COO-1748-15

NEUTRON - INDUCED MUTATION EXPERIMENTS

Progress Report

for Period March 1, 1975 - February 29, 1976

Seymour Abrahamson

University of Wisconsin
Madison, Wisconsin 53706

November, 1975

Prepared for

THE U.S. ENERGY RESEARCH AND DEVELOPMENT
ADMINISTRATION UNDER CONTRACT

NO. E(11-1) - 1748

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

Abstract

Experiments have been initiated 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 carried out to data are .68 MeV, 2 MeV, and 6 MeV (250 and 500 R), and 15 meV (250, 500, and 1000R). The data thus far collected from the recessive lethal test indicate that .68 MeV neutrons have the highest RBE among the energies tested, followed by 6 and 2 MeV. The specific locus mutation data also indicate the highest RBE for .68 MeV, followed respectively by 2 and 6 MeV. The 15 MeV data is as of now incompletely analyzed, as are some dose points of 2 and 6 MeV.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product or process disclosed or represents that its use would not infringe privately owned rights.

Introduction

Mutation-induction experiments have been started at the University of Wisconsin, with irradiations being carried out using the RARAF facility at Brookhaven National Laboratory, in collaboration with Prof. Harold Rossi and his group. The two main purposes of these experiments are (1) to determine the RBE for neutrons of different energies in gonial cells, and (2) to obtain information specific locus mutations induced by neutrons in gonads for purposes of interspecies comparisons.

With regard to the first aim, 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 months 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 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 dose² component of the equation.

Secondly, Abrahamson and Meyer's (1975) studies on the induction of mutations in Drosophila oogenesis by sparsely ionizing radiation over a dose range of 20-3000 Rad again demonstrates the quadratic relationship holds for this data and in addition from the knowledge of the coefficients of the D and D² 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.

Now, it is apparent that if the sparsely ionizing radiations produced a quadratic response and the more densely ion-

izing radiation response is a linear one, then the measure of the relative biological effectiveness (RBE) of the densely ionizing to the sparsely ionizing radiation will be variable one. Indeed at certain high doses the RBE should be less than 1 (where the curvilinear X-ray response exceeds the linear neutron response) this appears to be between 3000 and 4000 Rad X-rays relative to 15 MeV neutrons (see Figure 3). At doses below this the RBE will appear to be increasing for decreasing dose and in fact at low doses the RBE will be a constant. This will occur when the X-ray curve has the major contribution resulting from the αD component of the equation. Again in Drosophila oogonia we predict this to be at doses of 1000 Rad or below.

The best measure of RBE, as we see it, will be to compare the linear slopes of the X-ray curve to that of the more densely ionizing radiation energies as can be seen in Figure 3. Under these conditions the ratio of slopes will be a constant that will be a most meaningful expression in terms of population risk estimation! There are, however, several caveats to this suggestion; for example, the chronic X-ray or neutron mutation rate in the female mouse is probably unreliable as an estimate of RBE for the female, for reasons discussed in the Abrahamson Wolff paper. At high neutron doses saturation of the effect may be evident and could distort the RBE relationship.

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.

Finally, by making use of both the theoretical and empirical relationships for X-rays established by Kellereer and Rossi (1972), it has been possible to suggest that a biological constant of target diameter or site interaction distance exists for each species regardless of the genetic endpoint (Abrahamson and Meyer, 1975), i.e. gene mutation or chromosomal aberration (see Figure 4). While there are undoubtedly certain exceptions to the constancy, these exceptions highlight specific mechanisms or conditions which alter the expected results. If, as suggested by the data, target diameter is related to species DNA content, then we are in a position to provide an independent proof of the ABCW model of induced mutation relationship with DNA content. The Kellereer and Rossi (1972) study permits an independent check on target or interaction distance by neutron studies of different energies.

Irradiation Procedure

The original plan was to irradiate at 4-6 week intervals. However, it has been found possible to decrease this interval to 1 irradiation every third week, depending upon the level of use of the RARAF facility. Therefore, 13 irradiations have been carried out or are scheduled in this report period. It is planned to continue at this rate of 1 irradiation every three weeks 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 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 8 irradiations performed last year plus those analyzed of the 13 irradiations of this year are presented in Tables I-IV. The total number of appropriate flies scored, the number of variants detected and analyzed, the rate of mutation with standard error, and the mutation rate per rad per generation are indicated. The data are complete for 500R at .68 MeV, and will be completed for 500R at 2 MeV, when the existing variants have been analyzed. (It had been previously decided that 50,000 flies will be analyzed at each dose point. One additional irradiation is planned for 6 MeV, and several may be necessary for 15 MeV, depending upon analysis of irradiations now in progress. Based on the mutation rate per locus per rad of neutrons per generation at the 500R point, it appears that the RBE is greatest for .68 MeV neutrons, slightly less than that for 2 MeV neutrons, and least for 6 MeV neutrons. The 15 MeV data is not considered here, due to the low number of tests presently analyzed and the high standard error.

