies and believe they strongly refute the notion of repair of pre-mutational damage as envisaged by Russell. Instead they argue that the X-ray mutation response in mammalian germ cells, both male and female, is best represented by the quadratic relationship. The phenomenon of dose-rate mutation dependence, therefore, results from reduction in the interaction of independent ionization tracks which affects the D^2 component of the equation.

Secondly, Abrahamson and Meyer's (1975) studies on the induction of mutations in Drosophila oögonia by sparsely ionizing radiation over a dose range of 20-6000 Rad again demonstrates the quadratic relationship holds for this data and in addition from the knowledge of the coefficients of the D and D^2 terms they were able to predict remarkably closely the independently obtained

data of Muller et al. (1963) on the influence of dose rate on this stage.

We have until very recently assumed that mutation induction with low LET radiations was linearly proportional to dose. We have learned that this need not be true for all systems and that particularly for metabolically active cells, a quadratic response can be obtained. The region of inflection in the curve varies with systems because of target size, and dose rate. Again on the basis of experimental data with neutrons it has been found that for both mutation and chromosome aberration induction a linear relationship exists. It is by no means true however that the shape of the curve must be linear throughout the dose range. There may well be a quadratic contribution to these aforementioned genetic effects when investigated in appropriate systems over a wide enough dose range. Both mouse and silkworm data have appeared to show some neutron dose rate or 2 track contributions and whilst other interpretations are possible we believe that an adequate range of doses have not been studied (particularly high doses) to determine whether some proton track interactions are not possible. Again if this turns out to be the case we would expect that a rational basis for species differences in response to neutrons can be developed which may provide theoretical explanations for the RBE differences observed in Drosophila, mouse, tradescantia, etc.

The best measure of RBE, as we see it, will be to compare the coefficient of the linear term, α value of the X-ray curve, to that of the various neutron energies. This is discussed in the results.

Mutations induced in gonial cells are among the most important in considerations of human genetic risks. The specific locus mutation frequency is needed for an extrapolation from Drosophila to equivalent stages in other organisms, especially mammals and humans. This extrapolation is via the relationship discovered by Abrahamson, Bender, Conger and Wolff (1973), namely that the mutation rate per locus per rad of X-rays is directly proportional to the total amount of DNA per species haploid complement. It is hoped that a similar relationship can be shown for neutron-induced specific locus mutations.

Irradiation Procedure

Depending upon the level of use of the RARAF facility by other investigators, it has been possible to do 1 irradiation approximately every three weeks (15 per year). It is planned to continue at this rate next year.

Specific Locus Mutation Test

The breeding scheme for this test is shown in Figure 1. Virgin females are collected from a modified "Jynd" stock 24-48 hours preceding irradiation. They are mated to "Jynd" males 24 hours following irradiation. Matings are made en masse (20 females and 30 males) per culture bottle. The flies are kept at 25°C and transferred to fresh medium at 2-day intervals to

Only a fixed number of flies can be irradiated at the RARAF facility during the 15 irradiation dates assigned to this project. It was decided to concentrate on recessive lethal data during this grant period; therefore, plans to complete the 500 and 1000 R dose points at 15 MeV and to begin work on .34 and .43 MeV neutrons were postponed. The 7 completed experiments and those 8 in progress or planning were devoted to building a good baseline of data at the 0 R point. This year 118,309 tests have been completed and it is estimated that approximately 120,000 more tests are in progress or in planning, for a grant year total of 238,309 tests (1,906,472 loci). To date these experiments have resulted in a total of 397,150 specific locus mutation tests.

Note: The Principal Investigator, Seymour Abrahamson, has devoted and will continue to devote approximately 15% of his time to this project during its current term from March 1, 1976 to February 28, 1977.

14 days post-mating. These first broods are discarded, but three succeeding broods are kept for mutation scoring. Flies which hatch from these later broods are developed from cells irradiated as oogonia.

Upon hatching, the appropriate F_1 females are counted and examined for variant phenotypes, possibly due to mutation at one of the marked loci. Variants are mated to brothers and observed for viability and fertility and their progeny are observed for heritability of the variant phenotype. It has been decided, based upon genetic and phenotypic considerations, to include 8 loci in the scoring procedure.

