SEX-LINKED LETHAL MUTATIONS INDUCED BY THERMAL NEUTRONS IN MALE AND FEMALE DROSOPHILA MELANOGASTER[1]

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THE experiments to be described are concerned with a comparative study of the mutagenic effectiveness of thermal neutrons in producing sex-linked lethal mutations in male and female *Drosophila melanogaster*. Some of this work has appeared previously in abstract form (KING 1952a). Preliminary comparative studies of the mutagenic effectiveness of X-rays in producing sex-linked lethal mutations in male and female Drosophila have already been described (KING 1952b). The most extensive study to date on cytogenetic changes accompanying thermal neutron treatment is that of CONGER and GILES (1950) working with Tradescantia.

PHYSICAL METHODS

The thermal neutrons used in the experiments were produced in the thermal column of the Brookhaven nuclear reactor by moderation of fast neutrons from uranium fission by elastic collision with graphite. The exposure chamber is a 10 inch cubical space at the center of a 5 foot cube of graphite. Exposures are made in a lucite box having a cubical volume of eight inches on a side. The thermal neutron flux expressed as the number of neutrons crossing a surface area of one square centimeter per unit time is calculated from measurement of the induced radioactivity of gold foils exposed with the experimental material. The slow neutron flux in the thermal column varies linearly with pile power. During our experiments it amounted to $3 \times 10^{12} n_{th}/cm^2/hr$. The cadmium ratio is about 10^4 and the gamma ray contamination 125r/hr $\pm$ 30%. The temperature in the thermal column varies between 21 and 25°C.

For exposure in the thermal column groups of 100 male or female Drosophila are placed in small lucite chambers (outer dimensions $1\frac{3}{4} \times 1\frac{3}{4} \times \frac{1}{2}$ inches). These chambers consist of a lucite base and overlapping cover. The base is a rectangular frame $\frac{3}{8}$ inches high and $\frac{3}{16}$ inches thick enclosing a space $1\frac{1}{8} \times 1\frac{1}{8} \times \frac{3}{8}$ inches. The bottom of the frame is covered by a 0.003 inch (7.72 mg/cm²) polystyrene film. The inner walls of the frame are generally covered with a 1 mm thick layer of fly medium. In such containers flies can be maintained in a healthy condition for treatments of 72 hours or more, while flies often die from desiccation within 4 hours in food-free chambers. The covering frame holds taut a layer of cheese cloth over the top of the base frame, and thus flies are prevented from escaping. Drosophila are etherized through the cheese cloth. Polystyrene film (0.003 inch) glued over the top

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of the covering frame reduces evaporation and prevents the food within from drying out during long exposures. The floor of the thermal column holds a maximum of sixteen chambers at any one time.

BIOLOGICAL METHODS

The genetic setup used is a slight modification of that outlined by MULLER (1952), who has kindly supplied us with the necessary stocks for synthesis of the breeding systems used. The method has two advantages over the standard "M-5" technique. First, great numbers of the females used in the P_1 radiation crosses may be obtained with a minimum of effort, since these females are "automatic virgins". Second, the collection of lethals produced in the female germ line is facilitated, since lethals detected in the F_2 generation are balanced. The method makes use of modified X-Y conditions where the long or short arms of the Y (represented symbolically as Y^L or Y^S) have become attached to a region of the X chromosome to the right of the centromere. The remainder of the Y is present as a fragment, either a free short arm (Y^S), a free closed long arm (Y^{LC}), or the free long or short arm carrying the left distal region of the X containing the wild type allele of yellow (y^+ *sc*. Y^L or y^+ sc^{VI}. Y^S).

It has been known since the classical studies of BRIDGES (1916), STERN (1929) and NEUHAUS (1939) that males lacking a whole Y-chromosome or either arm of the Y are sterile. SHEN (1932) showed Y-deficient males produce immobile sperm which later degenerate, while STERN and HADORN (1938) demonstrated that testis transplants from Y-deficient males to normal males behaved autonomously, as did reciprocal transplants.

