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U.S. Atomic Energy Contract No. AT(11-1)-2001

GENETIC EFFECTS OF LOW X-RAY DOSES IN DROSOPHILA

(Period covered: December 1, 1968 to August 15, 1971)

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INTRODUCTION

This comprehensive report on the progress of our research on "Genetic effects of low X-ray doses in Drosophila" covers a period of approximately $2\frac{1}{2}$ years, from December 1968 through August 1971. Almost the entire time has been devoted to the large-scale experiment investigating the mutation frequency-dose kinetics in oögonia of adult Drosophila melanogaster females for very low X-ray doses, ranging from 20 to 500 R. During the last year, a high dose point (6000 R) was added in 4 experiments for comparison.

At the very beginning of the grant period, some of the research efforts of the principal investigator (S.A.) and one of his graduate students (Evelyn Sabol) were directed to conclude a study of dose-kinetics on the frequency of a special type of translocations induced by low X-ray doses (10 to 500 R) in mature oocytes of Drosophila (see bibliography).

PURPOSE OF INVESTIGATIONS

Information on the genetic effects of low X-ray doses is urgently needed for the assessment of radiation risks in everyday life at a time when sources of man-made radiation hazards are on the increase.

Radiation-induced mutagenesis has been studied in great detail ever since Muller (1927) and Stadler (1928) discovered this phenomenon. Chromosomes of different cell types, and also of various germ cell stages, each have their own characteristic radiosensitivity. For each of these germ cell stages, a linear

correlation of dose and mutation frequency was demonstrated for "one track ionization" events over a wide range of X-ray doses.

Most of the early studies were conducted with intermediate to high doses; low dose studies in Drosophila and other higher organisms have so far been limited mainly to mature germ cell stages. For mature spermatozoa of Drosophila, the linear correlation of X-ray dose and mutation frequency was shown to extend to doses as low as 25 R (Spencer and Stern, 1948), and even to 8 R (Shiomi et al., 1963) for sex-linked lethals; for a special type of small deletions (Minutes), linearity extended to doses as low as 5 R (Glass and Ritterhoff, 1961).

Traut (1963) then demonstrated that even translocations in mature sperm increase linearly at doses from 100 to 500 R. More recently, Abrahamson et al. (1971) found in mature oocytes of Drosophila that a special kind of translocation (detachment of attached X-chromosomes) follows linear dose kinetics for doses from 10 to 50 R, though effects increased exponentially at higher doses.

In all experiments with Drosophila cited above, the cells sampled were mature, or virtually mature germ cells. These represent a very special, and rather exceptional class of cells which are genetically inert in contrast to germ cells in other, earlier states of development. However, very little information is yet available for the chromosomes of immature, metabolizing and still dividing germ cells of Drosophila, the gonial cells. Since these cell stages persist for a long time during the life of most higher organisms, they may accumulate

significant radiation exposures during the long reproductive life span.

Oftedal (1964) irradiated immature germ cells in pre-adult stages of *Drosophila* with low X-ray doses, testing for sexlinked lethals induced in early spermatogonial cells. He observed non-linear dose kinetics, with doses from 55 to 160 R being several times more efficient in producing mutations than was expected on the basis of data obtained with higher doses. In contrast, he observed that a dose of 300 R gave a mutation frequency almost identical with that of the control, e.g. a value lower than the expectation based on linearity. Oftedal interprets these results as an indication that he is dealing with a heterogeneous population of spermatogonial cells, with the more sensitive stages eliminated non-randomly by doses above 160 R. If Oftedal's findings could be shown to be a general phenomenon, it would mean that the genetic risks from radiation exposures have been underestimated in the past. Since induced mutation rates for oogonia and spermatogonia of *Drosophila* are similar (Meyer and Muller, 1961), we expected that Oftedal's findings might also be confirmed for oogonia.

In addition to this possibility, there are two other alternatives: 1) We might be able to show that the linear relationship of X-ray dose to mutation frequency extends down to the very lowest dose (20 R) used in our experiments with oogonia, or 2) we might find mutation frequencies which are lower than expected when extrapolating on a linear model from data obtained with higher doses applied to oogonial cells. It is entirely possible that in actively metabolizing, immature

germ cell stages, repair of radiation damage might be more efficient than in mature stages. In this case, the per roentgen yield of mutations could be lower than expected on a linear model from data obtained for oogonia at higher doses. This might lead to a "threshold effect" for the low dose-mutation yield relationship. As a corollary to the threshold hypothesis it is possible to suggest that small amounts of radiation might initiate or activate repair systems such that both the induced and spontaneous mutation components may actually be reduced; in other words, that small doses of X-rays might be "anti-mutagenic".

These suggestions are in fact not incompatible with the observations of Russell and his colleagues at Oak Ridge. Russell has observed that in the mouse the induced mutation rate in spermatogonia and oocytes was dependent on the dose rate, dose spacing (i.e. fractionated doses) and most dramatically upon the time after irradiation that the oocytes were sampled. No induced mutations have been recovered from oocytes sampled 7 or more weeks after irradiation.

Also, a preprint of a paper by Rinehart and Lee (to appear in Mutation Research) was made available to us, in which they present some previously unpublished data from a large-scale study of dose rate effects on the sex-linked lethal rate in *Drosophila* oogonia, in collaboration with the late H. J. Muller. They interpret their data to show that relatively fewer mutations are obtained when radiation is given at a very low dose rate than at a high rate.

All of these observations are most simply reconcilable on the hypothesis that repair of induced damage is going on in

actively metabolizing cells and that factors such as low dose rate, or fractionation, permit the repair system to operate very efficiently. We have already discussed these points at length in our previous proposals, and no recent reports in this area are contradictory.

In our own present experiments, mutability is measured by studying the frequencies of newly arising sex-linked lethal mutations for X-ray doses ranging from 20 to 500 R. For comparison, a high dose of 6000 R has recently also been tested. Although our experiments are far from completion, at present our tentative conclusion would be that, at least for the conditions of our experiments with oogonia of adult *Drosophila* females, no support for the Oftedal model has been forthcoming. On the contrary, our results might even indicate that at low doses and very low doses the mutation rate is possibly below that expected on a linear model.

