STUDIES ON DIFFERENT CLASSES OF MUTATIONS INDUCED BY RADIATION OF DROSOPHILA MELANOGASTER FEMALES[1]

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DELETERIOUS mutations are produced in the germ plasm when animals are exposed to total body radiation. The ovaries of most higher animals contain germ cells at different stages of development, and these cells may be expected to be affected to different degrees by ionizing radiation. It is, therefore, of both practical and theoretical interest to study the mutation frequency in successive batches of germ cells produced by females subsequent to radiation exposure, since it is important to know whether or not mutated cells are eliminated from the germ line and if mutant-free eggs are eventually produced.

OÖGENESIS

The process of oögenesis in the fruit fly, *Drosophila melanogaster*, has been described recently (KING, RUBINSON, and SMITH 1956). Each of the two ovaries of this insect is comprised of an average of 12 ovarioles, and these tubes are differentiated in turn into an anterior germarium and a series of egg chambers. The sixteen cells (15 nurse cells and the primary oöcyte) making up each chamber result from four consecutive divisions of an oögonial cell located in the germarium. The development of an egg in an ovariole can be subdivided into at least 14 stages, and the number of the egg chambers in each ovary and the proportion of the egg chambers in the various stages depends primarily upon the age of the fly (cf. table 1). Mature eggs (stage 14) in the ovarioles show a Feulgen-positive micronucleus or karyosphere lying free in the oöplasm at the base of the dorsal appendages; whereas the oöcytes of egg chambers in stages 3–13 show a Feulgen-positive nucleolus or karyosome lying in a reticulate nucleoplasm which is surrounded by nuclear membrane. The work of PLOUGH (1917) indicates that genetic recombination has already taken place in chromosomes of the oöcytes in the most anterior egg chambers. Diakinesis and metaphase I occur subsequent to the time the egg leaves the ovariole (SONNENBLICK 1950; FAHMY 1952). Because the anatomical arrangement of the germ cells in the tubes is such that the most mature cells are closest to the uterus, successive batches of eggs laid subsequent to radiation exposure represent cells which on the average were at increasingly early meiotic and premeiotic stages at the time of treatment. Characteristically, the rates of mutation observed are highest in the germ cells first produced, and a decline is noted in the frequency of mutation in successive batches of germ cells (MAVOR 1924; PATTERSON, BREWSTER and WINCHESTER 1932; MAINX 1940; GALL 1950; MULLER, VALENCIA and VALENCIA 1950; KING 1952; NOVITSKI 1953; PARKER 1953; LÜNING 1954; MULLER and HERSKOWITZ 1954; HERSKOWITZ

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TABLE 1

Average number and distribution with respect to stage of developing eggs in the ovaries of flies of different ages

Age (hrs) of virgin female	Stage of development									Total
	1	2	3[x]	4[x]	5[x]	6[x]	7[x]	8[x]–13[x]	14[+]	
0–1	24	25	24	20	16	20	20	0	0	149
96–97	24	25	25	20	10	24	24	0	24	176

[x] Karyosome stages.
[+] Karyosphere stages.

1954; KUTSCHERA 1954; PARKER 1954, 1955; KING and WOOD 1955; GLASS 1955a, b; KING 1955a, b; and GLASS 1956). This paper will describe a study which was initiated to determine whether or not the rates of various types of radiation-induced mutations eventually decline to zero.