The diagram in figure 1 shows the type of breeding system used. Five stocks are kept which produce "B" males, "D" males, "E" males, "F" males, and "G" males. These are *y w*. Y^S/y^+ *sc*. Y^L and *y f*: = $/y^+$ *sc*. Y^L, *y v f*. Y^L/y^+ sc^{VI}. Y^S and *y f*: = $/y^+$ sc^{VI}. Y^S, *y In 49 v B*.Y^L/y^+ sc^{VI}. Y^S and *y f*: = $/y^+$ sc^{VI}.Y^S, *y In 49 v f*. Y^S/Y^{LC}, and *y In 49 v B*.Y^L/Y^S and *y f*: = $/Y^S$, respectively.

The tandem attached-X chromosome, *y f*: =, has been described by MULLER (1943). The cycling setup shown in the top of the diagram produces "A" and "C" females which are "automatic virgins", since their brothers are sterile. These females serve in the radiation experiments as shown in the lower part of the diagram. The "A" females are mates for treated "B" males and the "C" females are treated and mated to "E" males. In the case of "B", "D" and "E" males, these are always non-yellow, and the presence of yellow males indicates the loss of the Y fragment and the possible presence of a normal Y. This condition enables one to continually check whether or not the breeding system is behaving satisfactorily and to discard aberrant cultures. Patroclinous males are readily observed in the F_1 of all crosses since P_1 males differ in phenotype from F_1 males. "G" and "F" males do not contain the wild type allele of *y*, since the presence of the left hand tip of the X on the Y-fragment would mask mutation occurring in this region when the F_2 was observed. It should be noted that in the case of "F" males crossing over between the X and Y will produce dicentric chromosomes and consequently the stock will be a relatively stable one.

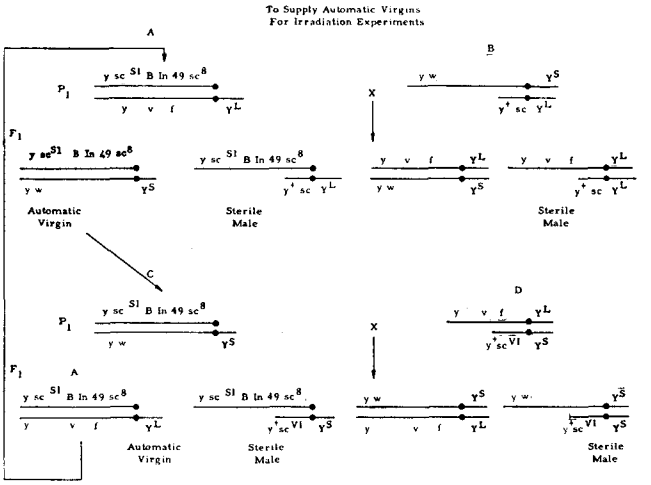

FIG. 1A

FIGURE 1.—The genetic system employed. Modified after MULLER's *Binscy* technique.

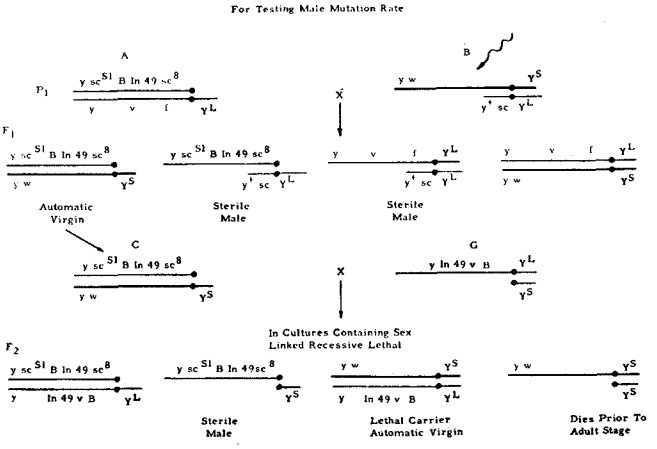

FIG. 1B

As a check on the above setup to see if "A" and "C" females were really automatic virgins *y sc*^{S1} *B In 49 sc.*^{8}/ *y*$^{+}$ *sc.* Y^{L}, *y v f.* Y^{L} / *y*$^{+}$ *sc.*Y^{L}, *y sc*^{S1} *B In 49 sc*^{8} / *y*$^{+}$ *sc*^{VI}.Y^{S}, and *y w.* Y^{S} / *y*$^{+}$ *sc*^{VI}. Y^{S} males were mated singly in creamers to 176, 106, 60, and 72 virgin *bw*; *e* females, respectively. The virgin *bw*; *e* females were obtained from the cross $X.Y^{S}/Y^{LC}$; *bw*; *e* males with *bw*; *e* females. The cultures produced no F_1 flies although many eggs were laid in all cultures.