EXPERIMENTAL METHODS

We decided to test oogonial cells of young adult females for sex-linked lethals, rather than to use an earlier developmental stage in case it should become necessary to give higher doses of radiation (which cannot be tolerated by early larval stages) during the course of the experiment. Since females of *Drosophila* are diploid for X-chromosomes we avoid the complication of a considerable degree of germinal selection against cell-lethals, which operates in the male since he is haploid for the X; this fact was reconfirmed by Abrahamson et al. (1966) in our laboratory.

Data for sex-linked lethals induced in oogonia would therefore be a more accurate measure of the mutation frequencies of pre-meiotic germ cells than if we would have tested spermatogonia; in the latter case it would be advisable to use autosomal lethals instead.

Irradiations are performed with a G.E. Maxitron-300 X-ray machine operating at 250 Kv peak, 20 mA, with 1 mm Al and 1/2 mm Cu filters and at a target distance of 68 cm. The same dose rate, between 70 and 75 R per minute (when corrected for temperature and barometric pressure) was used for all low doses, and also for the high doses given during the later part of our work.

Between 3000 and 4000 virgin females are collected for each experiment, kept on sugar-agar (proteinless) culture medium at 18°C from time of collection until time of irradiation (16-17 hours) to retard maturation of germ cells. The females are randomized and divided into lots receiving the various X-ray doses to be tested, and irradiated in gelatin capsules between two lucite plates (8 mm thickness) at the age of $16\frac{1}{2}$ to $24\frac{1}{2}$ hours after eclosion. Irradiations are always given at the same time of day (9:30 A.M.) to avoid any possible influence of circadian variations on metabolism.

During the first year of our grant period we tested 3 dose groups, 0 R (control), 20 R and 500 R; a fourth dose group receiving 100 R was added during the second year. All 4 doses are included in each experiment, though the proportion of females assigned to each dose group had to be gradually adjusted, as will be discussed later in this report.

The various X-ray doses are fractionated into 200 R + 80 R + 20 R + 200 R. The time needed for each interruption is about 30 seconds, during which capsules containing the females are added or removed from the radiation field. The 500 R dose is the sum of all fractions, 100 R are given as 80 + 20 R, and the 20 R dose is not fractionated.

A very high dose, 6000 R, was included in 4 of the regular experiments during the last year; it was given over a period of 8 days, in fractions of 3 X 2000 R with 4 and 3 day intervals, during which the females were kept on sugar agar medium at 18° C temperature.

In order to obtain offspring (F_1) from germ cells which were in oogonial stage at time of irradiation, the treated females (P_1) are mass-mated to an excess of males immediately after irradiation (25 females X 40 males per culture bottle), and then transferred to fresh culture media three more times to deplete their supply of post-gonial germ cells.

While this brooding period lasted 16 and 15 days in our first eight experiments, all later experiments were performed with 13 day brood periods, and in a few exceptional cases, a 14 day period.

On day 13 after irradiation the fertilized P_1 -females are separated from the males, and cultured individually in vials. Their offspring (F_1), to be tested for newly arisen lethals in the irradiated X-chromosome is therefore derived from eggs deposited on or after the 13th day following irradiation. It was shown for females bred in this manner and under similar

culture conditions (Meyer and Muller, 1961) that lethal mutation frequencies in offspring derived from the brood period between days 10 to 12 after irradiation were similar to the lethal rates from offspring from the period between days 15 to 18 after irradiation. This indicated that after the tenth day already most of the progeny is derived from cells which were oogonia at time of irradiation; this was also shown by some cases of "clusters" of allelic mutations.

In this present experiment, however, we want to avoid obtaining clusters of mutations of identical origin, since it would reduce the significance of the data by increasing the statistical error. Therefore only one single offspring (F_1) from each P_1 female is tested for lethality. Though this is a more time consuming procedure, we are assured that each new lethal is of independent origin. It also eliminates the need for keeping records on each of the P_1 females and their progeny, and localizing the lethals from "families" where more than one lethal are found. A very important precaution is the elimination of pre-existing lethals which may accumulate in the heterozygous females of the stock furnishing the P_1 females. About every 6 months this stock is cleared of lethals by re-introducing non-lethal X-chromosomes of the appropriate genotype. These are derived from at least 12 to 16 non-irradiated control cultures.

In spite of this periodic "rejuvenation" of the stock, a number of females still will be carriers of pre-existing lethals. We use a two-step procedure to eliminate such females: first,

inspection of all P_1 -cultures, without etherization, after emergence of the F_1 generation for presence of males representing the experimental (maternal) X-chromosome. All cultures with less than 2 such males are rejected, many of them with only a few offspring; those with no such males are suspected of pre-existing lethals. A second check is made again when the already pre-screened and accepted cultures are etherized to mate one single F_1 daughter to be tested for lethality of her maternal X-chromosome.

A record is kept on the various types of rejected P_1 cultures. ^{Those} ^ suspected of pre-existing lethals are retested, ever since experiment 50, for another generation.

Of course, this procedure eliminates only those cases where the original P_1 -zygote was heterozygous for a lethal. The latter might have arisen at an early cleavage stage, and would in that sense also be "pre-existing" i.e. before the gonial stage, which we irradiate in these experiments. Such cases will then be scored (erroneously) as "new" lethals and will be part of the spontaneous background mutation frequency.

Since gonial cells have a very low mutational response to and X-rays ^ because of the low doses used, the vast majority of "new" lethals which we find will be of spontaneous origin. It is therefore imperative to try to randomize environmental conditions which might influence the spontaneous frequency as much as possible. This includes randomization of the culture medium among the various dose groups, if it should happen not to come from the same batch of cooking; randomization of the P_1 females collected

for each experiment, if they had to be collected from more than one set of culture bottles. We ~~also~~ distribute these females among the various dose groups in similar proportions, which are related to the anticipated mutation frequencies. Equal numbers have so far (i.e. in experiments 1-90) always been allotted to 0 R and 20 R groups, fewer to the 100 R and the 500 R group. We have, however, lately increased the proportions assigned to these latter two doses, because induced lethal frequencies recovered for these doses so far have been lower than we had expected.

