FINAL TECHNICAL REPORT

Genetic Effects of Heavy Ions in Drosophila

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ABSTRACT

Drosophila sex-linked recessive lethal mutation test was used to study the dose response relation and relative biological effectiveness of heavy ions. The experiments were performed using the heavy ion beams at BEVALAC of Lawrence Berkeley Laboratory. These experiments were undertaken according to the proposed milestones and included Ne-20, A-40 and Fe-65 ions with respective energies of 600 MeV, 840 MeV and 850 MeV. At these energies several doses of these radiations ranging from 20 to 1280 R were used. Space radiation exposure to astronauts is supposed to be quite low and therefore very low dose experiments i.e. 20 R, were also performed for all the three ions. The mutation response was measured in all germ cell types i.e. spermatozoa, spermatids, spermatocytes and spermatogonia of treated Drosophila males. Even the very low dose of all these radiations induced significant numbers of mutations. A linear dose frequency relation was observed for most of the range except at high doses where the saturation effect was observed. Also, a very significant difference was observed among the sensitivity of the four germ cell stages where spermatozoa and spermatids were more sensitive. At the higher doses of this range, most of the spermatogonia and spermatocytes were killed. Although comparative and identical experiments with X-rays or neutrons have not been performed, the compassion of our data with the ones available in literature suggest that the heavy ions have a high rbe and that they are several times more effective than low LET X-rays. The rbe compared to neutrons however appears to be only slightly higher.

INTRODUCTION

There are several important reasons to investigate the genetic effects of heavy ions since it has been shown that the action of heavy ions on living cells is different from that of other radiation in several respects. Thus, i) unlike low LET radiation where several secondary electrons (several ionizations) cause the effect, the heavy ion effects are produced as a consequence of cell interaction with a single ion of high LET; ii) unlike low LET effects, which are modifiable significantly by intra-cellular enzymatic repair, the effects of heavy ions are not significantly modified by repair phenomena; iii) unlike low LET radiation, presence or absence or molecular oxygen dissolved in tissue does not significantly influence heavy ion effects iv) unlike low LET radiation, heavy accelerated particles can produce well defined range and depth distribution in tissue.

Among the two widely used genetic assays for eukaryotic intact animal experiments, Drosophila has a distinct advantage over mice, especially when large samples are required for statistical analyses leading to meaningful results. Experiments with mice may appear to be more relevant for extrapolation to man. However, mouse genetics still lacks comparable stocks to study various genetic end points such as sex linked recessive lethal mutations, heritable translocations and non-disjunction. Moreover, in order to obtain comparable large number of individuals required in heavy ion experiments, mouse specific locus test will be formidably expensive.

BACKGROUND

In the 1960's, several studies on mammalian cell cultures demonstrated that higher LET radiations induce higher rates of gene inactivation in irradiated cells. In 1970's, these investigations were extended to mutations in the belief that the data can be extrapolated to asses genetic risk to man and may also provide insights into mutagenesis mechanisms. Earlier studies (Bridges and Huckle 1970; Chu 1971), however, were less promising due to large variations in almost identical experiments. It was later known that these variations may be due to the methodological difficulties and the manner in which the data were analyzed. While extending the mammalian cell culture studies to the RBE/LET relation for mutations, it was thought that, it would be interesting to compare the results to those from other systems. In intact animals, the lowest RBE of one in insects and ninety in plants was reported. RBE in intact mouse was six. In mammalian cell cultures, this value came out to be eighteen (Cox et al, 1977). Thacker et al. (1979) also reported high RBE values. In these studies, the mutation frequency increased with dose for all LET radiations until the surviving fraction was reduced to 10 to 20%. At still higher doses (200 rad from 110 keV boron), at less than 10% survival, the mutation frequency fell below that induced by lower doses.

