NEUTRON-INDUCED MUTATION EXPERIMENTS

Progress Report

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Abstract

Experiments have been initiated to study the mutagenic effectiveness of neutrons of different energies. The genetic work is being done at the University of Wisconsin and the irradiations are being done at Brookhaven National Laboratory. The effects being scored are sex-linked recessive lethals and mutations at specific loci. The energies and doses used in the six irradiations carried out to date are .68 MEV (250 and 500 Rads) and 14 MEV (500 and 1000 Rads). The data collected to date, in five months of work, is mostly insufficient for discussion, but the recessive lethal data for .68 MEV neutrons does indicate an effectiveness much greater than that of X-rays at similar doses.

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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 on specific locus mutations induced by neutrons in gonial for purposes of inter-species comparisons.

With regard to the first aim, there is presently considerable inconsistency of results with regard to the RBE of neutrons when studies from different eukaryotic organisms are examined. The existing data (esp. that of the late Dr. Frank Gonzales, unpublished) suggest an increasing RBE with increasing LET, however; one problem is simply a general lack of detailed mutation studies. Secondly, experiments have been done at different dose levels and some were probably not on the linear portion of the dose:effect curve, i.e. were obtained from doses when the curve showed saturation. In addition, the studies have involved different biological end-points. Often the "mutation" end-points were chromosome aberrations (most of which would not be transmitted) and/or were mutations scored somatically or in eggs as in the case of the silkworm (Murakami, et al., 1965) without subsequent knowledge of the F1 adult viability.

In the present experiments, neutrons of different energies are or will be employed (.68, 2, 6(?), and 14 MeV) and at each energy at least 2 doses will be given, so as to ascertain linearity.
The biological endpoint is mutations in oogonia, so that few chromosome rearrangements will be involved and all mutations scored will be viable in the F₁ adults or transmissible to succeeding generations. Two types of mutations are being studied; X-linked specific locus mutations and recessive lethals.

Mutations induced in gonial cells are the most critical type in considerations of human genetic risks. Since there is almost no existing data for neutron treatment of gonia in Drosophila (the one published experiment being that of Lamb et al, scoring recessive lethals), the present experiments will provide useful risk-estimate information. The specific locus mutation frequency is needed for a still more exact extrapolation from Drosophila to equivalent stages in other organisms, esp. mammals and including humans. This extrapolation is via the relationship discovered by Abrahamsom, 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 mutations. The only existing data on specific locus mutations induced by neutrons in Drosophila is that of Muller and Valencia (1951) but the germ cell stage was mature sperm and the dose was high. For application of the "ABCW" hypothesis, gonial specific locus mutations are needed.
Report Period

Although the official starting date for this contract was March 1st, the additional funding which was necessary in order to begin the work was not obtained until June. Only then could arrangements be completed for carrying out the irradiations at Brookhaven National Laboratory. A preliminary visit was made June 19th to discuss procedure, and appointments were set up for irradiations. As a result of the late start, this report covers less than five months of actual work.

Irradiation Procedure

The original plan was (and still is) to irradiate at 4-6 week intervals, but in order to make up for time lost at the beginning of the grant year, the first 5 irradiations were done at much shorter intervals. The irradiation dates were:

<table>
<thead>
<tr>
<th>Irr.</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>July 23</td>
</tr>
<tr>
<td>&quot;</td>
<td>August 7</td>
</tr>
<tr>
<td>&quot;</td>
<td>August 27</td>
</tr>
<tr>
<td>&quot;</td>
<td>Sept. 13</td>
</tr>
<tr>
<td>&quot;</td>
<td>October 10</td>
</tr>
<tr>
<td>&quot;</td>
<td>November 19</td>
</tr>
</tbody>
</table>

For irradiations 1-3 we used .68 MEV neutrons and gave doses of 250 and 500 Rads. Irradiations 4-6 were with 14 MEV neutrons and doses of 500 and 1000 Rads. In each case, females were irradiated for both the specific locus mutation experiment and the sex-linked recessive lethal test.

Specific Locus Mutation Test

Virgin females are collected from a modified "Jynd" stock (figure 1) 24-48 (usually 36-48) hours preceding irradiation. They are mated to "Jynd" males approximately 24 hours after ir-
radiation. Matings are en masse (25 females and 40 males per bottle in earlier irradiations and 20 females and 30 males per bottle in later irradiations). The flies are kept at 25°C and transferred to fresh medium at 2-day intervals until 14 days post-mating. These first broods are discarded, but succeeding broods are kept for mutation scoring. Flies which hatch from these later broods are developed from cells irradiated as oogonia. In irradiations 1 and 2, two 3-day broods (A and B) were made, but in irradiations 3-6, three 2-day broods (A, B and C) were made, since the females were found to be surprisingly productive still at this age. (It was for this reason, too, that the number of flies per culture bottle was reduced.)

Upon hatching, the appropriate F1 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. Thereafter further characterization is done.

The data from the four irradiations scored to date are shown in table 1. Very few bonafide mutations at the specific loci have been found thus far, and these are presently in various stages of analysis. In none of the categories are the count numbers sufficiently large, as yet, to make even an estimate of results. In an experiment with the "Jynd" stock and 4000 R of X-rays R. Valencia (1961) found 32 oogonial mutations at the 10 loci considered "good" in a count of 43,828. Since none of our categories, nor even our grand total, approaches this sized count, and since our
dose levels are considerably lower (even allowing for high RBE),
the small number of bonafide mutants found is not too far from
expectation. (The cluster of 7 cuts of course represents one
event.)