The data from the 7 completed experiments of this year plus the data collected in the 2 preceding years are presented in Table I. The total number of appropriate flies scored (N), the number of variants detected and analyzed (m), and the frequency of mutation with standard error are indicated. It had been previously decided to analyze 50,000 flies at 500 R of each of the 4 neutron energies. This had been accomplished last year, with the exception of the 15 MeV point, which has an exceptionally high standard error associated with it. Based on the mutation rate per locus per Rad of neutrons per generation at the 500 R point, it appears that the RBE is greatest for .68 MeV neutrons (4.07×10^{-8} m/locus/Rad/gen.), slightly less than that for 2 MeV neutrons (3.54×10^{-8}), and least for 6 MeV neutrons (1.13×10^{-8}). The 15 MeV data aren't considered here, due to the low number of tests and the high standard error.

X-linked Recessive Lethal Mutation Test

The breeding scheme used for detecting X-linked recessive lethals is shown in Figure 2. It is the same as that used by Dr. Helen Meyer, whose experiment (also supported by an ERDA contract) concerns very low dose X-ray-induced recessive lethals. Since the data are similarly collected in the two experiments, results will be comparable and be able to be used to determine RBE of the different neutron energies.

Virgin females are collected 24-48 hours prior to irradiation and are mated to FM6 males approximately 24 hours post-irradiation (Figure 2). Matings are en masse, 25 females and 40 males per culture bottle. They are kept at 25°C and transferred to fresh medium at 2-day intervals until 14 days post-mating. At this time, the flies are etherized and the fertilized females placed individually in vials. Upon hatching, these cultures are scored for pre-existing lethals in the y dow chromosome. These lethals are discarded.

From each of the non-lethal containing vials two methods of test crosses have been made and are combined in the data.

1. All F_1 female progeny from each vial are mated individually to FM6 males, with the matings from each vial kept in a group. Lethals occurring in clusters (and presumably of common origin) can thus be identified. One female is also designated as a "random" mating. This "random" method is the one in use in Abrahamson and Meyer's (1975) experiment, and for the purposes of comparison this procedure is used.
2. The second method (and the one in use almost exclusively during this grant period)

is to mate 3 F_1 female progeny from each P_1 female. In each group of 3 matings, one is again designated as a "random" mating. This method was employed to reduce the chances of recovering clusters of lethals and thereby reducing the standard errors associated with the lethal frequencies obtained.

Table II gives the total data from the 24 irradiations from the 2 previous years as well as the data which has been analyzed for this year (7 irradiations completed, 3 in progress, and 5 in planning). It is estimated that approximately 71,000 recessive lethal tests will be carried out in this grant period.

Table II shows that number of X-chromosomes tested (N), the number of lethals detected (m), the lethal mutation induction frequency with standard error, and the number of clusters of each cluster size (in parentheses; for example (3-2, 4) means 3 clusters of 2 and 1 cluster of 4).

Table III shows the observed recessive lethal frequencies with standard errors for .68 MeV neutrons at 0, 250, 500, 1000 and 1500 R dose points. Also shown are those values expected based on both a weighted linear regression analysis (equation $Y = 1.34 \times 10^{-3} + 1.16 \times 10^{-5} D$) and a weighted quadratic regression analysis (equation $Y = 1.41 \times 10^{-3} + 8.52 \times 10^{-6} D + 2.70 \times 10^{-9} D^2$). These values are shown plotted in Figure 3. The data to date could fit either a linear or a quadratic relationship. Therefore, we will attempt to obtain a 3000 R dose point where there are expected values which differ by a substantial amount (3.61×10^{-2} for a linear relationship vs. 5.17×10^{-2} for a quadratic relationship).

Table IV shows the observed lethal frequencies and those expected based upon both a linear (equation $Y = 1.20 \times 10^{-3} + 5.79 \times 10^{-6} D$) and a quadratic (equation $Y = 1.25 \times 10^{-3} + 5.02 \times 10^{-6} D + 4.88 \times 10^{-10} D^2$) relationship. The values shown in Table IV are shown plotted in Figure 4. No conclusions can be drawn based on these data as to whether the observed data more closely fit a linear or a quadratic relationship.