As another precaution, we routinely rotate the manipulation of the various treatment groups among the members of our research team. This should randomize for personal differences in handling, or recognition of exceptional situations in the test cultures. In addition we now have begun to code the various dose groups, in order to exclude any (unrecognized) bias.

The frequency of new sex-linked lethals is determined in the F_2 generation by inspection of the offspring from each F_1 female (see Breeding Scheme). Absence, or scarcity of sons representing the experimental X-chromosome indicates a new lethal or sub-lethal mutation. All cases having less than 2 such males are kept as suspected lethals, sub-lethals or severe detrimentals and are tested for one more generation, usually in quintuplicate, with second broods made of each test. Reconfirmation and classification of suspected lethals is therefore based on counts of an extensive number of progeny. ✓

. While the majority of recessive sexlinked lethal mutations are characterized by a complete absence of males representing the experimental X-chromosome (complete lethals), one also finds

borderline cases in which a few such males survive, the so-called incomplete lethals, or sub-lethals, which we subdivide into the categories of near-lethals and partial lethals. We also keep a record of the cases of severe detrimentals. The definition of what to classify as a lethal mutation differs with various investigators and with different types of experiments. In some cases only "complete lethals" are scored but most frequently, also some cases of sub-lethals are included among the lethal mutations, with the cut-off line often times set at 10% of expectation. For the purposes of these experiments, a more narrow cut-off line was desirable. We therefore define as lethal mutations all cases in which the ratio of males with the experimental X-chromosome to heterozygous female sibs is 5% or less of expectation.

Theoretically, the ratio of males with the experimental X-chromosome to females heterozygous for it is equal to 1, (i.e. these two classes should appear with equal frequencies), but this does not take into account possible viability differences. The mutations y (yellow) and dow (downy) reduce the viability of males with the experimental X-chromosome so that this ratio is actually less than 1.

We therefore determine the "expected ratio" empirically from progeny counts of numerous unirradiated, non-lethal F_2 cultures (from the 0 R group) with obvious good viability for these classes. This also corrects for more subtle influences on viability, for instance for changes in culture conditions which

might favor one or the other class.

As mentioned before, we clear the basic stock periodically of pre-existing lethals; over the many years this will gradually change the genetic background somewhat. Therefore we do not have one overall mean value as "expectation" throughout the entire course of the investigations; instead, for each group of experiments which has utilized one of these slightly different stocks, we use the corresponding empirically determined "expectation" for the classification of sub-lethals and severe detrimental. Cases of "complete lethals" which comprise more than 80% of all lethal mutations, with zero surviving males are of course unaffected by this procedure. For experiments 1-90, which are the subject of this report, we have used 5 such "editions" of the basic stock, and lethality was measured and classified by 5 corresponding, empirically determined "expected ratios".

GENETIC BREEDING SCHEME

The breeding scheme used for detecting newly arisen sex-linked lethals in females has been described in detail in the original Research Proposal. This scheme has worked well, and we continue to use it without any changes. It is essentially a "Basc" (=Muller-5) type analysis, with a few refinements. We enclose here again a condensation of the procedure (next page).

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

$$\text{♀♀} \frac{y \text{ dow}^*}{Df(1^{JI} y \text{ ac}) sc^{SI} B \text{ Ins } w^a sc^8} \times \frac{Df, Basc}{y+sc^8 \cdot y} \text{♂♂}$$

P₁:

$$\begin{array}{l} \text{Virgin} \\ \text{♀♀} \end{array} \frac{y \text{ dow}^{**}}{Df, Basc^-} \times \frac{y^{3ld} w^{FM6} B, \text{ InsFM6}}{Y^+ (\text{normal } Y)} \text{♂♂}$$

P₁ females X-rayed, mated en masse in bottles and transferred every 3 or 4 days to fresh culture medium until day 13 after irradiation.

Surviving fertilized females bred individually in vial cultures. No males present.

F₁: To exclude preexisting lethals, each culture is inspected for presence of a reasonable proportion of y dow males; if satisfactory:

$$\begin{array}{l} \text{One single} \\ \text{F}_1 \text{ daughter} \end{array} \frac{Y \text{ dow}^{**}}{y^{3ld} w^{FM6} B, FM6} \text{ "automatically virgin" (*) } \times \frac{y^{3ld} w^{FM6} B, FM6}{Y^+} \text{♂♂}$$

Cultures raised at 25°C

F₂: Scored for y dow males; absence or scarcity indicates viability mutation (lethal or partial lethal).

F₃: Confirmation cross.

(*) Note: dow males are sterile. "Df,Basc" is lethal in combination with a normal Y (Y+), but not with the y⁺ sc⁸·y which compensates for the lethal effect of the deficiency.

(**) Broken lines indicate irradiated X-chromosomes.

Only 2 stocks are required for the main analysis. The first supplies the heterozygous females to be irradiated, which are phenotypically yellow. A special Y-chromosome ($y^+ sc^8 \cdot Y$) used in this stock provides the wild type y^+ allele so that exceptional females of XXY constitution can be recognized by their non-yellow phenotype and can be excluded. The genetic formula of this stock is given under "P₀" on the previous page. For explanation of the symbols and abbreviations, see Lindsley and Grell, 1968.

This stock is self-perpetuating, because only the parental types of males and females are able to breed. The females are heterozygous for the uninverted, "experimental" X-chromosome marked by y dow, and a "Basc" (=Muller-5) type multiply inverted chromosome with a deficiency for the loci of $l^{J1} y$ and ac which was X-ray induced in our laboratory by Brian Davis in 1961.