The low mutation frequency induced by higher LET radiation at low cell survival, was explained to be due to the number of tracks produced by different LET radiations. As a consequence, all recovered mutations do not suffer the same amount of radiation. A proportion of radiation induced mutations suffer more extensive damage and this damage is particularly greater at higher LET because of the differences in the track numbers of the two kinds of radiations passing through the nuclei of the treated cells. This explanation

was based on the observations of Goodhead et al. (1979). According to their studies, at 30% survival, for high LET radiation (with low track number), a mutant sub-population received 75% more dose. This increase is only 15% at low LET, where the track number is high. This higher dose to mutant cells by high LET kills more mutants (but not as many non-mutants) thus lowering the mutation frequency and the RBE. Additional factors, according to Thacker et al. (1979), are the less fitness of the mutant cells and the excessive chromosomal damage sustained by these cells.

Significance of such chromosomal damage was supported by the studies on Neurospora (de Serres et al 1967). In these experiments, 18 keV helium ions and 19-210 keV carbon ions were used. The over all RBE of 1.8 and 5.0 was observed. However, within the mutant population, the RBE for gene mutations was 5.5 and for extragenic mutations with excess chromosomal damage, it was 74.0. According to Thacker et al (1979), small shifts in the proportions of mutational damage considerably influence the over all RBE values obtained for mutation induction and the excess chromosomal damage to a mutant sub-population may explain the high maximum RBE reported in their work.

OBJECTIVES

One of the reasons Drosophila geneticists were reluctant to undertake the massive experiments with heavy ion research was the low RBE values reported in neutron experiments. In early neutron experiments, it was observed that neutrons are less effective than x-rays in inducing sex linked recessive lethal mutations in males. These results were taken as evidence that point mutations were caused by single ionizations, the lesser effectiveness of neutrons being explained as caused by wastage of ionizations because of their high density recoil proton tracks (Muller 1944). Later, in 1970's, a similar lesser effectiveness of neutrons was reported. At high doses of high energy neutrons, the RBE was somewhat greater than one (Sobels and Broerse, 1970; Gonzalez, 1972). The most comprehensive experiments were reported by Abrahamson et al, (1981). The lowest RBE was reported at the highest energy and these values increased progressively and linearly towards lower energies. It was suggested that, unlike x-rays, one densely clustered neutron track is capable of producing all the necessary hits in the target structure throughout the dose range resulting in a linear dose response for sex linked recessive lethals in oogonia.

In general, the RBE values in males of Drosophila were lower than that in mice and this might also have discouraged further Drosophila experiments using heavy ions. In interpreting neutron results, all Drosophila geneticists, including us (Kale and Baum 1980) appeared to have missed the consideration of a very important variable, the cell survival, which has now been shown to be of great significance in the analysis of RBE/LET relations. One of the reasons, this variable was not considered in Drosophila work was

perhaps the fact that it is impossible to know the absolute number of germ cells present in the testes. The sterility pattern observed at different doses was indeed discussed by Drosophila geneticists, but in the absence of any information on the absolute numbers of germ cells present in the testes at the time of irradiation, sterility pattern could not be directly related to observed mutation frequency.

One of the most frequently used biological observation to explain radiation action is the shape of the dose response curves induced by different qualities of radiation. However, the same shape of a curve can be attributed to different modes of radiation action depending on the model utilized in the interpretation. Thus, the typical linear quadratic response by low LET radiation is normally explained by the interaction of two sublesions (Keleerer and Rossi 1972). Goodhead et al (1982) explain this response to be due to a saturable repair phenomenon. Drosophila is well known for demonstating a linear relation between radiation quantity and mutation frequency. Such linear increase has been documented in very early literature for x-ray doses as low as 0 to 25 R and also for doses as high as 6000 R (see Stern 1973). A similar linear relation has also been observed later for a dose range of 0 to 2500 R (Gonzalez 1972). However, recently, Abrahamson et al (1981) reported a linear quadratic response in a dose range of 0 to 6000R. This suggested that the induced mutations are a result of two sublesions or hits either by single ionization tracts or by two independent tracks of low LET radiation. This interpretation supports the Rossi model which proposes an interaction of two sublesions to produce an effect. According to Goodhead et al (1982), the curvature observed at the high doses of low LET radiation can be explained by a saturable repair hypothesis rather than induction of two

sublesions. This model proposes that, all low LET radiation damage is repairable by a repair system whose efficiency decreases with increasing dose. At low doses, the repair system is more efficient and a lower mutation frequency is obtained (linear part of the curve). At high doses, the repair system is saturated (or is less efficient) and a high number of mutations and a quadratic relation is observed. This model can also explain the linear-quadratic relation observed in Drosophila oogonia.