It should be noted that in the male and female series the *y w.* Y^{S} chromosomes are balanced by chromosomes which differ structurally (*y sc*^{S1} *B In 49 sc*^{8} and *y In 49 v B.*Y^{L}). The Binscy (*y sc*^{S1} *B In 49 sc*^{8}) chromosome is similar to the M5 or Basc (*sc*^{S1} *B In S apr sc*^{8}) chromosome in general use today, differing mainly in that *In 49* is longer than *In S*. Crossing over is reduced to a greater extent in double inversion

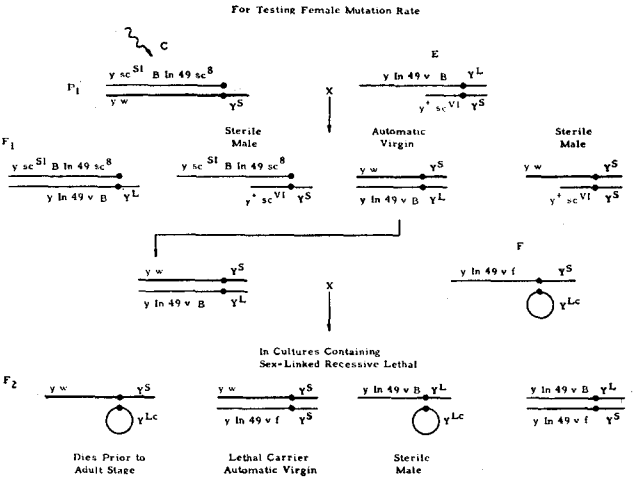

FIG. 1C

heterozygotes than in single inversion heterozygotes, and one should expect recovery of crossover products in the F_2 of the female series. These will include single crossovers (between *w* and *In 49* and B^+ and *In 49*) and double crossovers (between *w* and B^+). For example, a chromosome bearing a lethal located near *B* will yield crossover products including *y w v B* and *y w* v^+ *B*. Here the lethal still would be detected, since white Bar males obviously would be crossover classes. Double crossover class *y w v* B^+ would be viable in the case of X chromosomes bearing centrally located lethals and would be mistaken for the noncrossover class *y w*. However, it is assumed that this class, because of its low frequency of occurrence, will not introduce an appreciable error in mutation rate determinations.

THERMAL NEUTRON TREATMENT

Zero to five day old male and zero to four day old female *Drosophila melanogaster* with the identical X chromosome, *y w*. Y^S, were irradiated under identical conditions in the thermal column for varying periods of time. In the case of males it was found that 48 and 72 hour exposures (amounting to 134×10^{12} and 210×10^{12} n_{th}/cm^2 under the conditions of the experiment) produced sterile flies and consequently exposures were restricted to 24 hours or less. Six exposures of males and three of females were made. The flies were treated in groups of 100 each. In the male series 100 flies were treated at each of the three lowest exposures, 200 flies at the next two exposures and 300 at the highest exposure. In the female series 100, 200, and 300 individuals were treated respectively at the three increasing dosages. Irradiated males were mated with *y* sc^{81} *B In 49* sc^8/y *v f*.Y^L females in half pint bottles, ten pairs per bottle, and the parents removed and discarded on the tenth day. Irradiated females were mated with *y In 49 v B*.Y^L/Y^S males in a similar fashion, and then transferred to fresh bottles every second day for a total of five transfers. After the last transfer all the flies were removed from the bottles and discarded. The F_1 progeny from the five series of cultures thus represent eggs laid by treated females 0–2, 2–4, 4–6, 6–8, and

8–10 days after irradiation. These successive batches of eggs were at increasingly early stages of oögenesis at the time of exposure to thermal neutrons.

