MASTER

NEUTRON-INDUCED MUTATION EXPERIMENTS

Progress Report

for the Period March 1, 1977 - February 28, 1978

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November, 1977

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

UNDER CONTRACT NO. EY-76-S-02-1748.A001

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ABSTRACT

Experiments have been carried out 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 to study X-linked lethals are .43 MeV (500, 1000, 1500, 1900 R (in progress)), .68 MeV (250, 500, 1000, 1500 R), 2 MeV (250, 500, 1000, 1500, 2000 R), 6 MeV (250, 500, 1500, 3000 R) and 15 MeV (250, 500, 1000, 1500, 3000 R). .43 MeV neutrons appear to have an RBE in the range 1.9-4.7, .68 MeV 2.8-4.3, 2 MeV (incomplete data), 6 MeV 1.7-3.2, and 15 MeV 1.7-2.2. The data for .43 MeV and .68 MeV neutrons do not yet differentiate between a linear and a quadratic dose/frequency response curve for the doses studied, but suggest a quadratic relationship. The data for 2, 6 and 15 MeV are inconclusive. The specific locus mutation data indicate the highest RBE for .68 MeV neutrons, followed by 2 and 6 MeV respectively.

Introduction

Neutron-induced mutation experiments with Drosophila oögonia have been conducted at the University of Wisconsin, with irradiations being carried out at the RARAF facility at Brookhaven National Laboratory. These experiments are being undertaken in collaboration with Professor Harold Rossi. The three main purposes of these experiments have been (1) to determine the RBE with respect to X-rays for several energies of neutrons in the range of .34-15 MeV in gonial cells of Drosophila females, (2) to obtain information from specific locus mutations and/or recessive lethal mutations induced by neutrons in gonia for the purposes of interspecies comparisons (Abrahamson et al., 1973), and (3) to determine 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 (1976) have attempted a complete reanalysis 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 that 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 while 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-four weeks (15 per year). We will continue to irradiate at this rate next year.

X-linked Recessive Lethal Test

The breeding scheme used for detecting X-linked recessive lethals is shown in Figure 1. It is the same as that used by Abrahamson and Helen Meyer, whose experiment concerns X-ray induced recessive lethals. The data are similarly collected in the 2 experiments. Therefore, results should be comparable and should be able to be used to determine the RBE's 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. 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 preexisting lethal-containing vials are discarded.

Two types of test crosses have been made from non-lethal-containing vials. (1) All F_1 female progeny from each vial are mated individually to 2-3 FM6 males, with the matings from each F_1 vial kept in a group. Lethals occurring in clusters (and presumably of common origin) can thus be identified. One vial is designated as the "random" mating. This "random" method is in use for the purpose of comparison to Abrahamson and Meyer's

(1975) experiment. Large group matings such as described are no longer in use. (2) The second method (in current use) is to mate 3 F_1 female progeny from each P_1 female. In each group of 3 matings, one is designated as a "random" mating. This method was employed to reduce the probability of recovering clusters of lethals and thereby reducing the standard errors associated with the lethal frequencies obtained.

This year we have completed and analyzed the data from 7 irradiations, there are 4 in progress, and there are 4 planned, for a total of 15. This meets our goal as described in last year's proposal.

We proposed doing 5 irradiations using .68 MeV neutrons at a dose of 3000 R. This was impractical from 2 standpoints: (1) Even with measures taken to prevent the dessication of the flies, many died during the length of time needed to give 3000 R. (2) The dose was high enough to sterilize most of the flies which did survive the irradiation.

We did 8 irradiations at 2 MeV and 6 MeV with at least 2 high dose points in each case (5-6 were planned).

The remaining 6 irradiations will be devoted to getting a 1900 R dose point and more data at the 1500 R dose point with .43 MeV neutrons. We had planned to use .34 MeV neutrons during this time, but .43 MeV neutrons appear to be the best energy for trying to distinguish between a linear and a quadratic dose-frequency response relationship. .34 MeV neutron experiments will be postponed until next year.