The Bar eye in the "Jynd" stock does make eye color recogni-
tion more difficult and also there are quite extreme variations in
eye width and shape. At present, we are trying a new stock carrying
a similar chromosome without the Bar. This should remove much
of the "noise" from the system.

Sex-Linked Recessive Lethal Mutation Test

Virgin females are collected from the same stock bottles
being used by Dr. Helen Meyer's group in this laboratory. That
experiment concerns very low dose X-ray-induced recessive lethals
and is also supported by an AEC contract (see ref.). We are using
the same stocks and scheme, so that our results will be comparable.
By taking our females to be treated from the exact same stock
bottles, we can also use her control figures, thus saving a great
deal of work and expense. In our own experiment, we are carrying
only a small number of controls to monitor for any unusual cir-
cumstance.

The virgin females are collected 24-48 hours prior to irra-
diation and mated to FM6 males approximately 24 hours after irra-
diation (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
that time, the flies are etherized and the fertilized females placed
individually in vials. Upon hatching, these cultures are scored
for lethals in the \textit{y} dow chromosome. Any lethals showing at this time were pre-existing in the treated females. These cultures are discarded.

From each of the non-lethal-containing vials, two kinds of test crosses are made. In some cases, all the females in the vial are mated individually to FM6 males, with the matings from each vial kept in a group. These will be referred to as "family" matings. In this way, lethals occurring in "clusters" (and thus presumed to be of common origin) can be identified. It will be of interest to note whether clusters of induced lethals occur in oogonia and if so, how the number and size of clusters compares with those found in spermatogonia (data of R. Valencia et al, unpublished). In other cases, only one female is taken at random from each culture and mated for testing. (One randomly marked mating in each of the "family" groups will also be used in the "random" mating data.) This "random" method is the one in use in Dr. Meyer's experiment, and so it was thought that this same procedure should be used in part of the neutron work.

The results to date are sparse, due to the short time since initiation of the work plus the considerable time required from date of irradiation to final results (about 6 weeks to collection of lethals plus 2 weeks more for confirmation). Since the numbers are so small, table 2 shows the totals of the "family" matings plus the "random" matings. Only the .68MEV treated groups have sufficient numbers to warrant even preliminary comment. These figures indicate a rather high RBE, since the 500 Rad dose is nearly as effective as 3000R of X-rays, which have yielded 1.4% lethals in Dr. Meyer's experiments. RBE, however, calculated in
the usual way, taking the X-ray effect at any particular dose as 1, becomes meaningless at these low doses, since the X-ray dose: effect curve is not linear but falls off to nearly "no effect". It will be necessary to re-define "RBE", perhaps taking the neutron effect as the base.
Mated en masse and transferred every 2 days to day 14 to exhaust oocytes. Save cultures after day 14 to test oogonia.

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

Figure 1. Scheme for mutations at specific loci
Mated en masse and transferred every 2 days through day 14 to exhaust oocytes. Surviving fertilized females bred individually

Discard cultures lacking \textit{y dow} males

Mate females from other cultures:

\begin{align*}
\text{F}_1 & \text{ Df}(1\text{Jl} \ y \ \text{sc}) \text{ sc}^\text{Sl} \text{ R Ins w^n sc}^8 \text{ females X Ins "FM6", } y^{3\text{ld}} \text{ w B males} \\
& \text{ y dow} \\
\text{F}_1 & \text{ Ins "FM6", } y^{3\text{ld}} \text{ w B females} \quad \text{X} \quad \text{Ins "FM6", } y^{3\text{ld}} \text{ w B males} \\
& \text{ y dow} \quad \text{ mated singly} \\
\text{F}_2 & \quad \text{Score for } y \text{ dow males. If absent or scarce, mate} \\
& \text{ y B sisters X "FM6" males} \\
\text{F}_3 & \quad \text{Confirmation of lethals}
\end{align*}

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

Figure 2. Scheme for X-Linked Recessive Lethals
TABLE 1

Specific Locus Mutations

<table>
<thead>
<tr>
<th>Dose (Rads)</th>
<th>.68 MeV</th>
<th>14 MeV</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number Counted</td>
<td>Number Variants</td>
<td>Number Counted</td>
</tr>
<tr>
<td>0</td>
<td>1388</td>
<td>0</td>
<td>718</td>
</tr>
<tr>
<td>250</td>
<td>11,412</td>
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<tr>
<td>500</td>
<td>7,903</td>
<td>1$^1$</td>
<td>8972</td>
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<tr>
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<td>-</td>
<td>7752</td>
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<tr>
<td>Total</td>
<td>20,703</td>
<td>1</td>
<td>17,442</td>
</tr>
</tbody>
</table>

$^1$Mottled eye, locus not yet identified
$^2$Cluster of 7 cuts
$^3$Eye colors, not yet identified


TABLE 2

Recessive Lethal Mutations

<table>
<thead>
<tr>
<th>Dose (Rads)</th>
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<th>14 MeV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number Tests</td>
<td>Number lethals</td>
</tr>
<tr>
<td>0</td>
<td>212</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(846)¹</td>
<td>(1)</td>
</tr>
<tr>
<td>250</td>
<td>2184</td>
<td>8²</td>
</tr>
<tr>
<td>500</td>
<td>1453</td>
<td>17³⁴</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Meyer's controls from parallel experiments
² 4 unconfirmed
³ 7 unconfirmed
⁴ Includes 1 cluster of 2 and 1 cluster of 4
⁵ Unconfirmed