A similar support to both these models can also be interpreted from the high neutron RBE values reported by Abrahamson et al (1981). More densely clustered neutron tracks were more efficient than x rays in supplying all the necessary sub microscopic hits. If a single ionization (a single lesion) was required to produce a mutation, one would expect the neutron radiation to be more wasteful leading to lower RBE values. However, these values consistently increased with higher LETs ranging from 0.4 meV to 15 meV. These observations thus support the two sublesions model. However, saturable repair model can also be used to explain the high RBE values. thus, if the high LET induced lesions are less repairable, this will lead to higher mutation induction than that induced by low LET radiation. It should be noted that when repair deficient cells, (spermatozoa) are tested, the RBE values are low, whereas for repair proficient cells (oocytes and oogonia) the RBE values are higher. Both models can thus be used to interpret the oogonia data from Drosophila.

MATERIALS AND METHODS

Most mutagenic agents induce mutations, recombination and translocations suggesting that some primary step is common to all. This might be the effectiveness of contact of mutagen with DNA, but probably the association is more intimate, although complex. A common mode of origin of mutation and chromosome aberration induction is also indicated by similar kinetics observed for these phenomena (Abrahamson and Wolff 1976). Testing of the above three end points was planned. This yields advantages in the genetic breeding procedures, reduces labor required in these experiments and minimizes the number of visits to the heavy ion facilities located in California.

IRRADIATIONS

ION	ENERGY	LET RANGE	LET	DOSE(RADS)
Ne-20	600 MeV/n	25-70	25	200, 400, 800
A-40	840 MeV/n	0-200	100	200, 400, 800
Fe-65**	850 MeV/n	150-400	400	200, 400, 800

406 LET

Above doses of the respective radiations have been used. Exact dose and the dosimetrery has been provided by BEVALAC and will be used while publishing the results. At times as in case of Fe, the energy obtained was less than planned and had to be used since scheduled higher energies could not be reached. The exposures were performed in medicinal gelatin capsules which were taped to target spot marked by laser beam. The treated males were brought to A&M laboratory for genetic experiments.

Mating Intensity and Sample Size

Treated males were returned to the Drosophila laboratory and mated to virgins for 16 successive days in eight 2-day broods. This brooding procedure sections the germ line

precisely and permits the measurement of genetic damage to the difference cell types (Kale and Baum 1979). The sample size required for a particular heavy ion exposure was approximately determined by referring to a table for estimating samples sizes based on Kastenbaum-Bowman test (Wurgler et al 1977). Using our Canton-S stock with a spontaneous rate of 0.06% and referring to the table, we should be able to estimate the approximate number of chromosomes to be scored in a particular experiment.

Treatments were given to Canton-S adult males of uniform age. These were mated to base virgins at a ratio of 1 male to 6 virgins in each brood. The F1 progeny was pair-mated and the F2 vials were scored for presence, or absence of mutations.

Analysis of the Data

The scores from individual males in each successive brood were first subjected to Poisson analysis in order to know if the data contain clusters of identical events. A cluster, when detected, will be considered as one induced event for further analysis. Later, the data from different broods for a particular cell type were added and compared to those from other cell types.