GENERAL METHODS

A technique has been described (KING 1955b) which allows collection of almost all eggs laid by female flies over an extended period of time. This technique permits study of the effect of irradiation upon fecundity and fertility, and since the F_1 generation is obtained, studies of hereditary changes are also possible. The germ cells studied were produced by control and treated (2000r Co^{60} γ-rays delivered in ca. 40 seconds) female flies during periods up to 40 days subsequent to the time of treatment (T_0). All cultures were maintained at 25°C. Control and treated females were compared with respect to (1) fecundity (the average number of eggs laid per female for a given time interval); (2) the frequency of dominant lethals in samples of eggs laid (the induced dominant lethal frequency, L, is given by the equation $L = (a - b)/(1 - b)$ where the frequencies of eggs failing to hatch in treated and control series are a and b, respectively); (3) the frequency of X chromosome losses in eggs free of dominant lethals (the induced rate of X losses, $X - L$, is given by the equation $X - L = 2(p_2 - p_1)$, where p_1 and p_2 are the frequency of X0 males in the F_1 generation of the control and treated series, respectively); (4) the ratio of ring to rod X chromosomes in the F_1 offspring of ring-rod heterozygous females; and (5) the frequency of sex-linked, recessive, lethal mutations (in cells containing X chromosomes, but free of dominant lethal and sterility mutations). It is assumed that dominant lethal mutations and X chromosome losses result from gross chromosomal aberrations following chromosome breakage; whereas the sex-linked, recessive, lethal category of mutant is made up by a proportionally large number of point mutations.

EXPERIMENT 1

Methods

Criteria 1–4 were studied in the first experimental series. Here freshly hatched females of the genotype $X^{C2}/M5$ (sc^{81} *B InS apr* sc^{8})[4,5] were etherized, placed in

[4] See BRIDGES and BREHME 1944 and KING 1955a for descriptions of the mutants used.
[5] The X^{C2} stock was checked periodically to insure that the ring X-chromosome was still pres-

plastic tubes, irradiated, reëtherized, and placed in mesh-covered, plastic hoops with untreated males of the genotype *ac*^{3} *apr*/*sc*^{8}.Y. The hoops (each with 50 pairs of flies) were transferred daily for 35 consecutive days to petri dishes containing fresh medium. The number of eggs deposited through the mesh onto the medium was counted, and a day later the number of unhatched eggs was determined. Appropriate controls were maintained. Records were kept of the number of P_1 females surviving in the hoops and fresh males were added once a week. The phenotypic composition of the surviving adult F_1 population was determined. Four classes of regular and one class of exceptional offspring were obtained[6] (regular — *M*5/*sc*^{8}.Y (apricot, Bar males), X^{C2}/*sc*^{8}.Y (+ males), *M*5/*ac*^{3}*apr* (apricot, heterozygous Bar females), and X^{C2}/*ac*^{3}*apr* (+ females); exceptional — *ac*^{3} *apr*/0 (sterile achaete, apricot males).[7] To obtain data for cells treated as mature eggs, irradiated and control 3–4 day old females were studied. Zero-1 day old females were etherized, placed in plastic tubes, stored 3 days, irradiated, and then placed unetherized with males in hoops. Mating generally occurred at once and the eggs laid the first day after treatment were studied for criteria (2), (3) and (4).

Results

The results of experiment 1 may be summarized as follows. Over the 35-day period studied for the initial experimental series, the control and treated females showed no significant difference in fecundity (cf. fig. 1). Under the experimental conditions an average of six eggs was laid daily by each female. No eggs were laid the first day. After inspection of the data, the conclusion was reached that the hatchability of eggs laid by control and irradiated flies appeared to be the same subsequent to the thirteenth day. A standard analysis of variance showed that no significant difference in hatchability occurred from day to day for days 2–35 (eggs 1–205) in the control and days 13–35 (eggs 71–205) in the irradiated series. The average rates for these intervals were computed, and the treated and control values were shown not to differ significantly. The conclusion reached is that in both cases approximately the same proportion (11%) of all eggs laid fail to hatch for one of the following reasons: (a) they are unfertilized, (b) they die because they were improperly formed during oögenesis, or (c) they die during embryogenesis because of aneuploidy following

ent. Ring X-chromosomes were observed in larval salivary gland smears and crossing over studies on ring/rod heterozygotes showed the expected suppression of single crossover classes. The progeny recovered belonging to "single crossover classes" amounted to one-twelfth those normally found for the regions *apr-sn* and *sn-B*.