Females homozygous for Df,Basc are lethal, as are males hemizygous for it because of absence of a wild type allele for lethal-J1 (l^{J1}), unless one provides a duplication for this region of the X-chromosome. The $y^+ sc^8 \cdot Y$ chromosome fulfills this requirement by carrying the wild type alleles for $l^{J1} y$ and ac.

The Df, Basc chromosome is always of paternal and the experimental y dow X-chromosome always of maternal origin. Spontaneous mutations tend to accumulate in these heterozygous females, and we have previously discussed ("Methods") that lethal-free y dow chromosomes from control groups are periodically

re-introduced into this stock. We also mentioned the double-screening procedure used to eliminate P_1 females heterozygous for pre-existing lethals; this is also indicated in the "Breeding Scheme" under " F_1 ".

The second main stock is kept in homozygous condition and supplies the males to which both the P_1 and the F_1 females are crossed. Instead of the Basc-type balancer chromosome we use a modification of Lewis and Grell's (1956) multiply inverted FM6 chromosome. In this modification, sometimes referred to as FM6-K (Kidd, 1966) the female-sterilizing factor dm is replaced by its wild type allele, and a mutation to white eye color (w^{FM6}) greatly facilitates progeny classification and counts later on.

Since the FM6-K stock has a normal Y-chromosome, without a duplication for the deficient region of the Df, Basc chromosome, such males do not appear in the F_1 generation; the other class, y dow males, are sterile. Consequently all F_1 females are unfertilized, which greatly enhances the accuracy of the experiments and the determination of various classes of lethal, sub-lethal or severe detrimental in the F_2 and in all subsequent generations.

RESULTS OBTAINED TO DATE

The main objective of these investigations is to determine the frequencies of newly arisen X-chromosome lethals in the offspring of young adult females which were treated with low doses of 250 Kvp X-rays. The results from 90 experiments completed so far are presented in various tables.

The first table is a survey of survival and productivity of the P_1 females, according to dose received. The upper portion lists data from the first 30 irradiation experiments, performed from January to October 1969, before we had made appropriate adjustments in culture medium and improved the genetic background of the female-supplying stock. Ever since then, the overall efficiency of the treatments has essentially remained at the level represented in the lower portion of the table.

Table 1: SURVIVAL AND PRODUCTIVITY OF P_1 -FEMALES

X-RAY DOSE	INITIAL NO. P ₁ -FEMALES	INDIVIDUAL P ₁ -CULTURES NO.	%	EFFECTIVE F ₁ -TESTS NO.*	% OF P ₁ -CULT.
Exp. 1-30					
0 R	41,194	21,824	53.0	10,252	47.0
20 R	41,640	20,946	50.3	9,468	45.2
500 R	13,402	7,070	52.8	3,143	44.5
Exp. 31-90					
0 R	60,085	41,136	68.5	31,132	75.7
20 R	59,498	41,316	69.4	31,258	75.7
100 R	42,365	29,857	70.5	22,473	75.3
500 R	37,290	25,616	72.0	19,335	75.5

* Each F_1 test is derived from a different P_1 culture

The data show the reduction in the number of P_1 -females initially processed until the time when, after having been mated, brooded and aged, they are cultured individually without the presence of males. This period usually lasts 13 days except in the beginning of the work (experiments 1-8) when it lasted

15-16 days. The percentage of P_1 females surviving the brood period is given in column 4, and seems to be similar regardless of X-ray dose.

The last column gives information on the productivity of the individually cultured P_1 -females. Some die before producing progeny, some do not produce a sufficient number of progeny, and some have to be rejected because of presence of a pre-existing lethal in the y dow chromosome or for miscellaneous other reasons. As mentioned previously only cultures with a minimum of 2 y dow F_1 males are accepted, and from each accepted culture only one single F_1 female is tested for lethality of her y dow X-chromosome.

Once an F_1 test is set up, it is usually successful; only a small number are ineffective, for a variety of reasons, representing a very small proportion of the tests. For the doses of 0, 20, 100 and 500 R this was only 4.1, 4.3, 3.5, and 4.1%, respectively. This reduction is included in the percentages of F_1 cultures which yielded "effective" F_1 tests given in the last column.

The data show little variation for the different groups; we do not now have the impression that there is any relationship to X-ray dose.

The frequencies of sex-linked lethals obtained for the various treatment groups are presented in tables 2, 3 and 4 in several different ways. Before discussing these frequencies, it might be useful to restate at this point the values which could be expected at these doses, if one extrapolates from data

obtained with higher doses from genetic tests of gonial cells. On the basis of work of many investigators, including our own, approximately 0.5% of sex-linked lethals are expected to be induced in gonial cells per 1000 R. Assuming a linear relationship of dose and mutation frequency, we could expect, in addition to the control rate, an induced component of 0.25% for 500 R, 0.05% for 100 R and 0.01% for 20 R.

In Table 2 we present the data obtained for groups of consecutive experiments, summarized in such a way for each dose series that each contains approximately 5000 tested X-chromosomes in the 0 R and the 20 R series (these are always started with an equal number of P_1 females). We include this more detailed listing here to demonstrate the fluctuations of the frequencies obtained during the progress of the work. At the end of each dose series we have given the totals and the weighted mean mutation rates.

Results obtained with a high dose of 6000 R which had been included in 4 different experiments to check on the potential mutability of our particular test chromosome are given at the bottom of the table. The frequency obtained, 2.82% is in line with the above mentioned expectation.