Table 1 gives the total data from the 40 irradiations from the 3 previous years plus that which has been analyzed for this year. Table 1 shows the number of X-chromosomes tested (N), the number of lethal mutations detected (M), the lethal mutation frequency with standard error, and the number of clusters of each size (in parentheses; for example, (3-2, 4) means 3 clusters

of 2 lethals each and 1 cluster of 4 lethals).

Table 2 shows the observed recessive lethal frequencies with standard error for .43 MeV neutrons at 0, 500, 1000, 1500 and 1900 R dose points. Also shown are those values expected based on both a weighted linear regression analysis (equation $Y = 1.70 \times 10^{-3} + 1.23 \times 10^{-5} D$) and a weighted quadratic regression analysis (equation $Y = 2.13 \times 10^{-3} + 4.89 \times 10^{-6} D + 7.33 \times 10^{-9} D^2$). The data are suggestive of a quadratic relationship, however we feel we need more data at 1500 R and data at 1900 R to be more definitive. 1900 R is the highest dose point practical with the currently available targets.

Table 3 shows the same comparison for .68 MeV neutrons as did Table 2 for .43 MeV neutrons. The data is again suggestive of a quadratic relationship, but with the currently available targets it is not feasible to get the 2000 R dose point in a short enough time period. The 3000 R point is not possible for reasons discussed previously. If we obtain a target which delivers a sufficiently high dose rate, we will attempt to obtain a 2000 R dose point. The equations for this table are: linear $Y = 1.74 \times 10^{-3} + 1.11 \times 10^{-5} D$ and quadratic $Y = 1.80 \times 10^{-3} + 7.42 \times 10^{-6} D + 3.30 \times 10^{-9} D^2$.

The RBE's of the different energies of neutrons relative to that of X-rays can be obtained by comparing the α values of their equations. The following are the range of values of RBE's obtained by comparing the α 's of both linear and quadratic equations for each neutron energy to the α of X-rays (2.6×10^{-6}): .43 MeV 4.7-1.9; 68 MeV 4.3-2.8; 2 MeV (data not complete); 6 MeV 3.2-1.7; 15 MeV 2.2-1.7.

Specific Locus Mutation Test

The breeding scheme for this test is shown in Figure 2. Virgin females are collected from a modified "Jynd" stock 24-48 hours preceding irradiation. They are mated to "Jynd" males 24 hours post-irradiation. Matings are 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 14 days post-mating. These first broods are discarded, but the progeny of three succeeding broods of 2 or 3 days each are kept for mutation scoring, i.e. from days 14-20.

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 on genetic and phenotypic considerations, to include 8 loci in the scoring procedure.

The data from the completed experiments of this year plus that collected in the 3 succeeding years are presented in Table 4. The total number of flies scored (N), the number of variants detected and analyzed (M), and the frequency of mutation with standard error are indicated. Clusters are indicated in the same fashion as in Table 1. In order to get high dose points for the recessive lethal tests, all of our time at the RARAF facility was used for that purpose. Therefore, all specific locus data gathered this year was 0 R data gathered in experiments paralleling the recessive lethal experiments. Total chromosomes actually scored or in progress or in planning is projected to number 250,000-270,000. This corresponds almost exactly to plans put forth in our project proposal of last year.

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/generation) less 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 due to the high standard error.

Note: The Principal Investigator, Seymour Abrahamson, has devoted and will continued to devote 15% of his time to this project during its current term from March 1, 1977-February 28, 1978.

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Table 1

Neutron Induced X-linked Recessive Lethal Mutations in Oogonia

	.43 MeV		.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.	M/N	Frequency ± S.E.
0 R	79/44,304 (9-2,2-3)	.18±.02 x 10 ⁻²								
250 R			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±.14 x 10 ⁻²	7/5,117 (2)	.14±.06 x 10 ⁻²
500 R	47/7,152	.66±.01 x 10 ⁻²	57/9,043	.63±.08 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
1000 R	90/6,473 (3-2)	1.39±.15 x 10 ⁻²	109/8,721 (3-2)	1.25±.12 x 10 ⁻²	33/3,986 (2)	.83±.15 x 10 ⁻²			89/9,576 (11-2,4)	.93±.12 x 10 ⁻²
1500 R	53/1,971 (7-2)	2.69±.41 x 10 ⁻²	151/7,405 (10-2)	2.04±.17 x 10 ⁻²	67/3,596 (2)	1.86±.23 x 10 ⁻²	64/4,371	1.46±.18 x 10 ⁻²	130/13,466 (2)	.96±.08 x 10 ⁻²
1900 R					I N P R O G R E S S					
2000 R					41/1,970 (2-2)	2.08±.34 x 10 ⁻²				
3000 R							95/2,982 (8-2,3)	3.18±.36 x 10 ⁻²	63/3,053 (4-2,3)	2.06±.28 x 10 ⁻²