[6] The separation of *apr* and *B* occurs following certain types of double exchange between the X^{C2} and M5 chromosomes. Flies belonging to crossover classes occurred rarely (5.3, 7.9, 8.6 and 1.0 per 10,000 for heterozygous Bar females, apricot females, apricot males, and Bar males, respectively). Evidently *Bar*-containing crossover products are lethal (especially when hemizygous) more often than *apricot*-containing crossover products. No significant difference was noted between control and treated flies.

[7] Whenever achaete, apricot males were obtained, they were mated singly to two virgin females. No offspring were ever observed. XO, achaete, apricot males have a viability of 0.7, so that the values obtained for the frequency of XO males are underestimates.

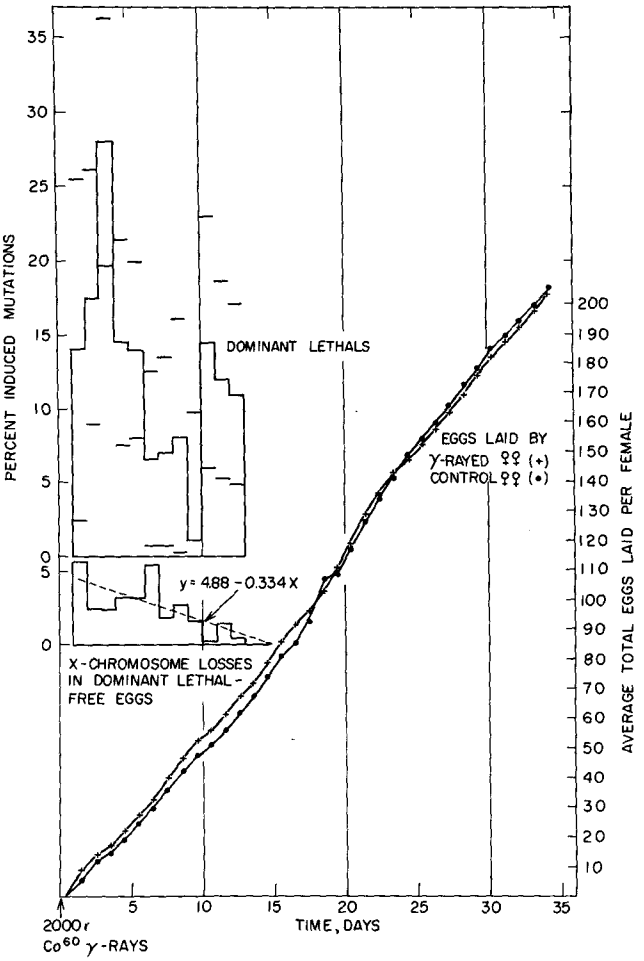

FIGURE 1.—Percent induced mutations and average total eggs produced per female (treated when freshly hatched) as a function of time. The percentage of eggs bearing induced dominant lethal mutations for each daily interval is shown with its 95% confidence limits (calculated from the analysis of variance).

attempted segregation of crossover products between the ring and the rod X chromosomes. With respect to the first 70 eggs laid (days 2 through 12), a statistical analysis showed that the induced dominant lethal rate decreases with time in a non-linear fashion. With respect to the induced rate of viable X chromosome losses, the situation is similar, except that the rate increases with time in a linear fashion (cf. fig. 1). Furthermore, no significant difference exists from day to day for the ring to rod X chromosome ratio for the series; γ-ray (2–12 days), γ-ray (13–35 days) and control (2–35 days). The average values for each series were not significantly different from each other. These data are summarized in tables 2 and 3 (columns B and C). Similar

studies on the eggs laid within one day after treatment by 3–4 day old females gave very different results (cf. tables 2 and 3—column A). The dominant lethal rate was much higher and so was the frequency of X0 males. The ring/rod ratio was somewhat lower, but the sample size was small because so few eggs hatched in this series. It is seen that our results differ from those of FABERGÉ (1952), who made no attempt to differentiate between mutation rates in more and less mature cells.