TABLE 2

DATA FROM EXPERIMENTS 1-90, GROUPED BY SUMMARIZING FOR APPROXIMATELY
5000 X-CHROMOSOMES TESTED IN CONTROL AND 20 R SERIES

X-ray dose	Exp. No.	No. ♀♀ Irrad.	No. ♀♀ Cultured	No. leth./Tot. No. tested	% X-Chrom. Lethals
0 R	1-23	28,072	13,612	10/4910	.204
	24-30	13,158	8,212	10/5342	.187
	31-39	9,336	6,558	19/5105	.372
	40-48	8,808	6,585	12/4948	.243
	49-59	12,295	7,371	9/5266	.171
	60-71	10,838	6,648	6/5112	.117
	72-81	10,678	7,257	10/5553	.180
	82-90	8,202	6,717	6/5148	.117
TOTALS	1-90	101,387	62,960	82/41,384	.198
20 R	1-23	28,186	13,078	9/4641	.194
	24-30	13,454	7,868	6/4927	.122
	31-39	9,121	6,480	9/5056	.178
	40-48	8,626	6,165	8/4722	.169
	49-59	12,221	7,672	10/5274	.190
	60-71	10,973	6,857	9/5177	.174
	72-81	10,436	7,465	9/5757	.156
	82-90	8,121	6,677	7/5172	.135
TOTALS	1-90	101,138	62,262	67/40,726	.164
100 R	1-23	--	--	--	--
	24-33	--	--	--	--
	*34-39	3,127	2,500	2/2059	.097
	40-48	5,826	4,453	10/3443	.290
	49-59	8,388	4,922	6/3473	.173
	60-71	9,052	5,747	4/4222	.095
	72-81	9,417	6,717	6/5155	.116
	82-90	6,555	5,418	5/4121	.121
TOTALS	*34-90	42,365	29,857	33/22,473	.147

* No data for Exp. 1-33.

Continued on next page

TABLE 2 (CONTINUED)

DATA FROM EXPERIMENTS 1-90, GROUPED BY SUMMARIZING FOR APPROXIMATELY
5000 X-CHROMOSOMES TESTED IN CONTROL AND 20 R SERIES

X-ray dose	Exp. No.	No. ♀♀ Irrad.	No. ♀♀ Cultured	No. leth./Tot. No. tested	% X-Chrom. Lethals
500 R	1-23	8,941	4,632	5/1664	.301
	24-30	4,461	2,438	4/1477	.271
	31-39	3,841	2,690	7/2015	.347
	40-48	4,483	3,495	3/2663	.113
	49-59	6,229	3,914	13/2742	.474
	60-71	8,773	5,175	17/3914	.434
	72-81	8,302	5,728	6/4454	.135
	82-90	5,662	4,614	11/3549	.310
TOTALS	1-90	48,978	32,686	66/22,478	.294
6000 R	64,67 68,70	9,675	4,779	103/3653	2.820

Judging from this table with its purely arbitrary grouping of the data, fluctuations in the lethal frequencies seem more extensive for the control than for the treated groups. They are, however, not unreasonable, and the mean spontaneous mutation rate (0.198%) is within the range of that obtained for laboratory stocks by many previous investigators. At the 2 lowest doses (20 and 100 R), most lethals found in the treated groups must also be of spontaneous origin, and we would expect similar fluctuations there, though not necessarily in the corresponding groups of experiments. In fact there seems to be less variation in the lethal frequencies of the treated groups. This might be a consequence of the arbitrary grouping of the data, and will be reinvestigated by alternative grouping when more data have been collected.

X-RAY DOSES

Exp. No.	0 R (control) No. lethals/No. tests	20 R No. lethals/No. tests	100 R No. lethals/No. tests	500 R No. lethals/No. tests
1-33	30/12,334 (.243%)	17/11,534 (.147%)	--	11/3,680 (.299%)
34-51	28/9,429 (.297%)	16/9,181 (.174%)	14/6,612 (.212%)	13/4,874 (.267%)
52-70	8/8,498 (.094%)	18/8,678 (.207%)	7/6,297 (.111%)	24/5,558 (.432%)
71-90	16/11,123 (.144%)	16/11,333 (.141%)	12/9,564 (.125%)	18/8,366 (.215%)
1-90	82/41,384 (.198%)	67/40,726 (.164%)	33/22,473 (.147%)	66/22,478 (.294%)

Table 3: Data from sex-linked lethal tests arbitrarily subdivided into 4 groups of approximately 10,000 chromosomes tested.

X-RAY DOSES

Exp. No.	0 R (Control)	20 R	100 R	500 R
1-33	.243±.044 (12,334)	.147±.036 (11,534)	--	.299±.089 (3,680)
1-51	.266±.035 (21,763)	.159±.028 (20,715)	.212±.056 (6,612)	.281±.0573 (8,554)
1-70	.218±.027 (30,261)	.174±.024 (29,393)	.163±.035 (12,909)	.340±.049 (14,112)
1-90	.198±.022 (41,384)	.164±.020 (40,726)	.147±.026 (22,473)	.294±.036 (22,478)

Table 4: Percent of sex-linked lethals based on accumulated totals of tested chromosomes with standard errors. Number of chromosomes tested in parentheses.

In Tables 3 and 4 the same data are divided into 4 subgroups of consecutive experiments with approximately 10,000 tests in each of the 0 R and 20 R series. Table 3 lists the frequencies of sexlinked lethals for each of the 4 subgroups, and a summary for all experiments (1-90) is given on the last line. In Table 4, the cumulative totals are presented; the total number of chromosomes tested is in parenthesis, and the observed lethal frequencies are listed with standard errors.

The lethal rates for 0 R, 20 R, and 100 R still overlap within the ranges of their standard errors. The rate for 500 R is significantly above the control frequency, but considerably lower than expected (on a linearity concept) from previous results with higher doses given to immature germ cells. We had expected an increment above the spontaneous frequency of approximately 0.25%, (i.e. 500 R was anticipated to be the "doubling" dose), but instead obtained only 0.096%.

If one were to determine the induced component for the 20 and 100 R doses in the usual manner, by subtracting the spontaneous frequency (0 R group) from that observed for the treated groups, one would obtain a negative value. This might be surprising at first glance, but can be explained by the fact, that the induced lethal frequencies expected on a linear model (from higher doses) for doses of 20 and 100 R are only 0.01 and 0.05%, respectively. With an expected induced component so close to zero, and with errors attached to these frequencies, the observed induced component could, just by chance, have a

EXPERIMENTS 1-100

FIGURE 1 : Linear regression analysis.

negative value.