Thus far, a total of approximately 258,200 of the appropriate females have been observed (or are calculated to be contained in experiments in progress) for specific locus mutations, for a total of approximately 2,065,600 loci scored.

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 (Figure 3).

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 kinds of test crosses are made. All females in the vial are mated individually to FM6 males, with the matings from each vial kept in a group ("family" mating). Lethals occurring in clusters (probably of common origin) can thus be identified. It will be of interest to note how the number and size of clusters compares with those found in spermatogonia (R. Valencia et al., unpublished). One female is also designated as a "random" mating. This "random" method is the one in use in

Dr. Meyer's experiment, and to compare data, this procedure is used.

Tables V-VII give the total data from the 8 irradiations of last year plus that data from the 13 irradiations this year which have been analyzed (2 irradiations are scheduled and 3 incompletely analyzed at present). The tables give the number of X-chromosomes tested, the number of lethals detected, the lethal mutation induction rate with standard error, and the lethal mutation rate per locus per rad per generation assuming 200 loci on the X-chromosome of Drosophila are capable of mutating to a recessive lethal. A total of approximately 43,000 tests are already analyzed or are currently in progress. This represents a net total of approximately 35,000 tests in the past year.

The recessive lethal frequencies for 2 dose points at each energy (.68, 2, 6, and 15) are plotted in Figure 3. Straight lines have been drawn to approximate those which would pass through the OR point plus the two dose points. The ratio of the slope of each of these lines to that of the linear component of the X-ray curve should indicate RBE. From these preliminary tests, .68 MeV has the highest RBE, followed by 6, 2, and 15 MeV among the neutron energies tested. It should be remembered that the data are not considered complete at all energies and at all dose points.

It is worth mentioning that the mutation rate/locus/rad/generation, corrected for control values seems to be consistent

between the two types of genetic tests for the one dose point (500R) at the one energy (.68 MeV) where those data are complete: 4.45×10^{-8} for recessive lethals vs. 4.07×10^{-8} for specific locus mutations. The total data on which a comparison of this type is made is still incomplete. If it were true that the mutation rate/locus/rad/generation for the two 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.

Alexander (1960) found an induced specific locus mutation rate in chromosome 3 of D. melanogaster larvae irradiated by 900R 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 RVE of between 3 and 4, the per/locus/R/generation rate found in the present experiment for .68 MeV neutrons appears to be in agreement with Alexander's data.

Note: The Principal Investigator Seymour Abrahamson, had devoted and will continue to devote approximately 15% of his time to this project during its current term from March 1, to February 29, 1976.

BIBLIOGRAPHY

- Abrahamson, S., M. A. Bender, A.D. Conger and S. Wolff, 1973
Uniformity of radiation-induced mutation rates among
different species. Nature 245: 460-462.
- Abrahamson, S. and H. U. Meyer, 1975 Quadratic analysis for
the induction of recessive lethal mutations in Drosophila
oögonia by X-irradiation. IAEA Symposium, Chicago, IL.
- Abrahamson, S. and S. Wolff, 1975 On radiation induced
specific locus mutations in the mouse. In manuscript.
- Alexander, M. L., 1960 Radio sensitivity at specific auto-
somal loci in mature sperm and spermatogonial cells of
Drosophila melanogaster. Genetics 45: 1019-1022.
- Edington, C. W., 1956 The induction of recessive lethals in
Drosophila melanogaster by radiations of different ion
densities. Genetics 41: 814-821.
- Kellerer, A. M. and H. H. Rossi, 1972 The Theory of Dual
Radiation Action. Current Topics in Radiation Research
Quart. 8: 85-158.
- Lea, D. E. A., 1955 Actions of Radiations on Living Cells,
Cambridge University Press, Cambridge (1st ed.).
- Muller, H. J., I.I. Oster, and S. Zimmering, 1963. Are chronic
and acute gamma irradiation equally mutagenic in Drosophila?
Repair from Genetic Radiation Damage (Sobels, F. H., Ed).
Macmillan, N. Y., 275.
- Webber, B. B. and F. J. De Serres, 1965 PNAS 53: 430.

(1) $\underline{1}^{J1}$ sc^{J1} pn w rb cm ct^6 oc^1 ras^2 v dy g^2 f od car sw

(2) Ins "FM6", y B dm

"Jynd"
Females

y sn^1 oc^1 ptg v $\cdot$ 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-chromosome #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

 $\underline{Df (1^{J1} y ac) sc^{S1} B InS w^a sc^8}$

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

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

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

X

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

y dow
mated singly

Y

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

Figure 3

Figure 4

TABLE I. NEUTRON-INDUCED SPECIFIC
LOCUS MUTATIONS

.68 MeV Neutrons

Dose	Number Chromosomes	Number Variants	p	per/locus/rad/generation ^a
0R	5,489	0	0	—
250R	11,412	0	0	—
500R	61,371	10 ^b	1.63×10^{-4} $\pm .80 \times 10^{-4}$	4.07×10^{-8}
1000R	—	—	—	—

a. Assuming 8 loci.

b. 1 garnet; 1 ruby; cluster 2 white; cluster 4 cut;
1 cut; 1 cut.