TABLE 2

Mutation as a function of cell class

P_1 female	Cell class	Series	Frequency of eggs not hatching $\pm s_{\bar{x}}$	N	Frequency of X0 males⊕ or X-recessive⊗ lethals	N	Average induced frequency of	
							Dominant lethal mutations = L	X-losses⊕ or X-recessive lethals⊗
Rod/Ring	A	γ	0.9924 ± 0.0170 (a)	2,485	0.0510 ⊕ (p_2)	764	0.986 ±	0.0972 ± ⊕
		C	0.4710 ± 0.281 (b)	3,107	0.0024 ⊕ (p_1)	1,236	0.032 (s_L)	0.0162 (s_d)
	B	γ	0.2232 ± 0.0129	42,140	0.0157 ⊕	17,510	0.129 (cf. figure 1)	0.0256 (cf. ⊕ figure 1)
		C	0.1079 ± 0.0101	36,960	0.0029 ⊕	16,397		
	C	γ	0.1265 ± 0.0084	59,644	0.0035 ⊕	29,137	0	0 ⊕
		C	0.1065 ± 0.0085	62,908	0.0032 ⊕	36,066		
Rod/Rod	A′	γ	0.7821 ± 0.0146	2,605	0.0226 ⊗	1,634	0.636 ±	0.0226 ± ⊗
		C	0.4010 ± 0.0267	2,597	0 ⊗	A′ + B′ pooled 1,778	0.029	0.0037
	B′	γ	0.2536 ± 0.0143	2,937	0.0109 ⊗	1,288	0.174 ±	0.0109 ± ⊗
		C	0.0969 ± 0.0114	2,380	0 ⊗	A′ + B′ pooled 1,778	0.019	0.0029
	C′	γ	0.2161 ± 0.0073	3,171	0.0125 ⊗	1,435	0	0.0125 ± ⊗
		C	0.1985 ± 0.0072	3,031	—	—		0.0029

A = Cells produced 0–1 days after T_0 by females 3–4 days old at T_0. A′ = 1st 26–30 eggs produced 0–4 days after T_0 by females 3–4 days old at T_0.

B = Cells produced 2–12 days after T_0 by females freshly hatched at T_0. B′ = 1st 29–33 eggs produced 12–15 days after T_0 by females 3–4 days old at T_0.

C = Cells produced 13–35 days after T_0 by females freshly hatched at T_0. C′ = cells produced 30–40 days after T_0 by females 3–4 days old at T_0.

γ = P_1 females given 2000r Co^{60} γ-rays in 40 seconds. C = P_1 females not treated.

Ring/Rod = $X^{C}2$/M5 females × *ac*^{3} *apr*/*sc*^{8}. Y males.

Rod/Rod = Oregon R females × M5 males.

T_0 = Time of irradiation. X-losses = 2 ($p_2 - p_1$).

N = The size of the population (of eggs, F_1 offspring, or X chromosomes) examined.

$$s_{\bar{x}} = \sqrt{\frac{pq}{N}} = \sqrt{\frac{\Sigma x^2 - \left(\frac{\Sigma x}{N}\right)^2}{N(N-1)}};$$

$$s_d = \sqrt{\frac{p_1q_1}{N_1} + \frac{p_2q_2}{N_2}};\ L = \frac{a-b}{1-b};\ s_L = \sqrt{\left(s_{\bar{x}_a} \cdot \frac{1}{1-b}\right)^2 + \left(s_{\bar{x}_b} \cdot \frac{(1-a)}{(1-b)^2}\right)^2}$$

TABLE 3

X chromosome composition of F_1 population resulting from the cross $X^{C2}/M5$ females × ac^3apr/Y: sc^8 males

Cell Class	Series	% of total F_1 flies from the regular classes†					
		Females		Males		F_1 ring/ rod ratio	
		Ring	Rod	Ring	Rod	Females	Males
A	γ	23.3	28.3	23.0	25.4	0.824	0.908
	C	25.8	29.1	22.4	22.7	0.886	0.986
B	γ	26.9	29.3	22.5	21.2	0.918	1.061
	C	27.6	28.0	22.8	21.7	0.986	1.051
C	γ	27.7	28.1	22.1	22.0	0.986	1.005
	C	27.1	29.2	21.7	22.1	0.928	0.982

† Cf. table 2 for explanation of abbreviations and data on the frequency of XO males in the total F_1 and the total number of F_1 offspring in each series.