Figure 1 shows linear regression analysis of data, prepared for us by Dr. Roy Milton, a biostatistician, who is at present analyzing our data. The graph indicates the observed frequencies of mutation for experiments 1-100 at 0, 20, 100 and 500 R. The solid vertical bars are 95% confidence intervals for the true frequencies. The dotted line connects the 4 data points. The solid regression line is based on these data, and the chance of observing a slope this, or more, disparate from zero, when in fact the true slope is zero, is about 10%.

The broken regression line is based on all the data, including those obtained for 6000 R, and its slope is significantly different from zero.

The frequencies for 20 and 100 R are not significantly different from the spontaneous (0 R) frequency; the 500 R frequency is significantly different from both the 0 R and 100 R frequencies.

The difference in the slopes of the 2 regression lines, one including the high dose point of 6000 R, the other one not, seem to contradict the assumption of linear dose kinetics for the entire range from 6000 R down to 20 R. However, the position of the 6000 R dose point and its confidence intervals has to become more secure by accumulation of more data; also, some intermediate dose points are needed.

If any conclusions can be drawn from the present state of the data, it is that they do not lend support to Oftedal's

hypothesis that very low doses of X-rays have a several-fold higher potential for mutagenicity than can be expected on linear extrapolation from work with higher doses. Oftedal reports this effect for doses of 55 to 162 R given to spermatogonia of early developmental stages, but not for 300 R. Our own results for 20 and 100 R, obtained from irradiation of oogonia of adult *Drosophila*, seem to show the opposite trend; we realize that this statement is premature until additional data for the 6000 R and another higher dose point have been obtained to relate these investigations more securely to the published data from work with higher doses, on which our expectations are based.

Our data can still be reconciled with the linearity concept for dose and mutagenicity. But it is also possible that further accumulation of data, especially for the 100 R dose, will continue to show the trend of depressed values below the control level for the lethal rates of the two lowest dose groups. If so, this would be support for the hypothesis that the normal repair processes going on in metabolizing cells could be stimulated to higher activity by low X-ray doses. However, on the basis of our results at the present time, this is still speculation, and much further work is needed.

Not all lethal mutations in the X-chromosome are characterized by complete absence of males with the experimental X-chromosome in the F_2 generation. In our experiments we classify the various types of viability impairing mutations on

the basis of a numerical index as "complete lethals" (class A), "near-lethals" (class B), "partial lethals" (class C) and "severe detrimentals" (class D). This numerical index, determined from extensive progeny counts of F_2 and F_3 cultures, is the ratio of males with the experimental X-chromosome to female sibs heterozygous for it.

As already discussed in the section entitled "Methods", we count as lethal mutations in this experiment all cases where this ratio is 5% or less of the "expectation", with the latter determined from the mean ratios found in non-lethal control cultures. While the theoretical expectation would be equal to one,--equal numbers of male and females with the experimental chromosome should appear,--this relationship is often distorted by the lower viability of the males. The empirically determined "expectation" in our experiments is less than 1, most often approximately .75.

For class A (complete lethals), this ratio is always zero since there are no survivors; for class B (near-lethals), it is greater than 0 but not more than 2% of expectation; for class C, greater than 2% but not more than 5%. For class D ("severe detrimentals") the ratio is greater than 5%, but not more than 10% of expectation. This last category is not included in the lethal frequency in these experiments, but ^{it} is desirable to determine this class too, since many other investigations have used the 10% line as a cut-off line for partial lethals.

The distribution of the different types, for each dose series, has been tabulated in tables 5 and 6, in order to relate

TABLE 5

CLASSIFICATION OF VIABILITY IMPAIRING MUTATIONS

X-RAY DOSE	EXP. NO.	CLASSES OF VIABILITY MUTATIONS				NO. OF X- CHROMOSOMES TESTED	
		CLASS:	A	B	C		D
		*SURVIVAL:	0	>0 ≤2%	>2% ≤5%		>5% ≤10%
0 R (control)	1-33		27	1	2	2	12,334
	34-51		25	3	0	0	9,429
	52-70		7	0	1	0	8,498
	71-90		14	1	1	0	11,123
	Totals 1-90		73	5	4	2	41,384
% of X-chromosomes			.176	.012	.010	.005	
20 R	1-33		16	0	1	1	11,534
	34-51		14	1	1	1	9,181
	52-70		15	1	2	1	8,648
	71-90		15	0	1	1	11,333
	Totals 1-90		60	2	5	4	40,726
% of X-chromosomes			.147	.005	.012	.010	
100 R	1-33		--	--	--	--	--
	34-51		12	1	1	0	6,612
	52-70		6	0	1	0	6,297
	71-90		10	2	0	2	9,564
	Totals 1-90		28	3	2	2	22,473
% of X-chromosomes			.125	.013	.009	.009	
500 R	1-33		8	2	1	1	3,680
	34-51		9	4	0	0	4,874
	52-70		22	1	1	0	5,558
	71-90		15	3	0	1	8,366
	Totals 1-90		54	10	2	2	22,478
% of X-chromosomes			.240	.044	.009	.009	
POOLED DATA							
0 R-500 R			215	20	13	10	127,061
% of X-chromosomes tested			.169	.016	.010	.008	
6000 R 64,67,68,70			99	3	1	9	3,653
% of X-chromosomes tested			2.710	.082	.027	.246	

*Survival measured in % of expectation; the latter is empirically determined from non-irradiated control cultures (see text).

TABLE 6

X-RAY DOSE	CLASSES OF VIABILITY MUTATIONS				NO. OF CASES
	A	B	C	D	
0 R	86.9%	6.0%	4.8%	2.4%	84
20 R	84.5%	2.8%	7.0%	5.6%	71
100 R	80.0%	8.6%	5.7%	5.7%	35
500 R	79.4%	14.7%	2.9%	2.9%	68
POOLED DATA					
0-500 R	83.6%	7.8%	5.0%	3.9%	257
6000 R	88.4%	2.7%	0.9%	8.0%	112

our data to those from other published reports with different cut-off limits. It is also of considerable theoretical interest.