RESULTS

During the last three years, experiments using heavy ions beams at the BEVALAC were performed according to the plans described in the original proposal. The following milestones were proposed, and have been reached:

YEAR	ION	ENERGY	LET RANGE	LET	DOSES(RADS)
I	Ne-20	600 MeV/n	25-70	25	20, 40, 80 and 160
II	A-40	840 MeV/n	0-200	100	20, 40, 80 and 160
III	Fe-65**	850 MeV/n	150-400	400	20, 40, 80 and 160

The data are given in the four enclosed tables for various ions and also in enclosed figures. The main trend of these experiments can be summarized as follows:

1. All radiations, even the lowest ones (20R) produced significant number of mutations.

2. The mutation frequency for all three ions types increased with the dose.

3. Higher LET radiations produced successively higher number of mutations in all germ cells. The RBE for all these radiations appears to be higher than that of X-rays and in some cases, higher than neutrons.

4. A pronounced germ cell sensitivity was observed. The spermatids appear to be the most sensitive stage followed by spermatozoa.

5. Spermatogonia appear to be least sensitive to all three kinds of radiation.

The above conclusions are drawn from data in tables 1 through 4 and figures 1 through 3. The dose response at the lower doses appears to be linear. It will be interesting to know if the response become quadratic at higher doses.

Table I. Induction of Sex-Linked Recessive Lethal Mutations by Neon-20 at 640 MeV in Drosophila

Germ Cell Stage	Dose (rads) 20R			40R			80R			160R		
	L	T	%	L	T	%	L	T	%	L	T	%
B_1 (Spermatozoa)	2	1105	.18	2	888	.23	5	1136	.44	3	635	.47
B_2 (Spermatids)	10	1148	.87	2	782	.26	00	454	.00			
B_3 (Spermatids)	5	782	.64	15	895	1.68	4	773	.52	7	1135	.62
B_4 (Spermatocytes)	6	914	.66	24	871	2.76	3	694	.43	4	440	.91
B_5 (Spermatocytes)	2	575	.35	10	876	1.14	12	334	4.0	4	570	.70
B_6 (Spermatogonia)	1	632	.16	9	725	1.24	0	560	0	3	519	.58

L=Lethal
T= Chromosomes Total

Table II. Induction of Sex-Linked Recessive Lethal Mutations by Iron-62 at 850 MeV in Drosophila

Germ Cell Stage	Dose (rads) 40R			80R			160R			320R		
	L	T	%	L	T	%	L	T	%	L	T	%
B_1 (Spermatozoa)	7	1665	0.42	14	1676	0.84	24	1507	1.59	34	1648	2.06
B_2 (Spermatids)	15	1484	1.01	11	1362	0.81	16	1287	1.24	30	1301	2.31
B_3 (Spermatids)	6	770	0.78	8	791	1.01	5	731	0.68	4	251	1.59
B_4 (Spermatocytes)	1	303	0.33	2	862	0.23	3	915	0.33	3	649	0.46
B_5 (Spermatocytes)	2	196	1.02	3	201	1.50	3	328	0.91	2	563	0.35
B_6 (Spermatogonia)	1	177	0.56	0	503	0.00	3	823	0.36	0	709	0.00

L= lethal
T= chromosomes total

Table III. Induction of Sex-Linked Recessive Lethal Mutations by Argon-40 at 840 MeV in Drosophila

	Dose (rads)											
Germ Cell Stage	40R			80R			160R			320R		
	L	T	%	L	T	%	L	T	%	L	T	%
B_1 (Spermatozoa)	8	2585	0.31	6	1136	0.53	6	1083	0.55	17	1397	1.22
B_2 (Spermatids)	2	1272	0.16	6	1201	0.50	8	1156	0.69	10	1162	0.86
B_3 (Spermatids)	18	2201	0.82	17	1797	0.95	21	1819	1.15	15	1440	1.04
B_4 (Spermatocytes)	3	1303	0.23	3	1179	0.25	11	1397	0.75	4	710	0.56

L= lethal
T= chromosomes total

Table IV. Differential sensitivity of Drosophila spermatozoa and spermatids at various doses of Neon, Argon and Iron ions. (L=lethals; T=total chromosomes scored; %=percent mutations)