In the control series the sperm from 120 "B" males were tested as shown in figure 1. In the female control the mutation rate in the *y w.* Y^S chromosomes from 210 females was tested as shown in figure 1. Here, however, the P_1 flies were allowed to remain in their culture bottles for ten days and were discarded on the eleventh. This means that the control rate is for eggs laid 0–10 days after treatment and is not broken down as in the treated series.

In all cases when the F_1 generation appeared, *y* heterozygous B females were allowed to mate freely for 24 hours in groups of 250–300 with 50 "F" males (female series) or "G" males (male series). Half pint milk bottles with 50 cc of "enriched" Drosophila medium served as mating bottles. (Formula (g component/1000g medium) propionic acid 3.8, agar 8.5, rolled oats 17.0, Anheuser Busch strain G primary, dried yeast 20.8, Karo white corn syrup 58.4, Grandma's molasses 58.6, yellow cornmeal 68.0, water 764.9. This medium remains free of mold for two weeks or more even if dispensed into unautoclaved containers. Growth of Drosophila on the medium is excellent.) Each fertilized female was then transferred to a separate culture bottle where it produced a second generation.

Three-quarter ounce creamers and 1 ounce Dixie sampling and restaurant cups containing 10–15 cc Drosophila medium served as F_2 cultures. The Dixie cups were covered by transparent acetate caps $1\frac{9}{16}$ inches in diameter and 0.03 inch thick. These containers had the advantage of being expendable and were discarded at the end of each experiment, while the acetate caps were saved.

The progeny of each F_1 female was examined through the side of the creamer or the top of the Dixie cup under a dissecting microscope. Magnifications of 6, 9, 10 or 12 × were used. The absence of white-eyed males in cultures containing a minimum of 10 males was generally taken as a criterion for the presence of a sex-linked recessive lethal mutation. Records were kept of the phenotype and number of individuals in each of the lethal cultures. Cases with no white males, less than 10 non-white males, but more than 30 females were classified as lethals. There were only a few of these cases, however.

RESULTS

The experimental data are shown in table 1 and figure 2. It is evident that very few F_1 test females were obtained from the 0–2 and 2–4 day groups although 100, 200, and 300 mature females, respectively, were laying eggs in the three increasing dosage series. All the available F_1 females were tested from the 0–2 and 2–4 day groups, whereas only a portion of the F_1 *y* heterozygous B females from the later groups were tested due to their large numbers. A similar rise in the productivity of X-irradiated females five days after treatment has been reported (KING 1952b).

The thermal neutron dose/mutation frequency relation for each gamete sample is assumed to be linear, and the lines of best fit were computed. The dose action curve for sperm computed from the seven experimental points is given by the equation $y = 0.003 + 1.49 \times 10^{-15} x$. The five dose action curves for the various egg samples were forced through the origin, since the control sex-linked recessive lethal frequency

TABLE 1

Dose-mutation frequency data for different gamete samples

	Thermal neutron exposure (n_{th}/cm^2)	Days between exposure and collection of gamete sample	Lethal frequency	Total X chromosomes tested
Sperm	0	0–10	0.0011	3623
	5.7×10^{12}	0–10	0.0099	1021
	15.6	0–10	0.0316	2439
	19.9	0–10	0.0344	1746
	33.7	0–10	0.0442	1356
	49.5	0–10	0.0801	936
	65.6	0–10	0.0992	534
Eggs	0	0–10	0.0003	6437
	16.6×10^{12}	0–2	0.0264	189
	33.2	0–2	0.0287	174
	49.8	0–2	0.1087	46
	16.6	2–4	0.0171	468
	33.2	2–4	0.0492	244
	49.8	2–4	0.0387	362
	16.6	4–6	0.0160	499
	33.2	4–6	0.0380	764
	49.8	4–6	0.0629	429
	16.6	6–8	0.0188	532
	33.2	6–8	0.0275	763
	49.8	6–8	0.0374	642
	16.6	8–10	0.0032	618
	33.2	8–10	0.0086	818
	49.8	8–10	0.0211	759
				25,399

was very low (0.0003). A standard analysis of variance revealed three facts about the egg data: (1) the difference in the regression lines for the pooled 0–6 day and 6–10 day egg data is very highly significant; (2) the regression line for the pooled egg data for 0–6 days gives as good a fit as the three separate regression lines; and (3) separate regression lines for 6–8 and 8–10 day egg samples give a significantly better fit for the data than does the regression for the pooled 6–10 day sample.