Table 5 gives details; the division of the data into 4 consecutive groups of experiments is the same as in tables 3 and 4. The total number of cases in each category is summarized for the various dose groups, and the frequency for each category given below the number of cases. The frequencies of class A mutations, for instance, means that the lethal frequencies for X-ray doses of 0, 20, 100, 500 and 6000 R, if one would only score for cases of complete lethals, would be, in that same order, 0.176, 0.147, 0.125, 0.240, and 2.710%. To compare the values of lethal frequencies with data from other investigations where 2% of expectation was used as criterium for lethality, one would have to add the frequencies for classes A and B. Similarly, for experiments with a 10% limit, one would summarize all frequencies, A through D. Dose^{and} lethal frequencies for complete lethals (A) alone show a similar pattern as for the data obtained with the 5% cut-off line.

The summary in Table 6 expresses the frequencies for all 4 classes as percent of total cases recorded (A+B+C+D). The results for 0-500 R seem similar and are pooled, and contrasted with those from the high dose, 6000 R. There is a suggestion that more severe detrimentals (D) are found at this high dose, which might be a manifestation of more drastic genetic changes (rearrangements) involving the X and/or irradiated autosomes.

In general, this survey is in agreement with previous information on different types of viability impairing mutations, which indicated that complete lethals represent by far the largest component (here, about 80%) and that the distribution of these mutations, from zero survival to zero impairment, is not evenly distributed; our data illustrate the clustering around the "zero survival" point.

X-chromosome lethals from the dose groups 0 through 500 R are tested for complementation with 4 different types of males, which carry duplications for certain regions of the X in their Y-chromosomes, or in one case, in an autosome. Complementation of the lethal by a specific duplication permits one to assign some of the lethals to a specific region. We have obtained a fair number of such cases; the relative frequency of lethals found to be covered by the various duplications seems to be related to the extent of the duplicated X-material. Some of the 4 tester stocks have duplicated X-chromosome regions in common, so that one gets complementation for the lethal in more than one type of test.

A number of *Drosophila* workers require mutations in specific regions of the X-chromosome for their studies, and we have supplied some of our lethals to those investigators, and will continue to do so. These regions are: the distal end of the X-chromosome, which is studied by Dr. Lim, the white region (Dr. Judd), the vermilion region (Dr. Lefevre), the maroon-like and the bobbed region (Dr. Schalet).

PLANS FOR FUTURE WORK

The main objective for future work will be accumulation of more data on the frequency of newly arising sex-linked lethals after irradiation of oogonia with X-ray doses from 0 to 500 R. At present, the rates obtained for 20 and 100 R still overlap the control frequency (0 R group) within the range of their standard errors. However, the frequency for 100 R might possibly, after more tests are completed, be lower than the control rate, while the results for the 500 R treatment already show a significantly higher rate than the control. We therefore plan to introduce an additional dose point, 300 R, to be tested along with the others. We will then investigate the following low doses: 0, 20, 100, 300 and 500 R.

We have not been able to confirm, in our material, Oftedal's results for doses of 55 to 162 R given to spermatogonia, in which he found that the yield of induced sexlinked lethals is several-fold that expected from extrapolation from higher doses, on a linear model. We are now testing the hypothesis that the relationship of dose to mutational yield is linear, or less than linear, for the dose range from 20 to 500 R.

Since the Oftedal hypothesis has been ruled out (for our material) a reduction of the proportion of females, assigned to the 20 R group in each experiment, is planned because of practical limitations. We feel, however, that this dose is still of interest, since it might also give a lethal frequency below the control rate. But we doubt that the difference will become statistically significant. This dose would also not be suited

to demonstrate a linear relationship to dose, since the "expected" induced lethal frequency should only be 0.01% above the control rate. Therefore, the emphasis will now be shifted to the 100 to 500 R range, with data for 20 R to be accumulated at a slower rate.

It will also be important to re-examine whether, for our genetic set-up, the lethal frequencies for high doses agree with the values from previous published work with oogonia or spermatogonia (among others: Abrahamson and Friedman, 1964; Meyer and Muller, 1958 and 1961; Muller et al. 1963; Oster, 1958 and 1959), i.e. whether the expected induced frequency of approximately 0.5% per 1000 R is correct.

We already have reassuring results from some 3500 tests for a high dose of 6000 R; we plan to double this number and at the same time also collect data for a dose of 3000 R. These high doses will again be incorporated in several experiments spread out during the year, and some of the low doses will be tested simultaneously. Data for these two high dose points will allow us to construct a more accurate regression line than if we have just one point available for the high dose range.

After the trends for the low dose range have become clearer, and the data from the 2 high doses will allow us to formulate a more securely founded "expected frequency", it might be important to test an intermediate dose, possibly 1000 or 1500 R. This should not be done now, and will only become necessary if the suspicion persists that the yield of mutations at the low dose range is lower than a linear model would predict. We should

then try to choose and test a dose point which represents the lower limit for the linearity principle of dose and mutagenicity.

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RECENT ADDITIONS TO LIST OF PUBLICATIONS OF PRINCIPAL
CO-PRINCIPAL INVESTIGATORS

This is an amendment to the lists of principal publications, which were included in the original grant request in 1968. Publications associated with this contract are marked with an asterisk(*).

- Abrahamson, S. and P. Gullifor 1968 A comparative study of induced half-translocations in stage 14 oocytes of *Drosophila*. (Abstr.) Proc. XII Intern. Congress of Genetics, Tokyo 1968, Vol. 1: 91.
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OPINION ON PRESENT STATE OF KNOWLEDGE IN THE FIELD OF RADIATION
GENETICS, ITS SIGNIFICANCE FOR BIOLOGY AND MEDICINE, AND NEEDED
FUTURE INVESTIGATIONS .