EXPERIMENT 2

Methods

The second series of experiments involved the determination of the frequency of X chromosomal, recessive, lethal mutations (criterion 5) in germ cell populations whose dominant lethal rate was known. Three to four day old Oregon R females were divided into two groups, one of which was irradiated. Females were mated individually with untreated M5 males in mesh-covered hoops. Records were kept of the fecundity and fertility of the flies at a series of time intervals subsequent to T_0. The survivors from the eggs laid during each interval developed into F_1 adults, and F_1 heterozygous Bar females were backcrossed to Oregon R males and then isolated singly in small culture bottles (¾ oz creamers). The population of creamers was scored for lethals (cultures with no + males) when the F_2 generation emerged. Records were kept which enabled us to tell which F_2 cultures originated from the same P_1 females. In this way it was possible to determine if the same P_1 female produced more than one lethal. The frequencies for recessive lethal mutation recorded are therefore free from errors due to duplicated lethals, but may be underestimates if many duplicated non-lethals were included.

Results

The results of the second group of experiments are summarized in columns A′, B′ and C′ of table 2. It is obvious that those cells (class C′) produced 30–40 days after T_0 by irradiated females show no difference in hatchability from those produced by untreated females. However, they are characterized by a rate of X chromosomal, recessive, lethal mutations which is not significantly different from those produced

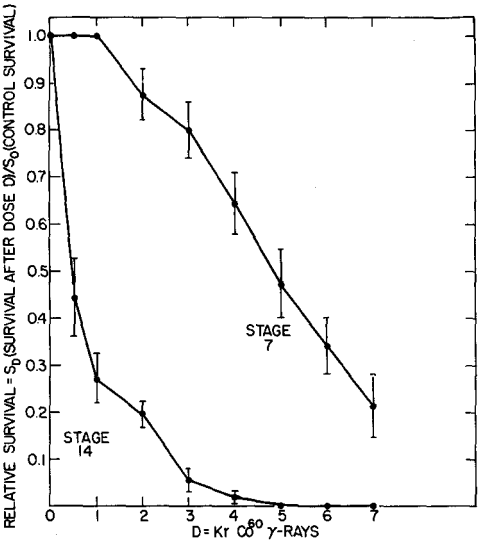

FIGURE 2.—The relation between relative survival and dose for oöcytes belonging to stages 7 and 14. Each point is bracketed by its 95% confidence limits

$$\left(\frac{S_D}{S_0} \pm 1.96 \sqrt{\frac{1-a}{1-b}\left[\frac{a}{N_a(1-b)} + \frac{b}{N_b}\right]}\right).$$

The frequencies of eggs failing to hatch in treated and control series are a and b, respectively.

12–15 days after T_0. It is also obvious that the first eggs produced by 3–4 day old females have a higher induced rate of dominant lethal mutations, if the female is a ring/rod heterozygote, than if the female had two rod-X-chromosomes. PONTECORVO (1942) has summarized the reasons why the chromosomal products resulting from the reunion of broken ends of ring chromosomes should be lethal to the eggs containing them. The fact that we observe the difference described above indicates that there is a genetic basis for the failure of treated eggs to hatch, and consequently the term "dominant lethal" is justified.