TABLE II. NEUTRON-INDUCED SPECIFIC
LOCUS MUTATIONS

2 MeV Neutrons c

Dose	Number Chromosomes	Number Variants	p	per/locus/rad/generation ^a
0R	6,047	0	0	—
250R	—	—	—	—
500R	42,408	6 ^b	1.41×10^{-4} $\pm .58 \times 10^{-4}$	3.54×10^{-8}
1000R	—	—	—	—

a. Assuming 8 loci.

b. 1 white; 1 white; 1 white; 1 garnet; 1 cut; 1 carmine.

c. Includes 1 irradiation with analysis incomplete:
1989-0R; 14,600-500 R.

TABLE III. NEUTRON-INDUCED SPECIFIC
LOCUS MUTATIONS.

6 MeV Neutrons

Dose	Number Chromosomes	Number Variants	p	per/locus/rad/generation ^a
0R	6,985	0	0	—
250R	—	—	—	—
500R	44,192	2 ^b	$.45 \times 10^{-4}$ $\pm .32 \times 10^{-4}$	1.13×10^{-8}
1000R				

a. Assuming 8 loci.

b. 1 garnet; 1 eye color, undetermined locus.

TABLE IV. NEUTRON-INCUCED SPECIFIC
LOCUS MUTATIONS

15 MeV Neutrons ^d

Dose	Number Chromosomes	Number Variants	p	per/locus/rad/generation ^a
0R	6,985	0	0	—
250R	—	—	—	—
500R	8,972	7 ^b	7.80×10^{-4} $\pm 7.80 \times 10^{-4}$	19.50×10^{-8}
1000R	7,752	2 ^c	2.58×10^{-4} $\pm 1.82 \times 10^{-4}$	3.22×10^{-8}

a. Assuming 8 loci.

b. Cluster 7 cuts.

c. 1 garnet; 1 carnation.

c. 2 irradiations in progress - approximately 40,000 more tests at 500R and 1000R dose points.

TABLE V. NEUTRON-INDUCED X-LINKED
RECESSIVE LETHAL MUTATIONS

.68 MeV Neutrons ^a

Dose	Number Chromosomes	Number Lethals	P	per/locus/rad/generation ^b (Corrected for Control)
250R	3,507	14	3.99×10^{-3} $\pm 1.06 \times 10^{-3}$	4.58×10^{-8}
500R	4,712	29 ^c	6.15×10^{-3} $\pm 1.42 \times 10^{-3}$	4.45×10^{-8}
1000R	—	—	—	—

a. Control frequency for all irradiations
 $1.70 \times 10^{-3} \pm .68 \times 10^{-3}$ (9 lethals/5306 chromosomes).

b. Assuming 200 loci capable of mutating to a recessive lethal.

c. 2 clusters of 2; 1 cluster of 4.

TABLE VI. NEUTRON-INDUCED X-LINKED
RECESSIVE LETHAL MUTATIONS

2 MeV Neutrons ^a

Dose	Number Chromosomes	Number Lethals	p	per/locus/rad/generation ^b (Corrected for Control)
250R	2,415	5 ^c	2.07×10^{-3} $\pm 1.37 \times 10^{-3}$	$.54 \times 10^{-8}$
500R	2,707	11 ^c	4.06×10^{-3} $\pm 1.52 \times 10^{-3}$	2.36×10^{-8}
1000R	—	—	—	—

a. For control frequency - see Table V.

b. See b. - Table V.

c. 1 cluster of 3

TABLE VII. NEUTRON-INDUCED X-LINKED
RECESSIVE LETHAL MUTATIONS

6 MeV Neutrons ^a

Dose	Number Chromosomes	Number Variants	p	per/locus/rad/generation ^b (Corrected for Control)
250R	3,154	16 ^c	5.07×10^{-3} $\pm 1.73 \times 10^{-3}$	6.54×10^{-8}
500R	3,064	14 ^d	4.57×10^{-3} $\pm 1.46 \times 10^{-3}$	2.87×10^{-8}
1000R	—	—	—	—

a. For control frequency - see Table V.

b. See b. - Table V.

c. 1 cluster of 4; 1 cluster of 2.

d. 1 cluster of 3.

TABLE VIII. NEUTRON-INDUCED X-LINKED
RECESSIVE LETHAL MUTATIONS

15 MeV Neutrons ^{a,d}

Dose	Number Chromosomes	Number Lethals	p	per/locus/rad/generation ^b (Corrected for Control)
250R	—	—	—	—
500R	2,275	8 ^c	3.52×10^{-3} $\pm 1.38 \times 10^{-3}$	1.82×10^{-8}
1000R	1,462	10	6.84×10^{-3} $\pm 2.16 \times 10^{-3}$	2.57×10^{-8}

a. For control rate - see Table V.

b. See b. - Table V.

c. 1 cluster of 2

d. 3 irradiations in progress - approximately 20,000 tests
at 250, 500, and 1000 R.