1. When clusters were obtained (indicated by parentheses) a special S.E. formula was used (Muller, 1952).
2. The 0 R mutation rate is cumulative for all experiments.

Table 2

Observed Lethal Frequencies with .43 MeV Neutrons Plus Expected
Values Based on Computed Linear and Quadratic Dose - Frequency Relationships

Dose	Observed lethal frequency	Expected lethal frequency-linear	Expected lethal frequency-quadratic
0 R	$.18 \pm .02$	$.18 \times 10^{-2}$	$.21 \times 10^{-2}$
500 R	$.66 \pm .01$ $\times 10^{-2}$	$.79 \times 10^{-2}$	$.64 \times 10^{-2}$
1000 R	$1.39 \pm .15$ $\times 10^{-2}$	1.40×10^{-2}	1.44×10^{-2}
1500 R	$2.69 \pm .41$ $\times 10^{-2}$	2.01×10^{-2}	2.59×10^{-2}
1900 R	in progress	2.50×10^{-2}	3.78×10^{-2}
2000 R		2.63×10^{-2}	4.12×10^{-2}

Table 3

Observed Lethal Frequencies with .68 MeV Neutrons Plus Expected Values
Based on Computed Linear and Quadratic Dose - Frequency Relationships

Dose	Observed lethal frequency	Expected lethal frequency-linear	Expected lethal frequency-quadratic
0 R	$.18 \pm .02 \times 10^{-2}$	$.17 \times 10^{-2}$	$.18 \times 10^{-2}$
250 R	$.40 \pm .11 \times 10^{-2}$	$.45 \times 10^{-2}$	$.38 \times 10^{-2}$
500 R	$.63 \pm .10 \times 10^{-2}$	$.73 \times 10^{-2}$	$.63 \times 10^{-2}$
1000 R	$1.25 \pm .12 \times 10^{-2}$	1.29×10^{-2}	1.25×10^{-2}
1500 R	$2.04 \pm .17 \times 10^{-2}$	1.84×10^{-2}	2.04×10^{-2}
2000 R		2.40×10^{-2}	2.99×10^{-2}
3000 R		3.51×10^{-2}	5.38×10^{-2}

Table 4

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.
0 R	11/386,710 (2,4)	2.8±1.3 x 10 ⁻⁵						
250 R	0/11,412	0						
500 R	10/61,371 (2,4)	16.3±8.0 x 10 ⁻⁵	8/57,008	14.0±5.0 x 10 ⁻⁵	2/57,861	3.4±2.4 x 10 ⁻⁵	8/31,380 (7)	25.5±22.5 x 10 ⁻⁵
1000 R							3/14,864	20.1±11.6 x 10 ⁻⁵
3000 R					7/12,412 (2-2)	56.4±26.7 x 10 ⁻⁵		

1. When clusters were obtained, a special S.E. formula was used (Muller, 1952).
2. The 0 R mutation rate is cumulative for all experiments.

P_1
Df (1^{J1} y ac) sc^{S1} B InS w^a sc⁸
Ins "FM6", y^{3ld} w B
y dow
females X
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
Ins "FM6", y^{3ld} w B females (1)
X
Ins "FM6", y^{3ld} w B
y dow
mated singly
Y
males

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 1. Scheme for Detecting X-Linked Recessive Lethal Mutations.

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

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

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-chromosome #1. Mate putative mutants to y v sn oc brothers for further study.

Figure 2. Genetic Scheme for Detecting Mutations at Specific Loci.