EXPERIMENT 3

Methods

In order to obtain further information about the A and B categories of cells in the ovaries of *Drosophila melanogaster*, we decided to determine the dose-response relations for dominant lethal mutations induced in cells belonging to stages 14 and 7. Eight exposures to Co^{60} γ-rays (ca. 50r/sec) were used: 500, 1000, 2000, 3000, 4000, 5000, 6000, and 7000r. Virgin, Oregon-R females (0–1 hour and 96–97 hours old) were treated and mated to untreated males. Care was taken to collect less than the first thirty eggs laid subsequent to treatment, and in this way we sampled in most cases only the posterior egg in each ovariole. It is apparent from table 1 that we were therefore determining the induced rate of dominant lethals in cells treated at stages 7 (P_1 females 0–1 hour old) and 14 (P_1 females 96–97 hours old).

TABLE 4

Egg hatch vs dose

Dose (KR)	Stage 14		Stage 7	
	N	S	N	S
0	941	0.849	1,478	0.777
0.5	172	0.378	326	0.807
1.0	314	0.232	404	0.800
2.0	940	0.171	1,008	0.681
3.0	431	0.049	508	0.618
4.0	719	0.019	357	0.501
5.0	99	0.000	215	0.367
6.0	132	0.000	260	0.265
7.0	127	0.000	234	0.167
Total.....	3,875		4,790	

N = total eggs observed.
S = frequency hatching.

Results

The dose-response relations obtained from experiment 3 are shown in table 4 and figure 2. The dose-response relations for stage 14 oocytes and for stage 7 oocytes can be described by a one event curve and by a multiple event curve, respectively (cf Parker 1955). We assume that dominant lethality requires isochromatid breakage in at least two different bivalents followed by sister union of the broken chromatids and passage of the fused bivalents so produced to opposite poles at anaphase 1. The "event" is visualized as the passage of an electron tail through the genetic material. In the case of the mature egg a single electron tail traversing the densely packed bundle of chromatids contained in the karyosphere presumably causes multiple isochromatid breaks; whereas multiple tails are required in the case of the stage 7 oocyte nucleus to produce the same effect. The resulting anaphase 2 chromatid bridges tie the terminal ootid nuclei to the inner ootid nuclei with the result that the female pronucleus receives an aneuploid chromosome complement.

DISCUSSION AND CONCLUSIONS

Our conclusions are summarized in table 5. The germ cells studied in experiments 1 and 2 can be subdivided into three categories (A, B and C) on the basis of their mutational responses. Cells of class A show a high rate of dominant lethal mutation and of X chromosome loss and recessive mutation. When treated, these cells were stage 14 primary oöcytes containing a free karyosphere. Cells of class B show lower rates of all three types of genetic change. Under the experimental conditions employed, these cells at the time of treatment were generally the posterior-most three egg chambers in the ovarioles of the exposed females. Therefore, these cells were less mature chambers of varying size (stages 3–7 were represented) with their oöcytes characterized by a karyosome-containing nucleus. Since the fecundity of treated females was unaltered, and since the primary oöcytes of similarly treated flies ap-

TABLE 5

The correlation of mutational behavior with cellular morphology

	Class of induced mutants	Germ cell class		
		A	B	C
Mutational response to 2000 r of gamma rays	Dominant lethal mutations	++++++	+	0
	X chromosome losses	+++	+	0
	X chromosome recessive lethal mutations	++	+	+
Stages of oogenesis represented		14	3–7.	1, 2 and oogonia
Nuclear morphology		Karyosphere	Karyosome	No karyosome
Genetic recombination occurs		Prior to	Prior to	During and after