The mutation rates for the different gamete samples are given in table 2. The data indicate that the sex-linked lethal mutation rate for viable eggs laid after exposure to thermal neutrons remains fairly constant for the first six days. Subsequently the rate decreases. Eggs laid 6–8 and 8–10 days following exposure have mutation rates 70% and 30% the original value.

Patroclinous *y v B* males appeared in many F_1 populations of the 0–2, 2–4 and 4–6 day female series. A decrease in the frequency of patroclinous males was noted in the 6–8 and 8–10 day series. Such males may be produced by fertilization of an X-deficient egg by X-bearing sperm resulting in an X0, sterile male. Patroclinous males may also arise by union of a *y In 49 v B*.Y^L-bearing sperm with an egg bearing

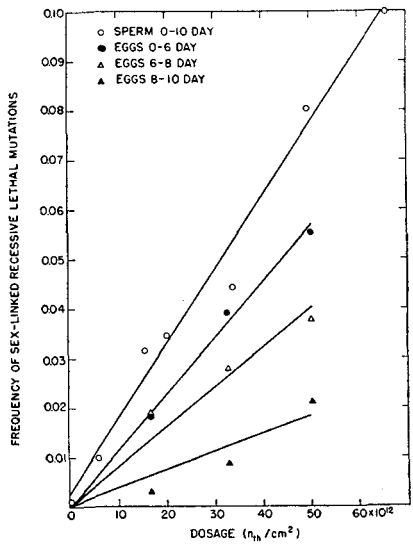

FIGURE 2.—The relation between thermal neutron dose and lethal mutation frequency in surviving X chromosomes for various gamete samples.

TABLE 2

Thermal neutron-induced mutation rates for different gamete samples from Drosophila melanogaster

Gamete	Days between exposure and collection of gamete sample	Mutation frequency per thermal neutron
Sperm	0–10	1.49×10^{-15}
Eggs	0–6	1.13×10^{-15}
	6–8	0.80×10^{-15}
	8–10	0.36×10^{-15}

the short arm of the Y resulting from radiation-induced breakage of the *y w*.Y^S chromosome in the proximal region of the X. Such males would be fertile. The fact that a second generation appeared in cultures and that the F_2 population contained *y v B* females supports the conclusion that some patroclinous males were fertile.

In the male series *y v f* males were found, and some of these were undoubtedly X0 individuals. However, a second generation containing *y v f* females was produced in cultures where all males were supposedly sterile. This means that some of the *y v f* males were fertile and indicates the fertilization of *y v f*.Y^L-bearing eggs by Y^S-bearing sperm. Thus the treatment given the males and females produced fragmentation of the *y w*.Y^S chromosome as well as its complete loss. A sterile F_1 male phenotypically *y* heterozygous *B* was probably of the genotype *y* sc^{sl} *B In 49* sc^s/B^+ .Y^S.

DISCUSSION AND CONCLUSIONS

From the thermal neutron dose/mutation frequency relation for sperm one can calculate that a thermal neutron exposure of $1 \times 10^{13} n_{th}/cm^2$ produces 1.8% sex-linked lethal mutations. Substitution of this value in the X-ray dose/mutation fre-