The RBE's of these two energies of neutrons relative to X-rays can be obtained by comparing the α values of their equations. For .68 MeV neutrons the RBE calculated on the basis of linearity is 4.5 (α of x-rays = 2.6×10^{-6}) and that calculated on the basis of a quadratic relationship is 3.3. For 15 MeV neutrons, the RBE calculated on the basis of linearity is 2.2 and that calculated on the basis of a quadratic relationship is 1.9.

It is worth mentioning that the mutation rate per locus per Rad per generation seems to be consistent between the recessive lethal and the specific locus mutation tests. Alexander (1960) found an induced specific locus mutation rate in chromosome 3 of D. melanogaster larvae irradiated with 900 R of x-rays to be 1.28×10^{-8} /locus (95% confidence limits were $0.75 - 2.03 \times 10^{-8}$). Assuming that .68 MeV neutrons have an RBE between 3.3 and 4.5 and that there are approximately 200 loci on the X-chromosome capable of mutating to lethality, the per locus/R/generation rates found for 500 R (6.30×10^{-8}) and 1000 R (7.5×10^{-8}) appear to be in general agreement with Alexander's data as does the specific locus mutation data at

500 R (4.07×10^{-8}) for .68 MeV neutrons. If it were true that the mutation/locus/R/generation rates for recessive lethal and specific locus mutation tests were consistent, an experimenter could choose the test which was least expensive of time and effort to get an estimate of mutation rate per locus per Rad for the purposes of interspecies comparisons.

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(1) 1^{J1} sc^{J1} pn w rb cm ct⁶ oc¹ ras² v dy g² f od car sw

(2) Ins "FM6", y B dm "Jynd"
Females

y sn¹ oc¹ ptg v . y^S "Jynd"
Males

Mated en masse and transferred every 2 days to day
14 post-irradiation (p.i.) to exhaust oocytes. Save
cultures after day 14 p.i. to test oogonia.

Observe non-disjunctional daughters for mutations
induced in X-chromosome #2 at loci marked in X-chro-
mosome #1. Mate putative mutants to y v sn oc
brothers for further study.

Figure 1: Genetic Scheme for Detecting Mutations at
Specific Loci

P_1

 $\underline{Df (l^{Jl} y ac) sc^{S1} B InS w^a sc^8}$
y dow

 females X

 $\underline{Ins "FM6", y^{3ld} w B}$
y

 Males

Mated en masse and transferred every 2 days
 through day 14 to exhaust oocytes. Surviving
 fertilized females bred individually

Discard cultures lacking y dow males,
 Mate females from other cultures:

F_1

 $\underline{Ins "FM6", y^{3ld} w B}$ females⁽¹⁾
y dow

 X

 $\underline{Ins "FM6", y^{3ld} w B}$
y

 males

mated singly

F_2

 Score for y dow males. If absent or scarce, mate
y B sisters X "FM6" males

F_3

 Confirmation of lethals

(1) "Automatic" virgins, since dow brothers are sterile and
 the "Df Basc" chromosome is lethal with normal Y

Figure 2. Scheme for Detecting X-Linked Recessive
 Lethal Mutations

MUTATION RESPONSE IN OOGONIA WITH .68 MeV NEUTRONS

Figure 3

MUTATION RESPONSE IN OOGONIA WITH 15 MeV NEUTRONS

Figure 4

Table 1

Neutron-Induced Specific Locus Mutations in Oogonia

	.68 MeV		2 MeV		6 MeV		15 MeV	
Dose	m/N	Frequency + S.E.	m/N	Frequency + S.E.	m/N	Frequency + S.E.	m/N	Frequency + S.E.
OR	5/163,254 (4)	3.1 ± 2.4 $\times 10^{-5}$						
250R	0/11,412	0						
500R	10/61,371 (2,4)	16.3 ± 8.0 $\times 10^{-5}$	8/57,008	14.0 ± 5.0 $\times 10^{-5}$	2/57,861	3.4 ± 2.4 $\times 10^{-5}$	8/31,380 (7)	25.5 ± 22.5 $\times 10^{-5}$
1000R							3/14,864	20.1 ± 11.6 $\times 10^{-5}$

1. When clusters were obtained, a special S.E. formula was used (Muller, 1952).
2. The OR mutation rate is cumulative for all experiments.