peared cytologically normal[8], it is unlikely that mutated oöcytes in egg chambers are prevented from maturing and being laid as eggs. Therefore, the difference in sensitivity to mutation between cells of groups A and B must be real and not a result of germinal selection. The cells of group C show no induced dominant lethals and X chromosome losses, but significant numbers of sex-linked recessive lethal mutations. The class C group of cells includes oögonia, each of which must subsequently undergo four consecutive divisions to produce the sixteen components of the egg chamber. Possible oögonia containing genetic lesions which would later be detected as dominant lethals or X losses cannot produce a normal cyst of sixteen orthoploid cells. Perhaps such cells are resorbed at a very early stage and replaced by viable cells. If this actually occurs, one would expect to see pycnotic nuclei in the germaria of treated females. Our cytological studies[8] support this suggestion. However, the class C group of germ cells must *also* contain developing eggs which at the time of treatment had passed the four divisions and were in stages 1 and 2. The above considerations cannot apply to these cells. It is also possible that a damaged, potential oöcyte nucleus may be resorbed and its place is taken by an undamaged nucleus which would normally differentiate into a nurse cell nucleus. However, we find no evidence of chambers with less than the normal number of nurse nuclei in irradiated females[8]. The foregoing statement applies only to females which received radiation exposures of 2000r or less. At higher doses one sees that the nuclei of the oöcyte, nurse cells and follicle cells lose their ability to stain darkly following the Feulgen procedure, egg chambers are found which appear normal except that they contain more or less than 15 nurse cells, and degenerating chambers are often observed. Most germaria degenerate completely following exposure to 6000r of γ-rays or more. At lower doses the ovary often recovers, but ovarian tumors appear subsequently (KING, RUBINSON and SMITH 1956).

The simplest explanation for the failure to recover dominant lethals or X losses from stage C cells is that (1) the probability of chromosome breakage is very low or

[8] A manuscript describing these studies is in preparation.

(2) that the probability of restitution very high in chromosomes at early stages of oögenesis; or to put it more specifically, in chromosomes prior to the karyosome stages. If explanation (1) is correct, then sex-linked, recessive, lethal mutations recovered from class C cells may not have required chromosome breakage for their origin. If explanation (2) is correct then the high probability of restitution may be in some way related to the nuclear conditions which allow genetic recombination.

Dominant lethal mutations and X chromosome losses are taken as a measure of chromosome breakage in these experiments. The data show that the sensitivity of the germ cells to radiation-induced chromosome breakage varies during oögenesis in *Drosophila melanogaster*. Unlike dominant lethal mutations and X chromosome losses which under the experimental conditions are not recovered in significant numbers from oöcytes which were at stages of oögenesis earlier than the karyosome stages, sex-linked, recessive, lethal mutants are found in eggs which were in these and even more immature stages at the time of treatment[9]. It is probable, therefore, that mutations of this category (mostly point mutations) are *never* completely eliminated from the germ tract, whereas dominant lethals and X losses (mostly the result of gross chromosomal aberrations) are eliminated once the germ cells (which at the time of treatment were karyosome-containing oöcytes) complete development and are laid.

When attempting to assess the genetic damage from radiation exposure, one must take into account the interval between the exposure and fertilization in order to predict the type of genetic change which is likely to persist. In female *Drosophila melanogaster* and higher animals the dominant lethal category of genetic changes is the easiest to study. In the fruit fly this category of mutant is also the class most readily eliminated from the ovary, whereas recessive lethal mutants persist. Therefore, if no significant difference in the rate of dominant lethal mutations were noted between females of irradiated and non-irradiated populations, an investigator could not conclude that the exposure to ionizing radiation failed to damage the germinal material of the members of the irradiated population unless the attendant conditions made it unlikely that in the average female a large proportion of the germ cells bearing dominant lethals was naturally eliminated before fertilization occurred.

SUMMARY

In *Drosophila melanogaster* the sensitivity of the germ cells to radiation-induced chromosome breakage (measured by dominant lethality and X chromosome loss) varies strikingly during oögenesis. These changes are correlated with the cytology of the developing primary oöcytes. Following a single exposure to 2000r of Co^{60} γ-rays (delivered in 40 seconds) such gross chromosomal aberrations are quickly eliminated, but recessive, lethal mutations appear never to be completely eliminated from the germ tract.

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[9] A mutant cluster occurred in the C group of germ cells which indicates that a mutated oögonium may give rise to duplicate, sex-linked, recessive, lethal mutants.

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