RADS	20	40	80	160	320
Spermatozoa	**LT %**	**LT %**	**LT %**	**LT %**	**LT %**
Neon, 585 MeV	2/1105;0.18	2/888; 0.23	5/1136; 0.44	3/635; 0.47	-
Argon, 840 MeV	-----	8/2585; 0.31	6/1136; 0.53	6/1083; 0.55	17/1397; 1.22
Iron, 850 MeV	-----	7/1665; 0.42	14/1676;0.84	24/1507;1.59	34/1648; 2.06
Spermatids					
Neon	10/1148;0.67	2/782; 0.26	0/454	-----	-----
Argon	-----	2/1272;0.16	6/1201; 0.50	8/1156;0.69	10/1162; 0.86
Iron	-----	15/1484;1.01	11/1362; 0.81	16/1287;1.24	30/1301; 2.31

FIGURE I: Induced Sex-Linked Recessive Lethal Mutation by Neon-840 MeV

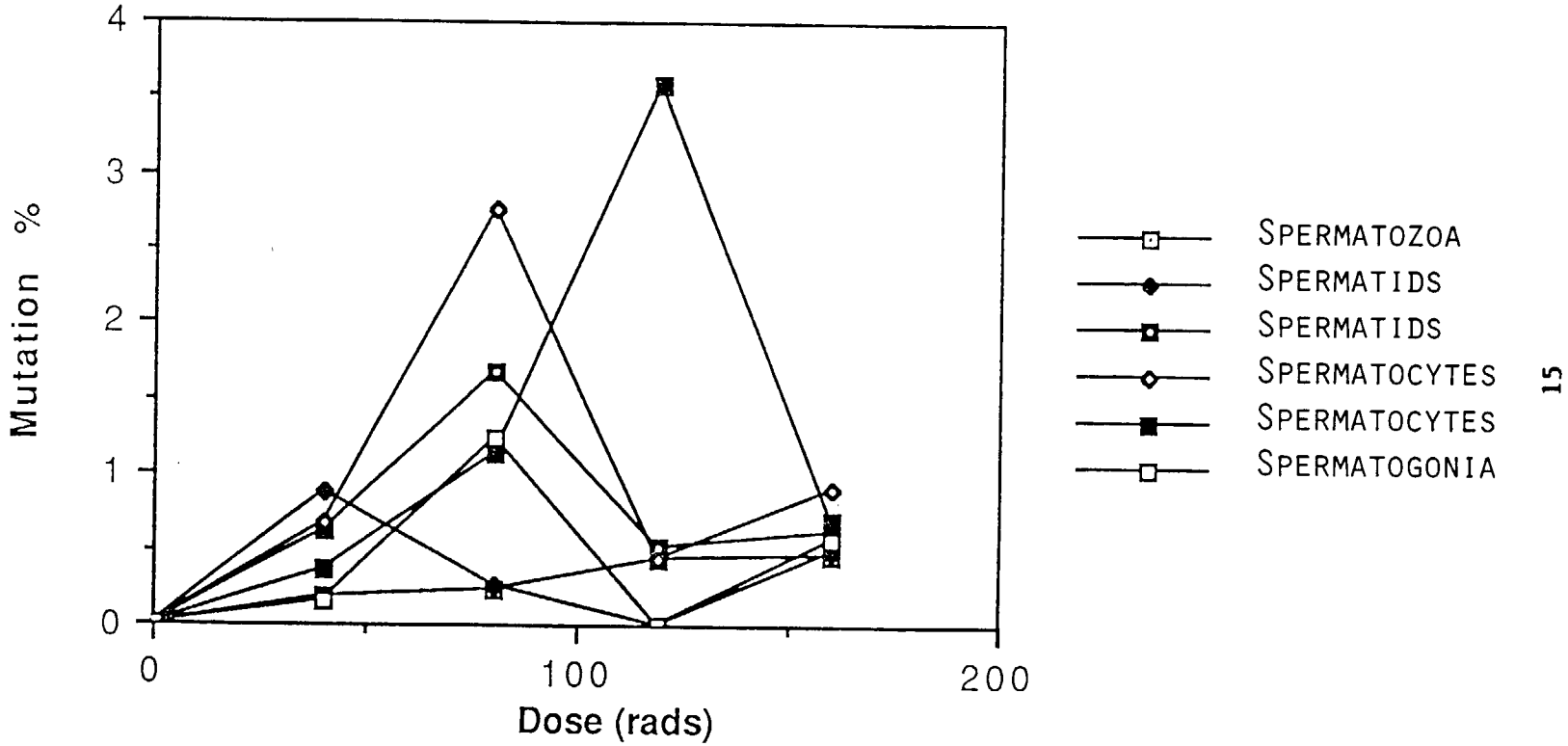


FIGURE II: Induced Sex-Linked Recessive Lethal Mutation by Argon - 840 MeV

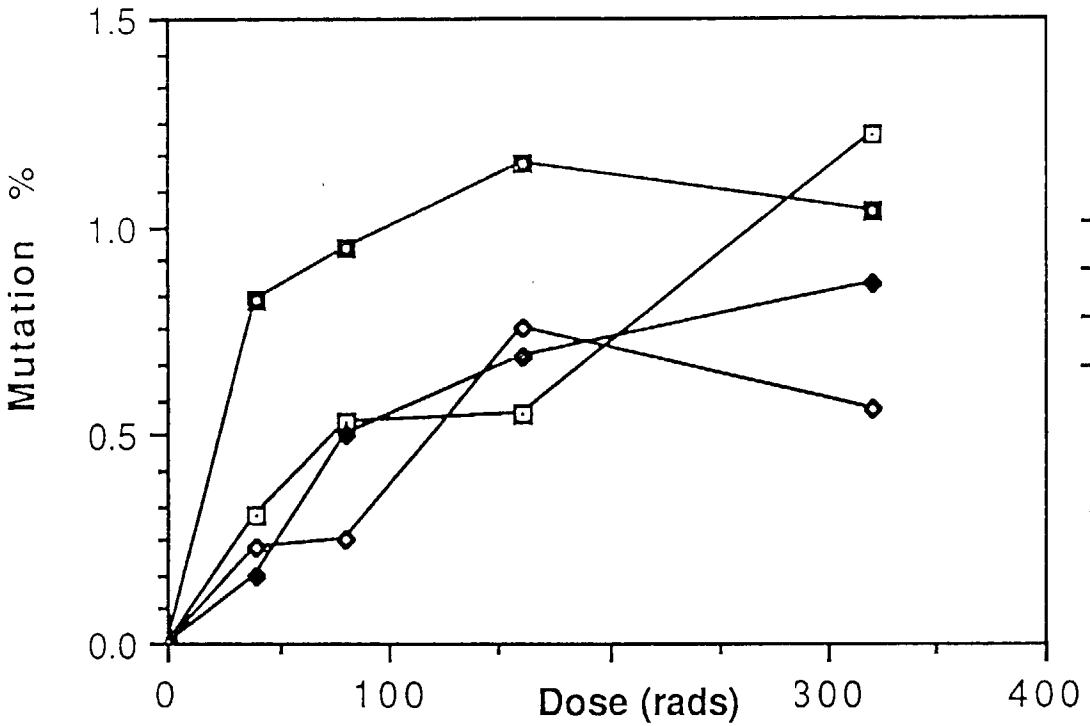
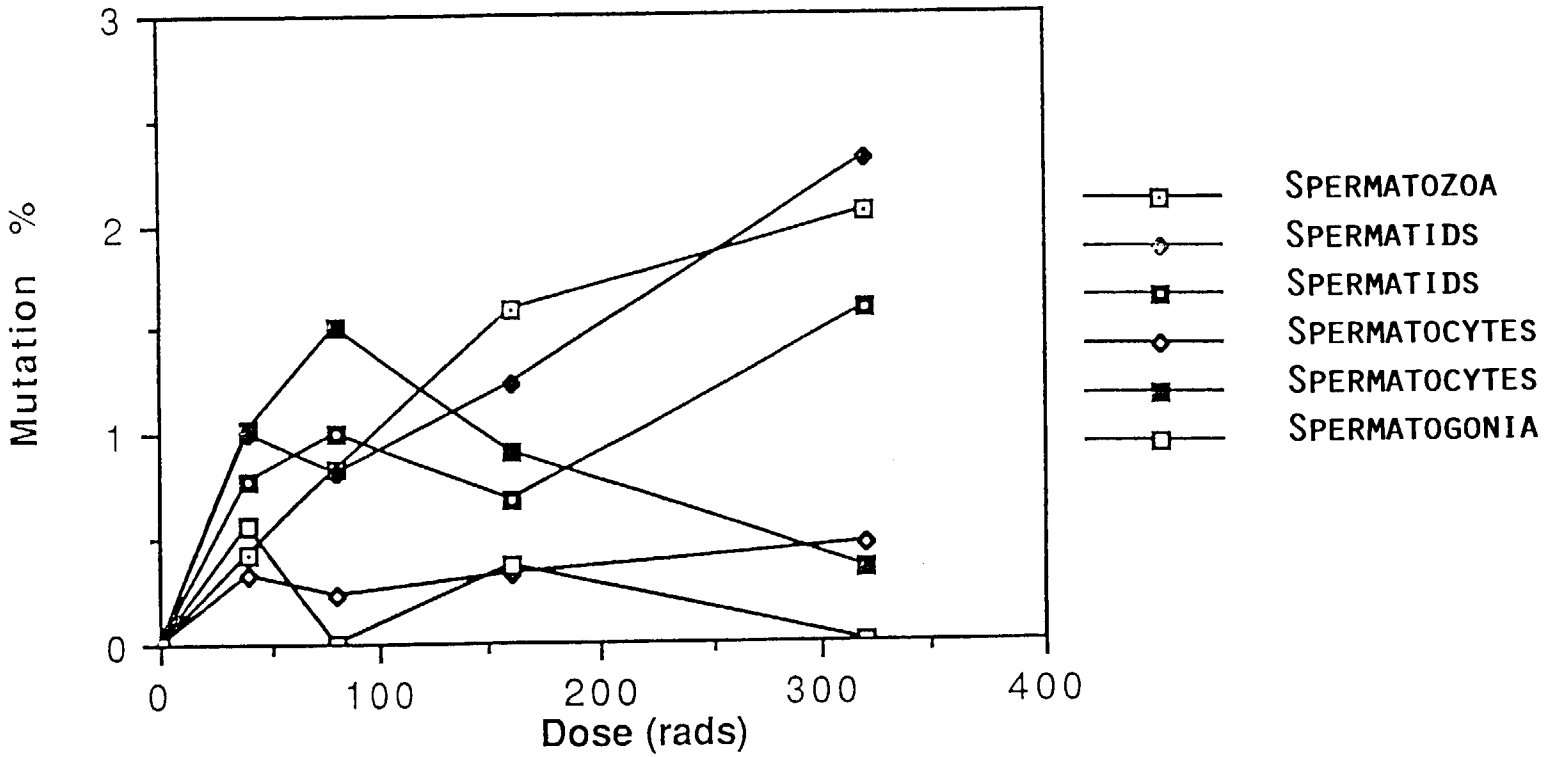


FIGURE III: Induced Sex-Linked Recessive Lethal mutation by Iron - 840 MeV



Future work on Genetic Effects of Heavy Ions in Drosophila

It will be interesting to study the end points such as translocation, somatic mutations, etc. using the heavy ion beams with different enemies. However, the BEVALAC is now closed and therefore these experiments can not be undertaken. It is possible that a comparable heavy ion facility may be available at Brookhaven in future and if this happens these experiments can be considered.

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