quency relation determined by SPENCER and STERN (1948) yields a value of 740r. Thus 740r of X-rays and $1 \times 10^{13} n_{th}/cm^2$ produce equivalent mutation frequencies. Data are available (KING 1954a) which allow calculation of the radiation dose to Drosophila tissue accompanying exposure to $1 \times 10^{13} n_{th}/cm^2$. With respect to radiations of high specific ionization the $N^{14}(\eta, p)C^{14}$ reaction yields 293 rep of protons and 23 rep of recoiling carbon nuclei, while the $H^1(\eta, \gamma)H^2$ reaction yields 5 rep of deuterons. With respect to sparsely ionizing radiations, 7 rep of 2.23 Mev maximum energy gamma rays ($H^1(\eta, \gamma)H^2$ reaction) are produced and a dose of 430 rep is delivered by 5 Mev maximum energy gamma rays which contaminate the thermal column. If, as has been shown for another radiation of low specific ionization (KING 1954b), these hard γ-rays are 60% as efficient as X-rays in producing sex-linked lethals, then the densely ionizing radiations must be roughly 1.5 times as effective mutagenically as X-rays to account for the observed mutation rates.

The radiation doses delivered to Drosophila tissue by the $Li^6(\eta, \alpha)H^3$ and $B^{10}(\eta, \alpha)Li^7$ reactions are omitted in the above calculations. These reactions are believed to be unimportant, since treatments which increase the average lithium and boron contents of Drosophila tissue by factors of 2 and 100 respectively, are ineffective in increasing the thermal neutron-induced mutation rate (KING 1952a, 1954a).

The yield of mutations does not vary for equal radiation doses delivered at different intensities. It is assumed, therefore, that the large differences in the length of time necessary for delivery of mutagenically effective thermal neutron exposures as opposed to X-ray exposures does not introduce a significant variable into the experiments described above.

It seems unlikely at first thought that the decline in the sex linked lethal rate in successive batches of eggs can be due to selection of lethal-free eggs during oögenesis, since the developing eggs are diploid and the lethal mutations are recessive. It may be, however, that fewer viable restitutions of broken chromosomes occur in pre-meiotic and early meiotic stages than in late meiotic stages and consequently many potential recessive lethals are drawn into chromosome configurations which function as dominant lethals. It is known that oögonia are very sensitive to X-irradiation (BUCHER 1951) and that few gross chromosomal aberrations are recovered from the eggs of irradiated females (SHAPIRO and NEUHAUS 1933; GLASS 1940). If elimination of potential recessive lethals does occur then one might expect to find the reverse of what actually happens, namely, a decrease in the productivity of irradiated females as the mutation frequency in successive batches of eggs decreases. However, this lowered productivity might be masked, if the unaffected oogonia multiply rapidly enough. A second explanation for the lower mutation rates observed in pre-meiotic and early meiotic stages is that the mutation process actually occurs at a lower frequency in these less mature cells because of physiological differences between these and more mature nuclei.

The above calculations depend upon the assumption that the nitrogen concentration per unit weight of Drosophila gonad is the same as the mean nitrogen concentration per unit weight of fly. The difference in the mutation rates induced in the male and female germ line may point to a difference in mutability between sperm and egg chromosomes during late stages of gametogenesis or may simply be due to differences in nitrogen concentration between the male and female gonad.

SUMMARY

A study was made of the sex-linked lethal mutation rates induced in the germinal tissue of *Drosophila melanogaster* males and females by thermal neutrons. Nitrogen capture protons (which are primarily responsible for the biological effects of thermal neutrons in the fruit fly) are approximately 1.5 times as effective in producing sex-linked lethal mutations in sperm as are 90 kv X-rays. Over the range of doses used the lethal mutation rate/dosage relation for X chromosomes of sperm appears to be linear. The mutation rate detected in viable eggs laid following treatment remains fairly constant for the first six days. Eggs laid 6–8 and 8–10 days following exposure have mutation rates 70% and 30% the original value. This original rate is only 75% the rate for sperm. The difference in the mutation rates induced in the male and female germ line may be due to a difference in mutability between sperm and egg chromosomes treated in late stages of gametogenesis or to differences in the nitrogen concentration between the male and female gonad. In the female germ line the lower frequency of mutations recovered from pre-meiotic and early meiotic stages than from late meiotic stages may mean that the mutation process occurs at a lower frequency in less mature cells or that a larger fraction of the potential lethal mutations are drawn off into inviable chromosome recombinations. Exposure of males and females to thermal neutrons also produces loss and fragmentation of X chromosomes in germ cells.

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