The field of radiation genetics is in the unique position of receiving continuous review by both national and international agencies. Both the Advisory Council to the Federal Radiation Council (presently part of EPA) and the United Nations Scientific Committee on the Effects of Atomic Radiation are completing their independent and updated reviews of the state of knowledge on this subject. Since one of us, (S. A.) is presently serving on both committees, we will not attempt to give a detailed review here, but will delineate the highlights of new developments in this area.

Through the period of the 1930's, 40's and 50's most of the basic principles concerning the induction of mutations and chromosome aberrations appeared to have been established. Most workers in the field believed that little would be added to our understanding of the basic principles, save for extending the observations to other organisms, and for using radiation as a tool to provide desired genotypes for further experimentation.

A major development started however in the late 1950's, when William L. Russell and his colleagues demonstrated that the yield of induced mutations was not only dependent on the dose of radiation given, but was also dependent on the dose rate. This appeared to violate one of the closest held tenets of radiation genetics, namely that the yield of mutation was

independent of the dose rate. Russell and his colleagues also demonstrated in their pioneering work on mammalian oocytes, that there are stages which appear completely refractory to mutation induction (i.e., no mutations are recovered from these irradiated stages, and selection does not appear to be able to account for a substantial portion of mutation loss). Finally, their observations also suggested that at lower doses, even with the dose rate remaining constant, fewer mutations are recovered than expected on a linearity concept. On the basis of all this information a hypothesis was constructed, proposing that metabolically active cells are capable of repairing premutational damage induced by irradiation, when low doses or a low dose rate is used. Simultaneously with these investigations in higher organisms, workers in the field of radiation genetics using microorganisms were also providing evidence for mutation repair.

Another area in which recent results seem to contradict an established belief is that of dose kinetics of translocations. Recently, the mammalian workers have been able to demonstrate that translocations, induced in spermatogonia and cytologically observed in meiosis, follow a linear induction pattern over a wide range of doses. Similar observations have been made, at the low dose range, for mammalian somatic cells in culture and also for both male and female *Drosophila* germ cells at low doses. This challenges the widely held view on the non-linear dose kinetics of ^{all} chromosome rearrangements. Thus we are presently in a critical period of reevaluation and

renewed uncertainty of the genetic risks from high energy radiation. Recent information from low dose irradiations, and the discovery of dose rate differences might make us less dogmatic about the genetic effects of low doses of radiation than we were 15 years ago.

In this perspective our own studies of low dose effects in metabolizing germ cells in *Drosophila*, though far from completion, seem to weaken further those early views on the dose kinetics of induced mutational events (at least judging from our data accumulated up to the present time) and might even provide support for the Russell hypothesis of repair. Our observations, so far, suggest that low doses of radiation (low by *Drosophila* standards) produce fewer mutations than predicted on a linearity hypothesis. Indeed, doses of 20 R, and in particular those for 100 R, so far, do not show evidence of mutation induction, and may even be responsible for a depression in mutations yield below the control level. We have to be extremely cautious and avoid conclusions at this time, when the data are still incomplete and inconclusive. But should our future experiments continue to show this trend, we will be forced to conclude either that low doses of irradiation stimulate a repair process which reduces the yield of both the induced and the spontaneous mutations, or that in some way, the mutated cells are selectively removed from subsequent sampling.

In view of these new developments it would seem prudent that top priority for future investigations should be given to

precise explorations of the genetic effects of low doses of ionizing radiations on metabolizing cell systems, and re-assess the genetic risks involved, since low dose effects represent an increasing hazard in today's environment.

Much can be learned from mammalian cell culture systems, where the techniques are available and the costs are considerably lower than for an equivalent study performed on the germ line of mammals. But for the exact appraisal of truly genetic effects, such studies need also to be carried out on cells of the germ line of various higher organisms. We hope that our own studies on low dose effects in metabolizing, immature germ cells of *Drosophila*, as well as those of other investigators in this field, may serve as a bridge between somatic cell culture techniques and mammalian germinal material, in providing the basis for some new generalizations for the genetic response of metabolically active cells to low dose irradiations.

STATEMENT ON PRESENT DIVISION OF FEDERAL SUPPORT FOR OUR OVERALL RESEARCH PROGRAM.

The overall research program of the Research Laboratory headed by S. Abrahamson (Professor of Zoology and Genetics, and presently also Chairman of the Department of Zoology) is supported by two completely independent research contracts with the Atomic Energy Commission. One is the contract for which this report is being submitted, Contract AT(11-1)-2001, S. Abrahamson and H. U. Meyer, entitled "Genetic effects of low x-ray doses in *Drosophila*", which was supported by \$40,000 during 1970/71.

The other one is Contract AT(11-1)-1748, S. Abrahamson and R. M. Valencia, entitled "Total radiation-induced genetic damage in entire genomes of *Drosophila melanogaster*". It was funded during 1970/71 with approximately \$27,000 and has been renewed for 1971/72 with approximately \$24,000.

The two projects are entirely separate and are staffed separately as far as the scientific work and research staff is concerned. The expenses for laboratory equipment, glassware, and the supplies for the preparation of the culture medium are shared by both groups.

Some additional financial support involving Federal sources: One of the full-time project specialists who devotes her entire time to the "low x-ray dose" project, could only be supported by this contract for 3 months, because of the reduction in last

year's proposed budget (1970/71). For the remaining 9 months she has been paid by a Research Grant of the NIH to the Laboratory of Genetics.

Several students, supported by the Work-Study Program have also assisted in this work, on an hourly basis, when available. 80% of the Work-Study Program is funded from Federal sources.

STATEMENT ON STUDENT SUPPORT.

No funds from this contract were used for the training of graduate students, nor to support work of graduate students towards a degree, with the exception of 2 summer months in 1969, when a graduate student, Evelyn Sabol Rabin, was paid a halftime research assistant salary while concluding a low dose study of induction of translocations in *Drosophila* oocytes (see bibliography, S. Abrahamson et al., 1971). No post-doctoral tenures were supported.