Table II

Neutron-Induced X-linked Recessive Lethal Mutations in Oogonia

	<u>.68 MeV</u> ave. = 650R/hr.		<u>2 MeV</u> ave. = 650R/hr.		<u>6 MeV</u> ave. = 800R/hr.		<u>15 MeV</u> ave. = 900R/hr.	
Dose	m/N	Frequency + S.E.	m/N	Frequency + S.E.	m/N	Frequency + S.E.	m/N	Frequency + S.E.
OR	32/23,413	.14 ± .00						
250R	14/3,507	.40 ± .11 x 10 ⁻²	11/5,066 (3)	.22 ± .08 x 10 ⁻²	38/7,062 (4-2, 3, 4, 7)	.54 ± .16 x 10 ⁻²	7/5,117 (2)	.14 ± .06 x 10 ⁻²
500R	57/9,043 (3-2, 4)	.63 ± .10 x 10 ⁻²	23/5,693 (2-3)	.40 ± .10 x 10 ⁻²	17/5,472 (3)	.31 ± .09 x 10 ⁻²	41/10,105 (3-2)	.40 ± .07 x 10 ⁻²
1000R	109/8,721 (3-2)	1.25 ± .04 x 10 ⁻²					89/9,576 (11-2, 4)	.93 ± .12 x 10 ⁻²
1500R	148/7,248 (9-2)	2.04 ± .19 x 10 ⁻²					77/8,829 (2)	.87 ± .02 x 10 ⁻²
3000R							42/1,988 (3-2, 3)	2.1 ± .23 x 10 ⁻²

1. When clusters were obtained; a special S.E. formula was used (Muller, 1952).
2. The OR mutation rate is cumulative for all experiments.

Table III

Observed and Expected Recessive Lethal Frequencies of .68 MeV
Neutrons Based on a Linear Dose-Response Relationship vs.
a Quadratic Dose/Response Relationship

Dose	Observed	Expected Y if Linear	Expected Y if Quadratic
0R	$.14 \pm .00$ $\times 10^{-2}$	$.13 \times 10^{-2}$	$.14 \times 10^{-2}$
250R	$.40 \pm .11$ $\times 10^{-2}$	$.42 \times 10^{-2}$	$.37 \times 10^{-2}$
500R	$.63 \pm .10$ $\times 10^{-2}$	$.71 \times 10^{-2}$	$.63 \times 10^{-2}$
1000R	$1.25 \pm .04$ $\times 10^{-2}$	1.29×10^{-2}	1.26×10^{-2}
1500R	$2.04 \pm .19$ $\times 10^{-2}$	1.87×10^{-2}	2.03×10^{-2}
3000R	--	3.61×10^{-2}	5.13×10^{-2}

Table IV

Observed and Expected Recessive Lethal Frequencies of 15 MeV
Neutrons Based on a Linear Dose/Response Relationship ...
vs. a Quadratic Dose/Response Relationship

Dose	Observed Y	Expected Y if Linear	Expected Y if Quadratic
0R	$.14 \pm .00$ $\times 10^{-2}$	$.12 \times 10^{-2}$	$.12 \times 10^{-2}$
250R	$.14 \pm .06$ $\times 10^{-2}$	$.26 \times 10^{-2}$	$.25 \times 10^{-2}$
500R	$.40 \pm .07$ $\times 10^{-2}$	$.41 \times 10^{-2}$	$.39 \times 10^{-2}$
1000R	$.93 \pm .12$ $\times 10^{-2}$	$.70 \times 10^{-2}$	$.68 \times 10^{-2}$
1500R	$.87 \pm .02$ $\times 10^{-2}$	$.99 \times 10^{-2}$	$.99 \times 10^{-2}$
3000R	$2.1 \pm .23$ $\times 10^{-2}$	1.86×10^{-2}	2.07×10^{-2}