INDUCED CHANGES IN FEMALE GERM CELLS OF DROSOPHILA. II. OVIPOSITION RATE AND EGG MORTALITY IN RELATION TO INTENSITY AND DOSAGE OF X-RAYS APPLIED TO OOCYTES¹

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AT the time gross chromosomal rearrangement frequency in *Drosophila* oocytes was found to increase with X-ray intensity, a significant decrease in the fecundity of the treated females was simultaneously noted (HERSKOWITZ and ABRAHAMSON 1956a). It seemed desirable to study this change in fecundity to learn to what degree it was based on a decrease in the number of eggs laid as opposed to an increase in the number which fail to hatch as larvae. The latter event, called egg mortality, may result from changes in the egg which are nongenetic, at least so far as the genes of the egg or zygote themselves are concerned, or genetic (dominant lethal mutations). In the experiments reported here oviposition rate and egg mortality were determined following treatment of oocytes with the same dosage of X-rays delivered at different intensities as well as after different dosages given in the same time interval. The present results with *D. melanogaster* on egg mortality and those obtained earlier by SONNENBLICK (1940) are discussed in relation to dominant lethal mutations induced in the female germ line. Some of the present work has been summarized elsewhere (ABRAHAMSON and HERSKOWITZ 1955).

MATERIAL AND METHODS

Wild type (Oregon-R) virgin females were collected and aged under excellent nutritional conditions for at least three days prior to treatment. The irradiations were given to motile, well aerated flies at 20 ma at a peak of 200 kv with 1 mm Al filtration. A Victoreen dosimeter was employed to determine the output of the X-ray machine just before the first irradiation of a series, and sometimes also between and/or after treatments. The delivery rate of the X-rays was varied either by changing the target distance or by stopping the machine periodically before the total dose was delivered or by both methods.

Simple, especially constructed, egg-laying units, modified from those used by MULLER (unpub.) and by TELFER (1954), were employed, each composed of 19 glass cylinders (25 mm in diameter and about 6 cm long) with one end covered by dacron netting held taut by cementing the netting to the wall of individual cylinders and with the cylinders held together in parallel in the shape of a hexagon by rubber bands. One female was placed in each cylinder with two males and the open end

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plugged with cotton. The unit was then placed with the netting side down firmly on the surface of a large petri dish (15 cm in diameter) containing about one quarter of an inch thickness of preyeasted sugar-agar food. The females were then permitted to oviposit for about 12 hours, after which the egg-laying unit was removed, its netting brushed free of adhering eggs or food after about 20 minutes, and placed on a fresh nutrient-containing petri dish. One cylinder of each unit was labelled as was its position on the petri dish, so that from the imprint of the rims and netting of the cylinders on the medium it was possible to identify and keep a record of the eggs laid by individual females. As an aid in scoring the eggs from different cylinders a translucent plastic sheet, on which the outlines of the cylinders of a unit were traced, sectoried, and numbered with India ink, was aligned under the petri dish.

Observations of the petri dishes were made, using transmitted light and a dissecting microscope, soon after the units were removed. At this time egg counts were made, and 36-48 hours later counts were again made, this time of unhatched eggs only. The petri dishes, whether carrying the laying units or not, were kept sealed from the outside in paper sacks except during scoring.

No attempt was made in these experiments to assure beforehand that the treated females carried lethal-free X chromosomes and no Y chromosome. As a means of compensation, at least 19 irradiated females were used per treatment.

It may be recalled that the most usual spontaneous mutation frequency of sex-linked lethals arising in either males or females is about .2 percent and that they tend to accumulate to only triple their frequency of origination, i.e. to .6 percent, in the chromosome that a female derives from her mother. Hence the usual chance of a female having such a lethal in one of her two X's is .8 percent, that is, only about one in 125 females carry a sex-linked lethal. Since only one quarter of the F_1 (one half those that were to produce males) of a lethal-bearing female are killed by the lethal, one in (4×125) , that is, .2 percent, of the eggs laid by a large group of unrelated females collected at random will be killed by a sex-linked lethal, on the average. For counts of eggs derived from batches of 125 such females, the Poisson expectation is that in 37 percent of the batches no lethal-bearing female would have been present; in 26 percent one such female, resulting in .2 percent of egg mortality from this cause; in another 26 percent two such females, giving .4 percent mortality; in 8 percent three such females, giving .6 percent mortality; and in two percent four, giving .8 percent mortality. This gives .02 confidence limits at egg mortalities of .5 percent more and .15 percent less than the average, for counts from 125 unrelated mothers, and a correspondingly larger or smaller range for smaller or larger numbers of mothers, in inverse proportion to the square root of their number. The range would of course be greater for a given number of related mothers, since relationship would introduce the possibility of runs of lethals of common origin, and it would also be greater, by as much as ten-fold, if mutator genes were present in some or all of the individuals. (We are indebted to Dr. H. J. MULLER for the clarifications presented in this paragraph.)

In all our experiments, the females were distributed randomly among the treated and control groups. Moreover, none of 103 control females ever gave egg mortalities which could be interpreted as resulting from any having carried a recessive sex-linked

lethal. Thus it is very unlikely that pre-existing lethals were frequent among the females. In a number of experiments performed later, which are not reported here, in which the female parents had been verified as being free of pre-existing lethals, it was found that if these experiments covered an aspect dealt with in the present experiments there was complete agreement in the results obtained.

Precaution was taken to avoid any non random effect of dehydration and starvation in flies exposed to treatment for different lengths of time. The longest treatment covered less than seven and one half hours. Flies which were given more concentrated treatments were irradiated either about midway in the period of time required for the diluter treatment, or were treated as two groups with one group receiving treatment toward the beginning and the other toward the end of the interval for the diluter treatment.

Experiments were performed at room temperature. Other procedural details or modifications of those already described are given with discussions of the results of specific experiments.

RESULTS AND DISCUSSION OF SPECIFIC EXPERIMENTS

In Experiment I 2000r were delivered either continuously at about 1266r/min (requiring only one hour 30 minutes for delivery) or at about 32r/min in eight equal parts with 24 minutes between the successive 8-minute long irradiations (taking three hours 52 minutes to deliver). After treatment females were placed in culture bottles for about 24 hours with an excess of Basc males, approximately 60 females and 120 males per bottle since the presence of numerous flies promotes mating. At the end of this time the males and females were placed into the cylinders of egg-laying units which were then transferred to new petri dishes in the manner and according to the schedule already described. The results are summarized in table 1.

In all experiments (as in Experiment I) in which males were present in the egg-laying units with the females, the first usable results for each individual female begin with the first one-half-day egg-laying period *following* the one in which collapsed eggs were first found (hence starting with an egg-laying interval after females had been inseminated). Accordingly, there are no data for the first 1 to 1.5 days after treatment for the females in Experiment I.

From 263 females in the diluted treatment test, 260 in the concentrated and 18 in the control, there were 11,902, 12,119, and 801 eggs oviposited, respectively, from days 1.5 through 3.5 after completion of the irradiation. Here, in the control, the difference due to recessive lethals, with a .01 confidence limit, would be 2.8 percent, that is, the control percent would have only one chance in 100 of being 2.8 percent too *high* if the mothers were unrelated. However, the data from these females proved that none carried a lethal from a previous generation. Hence this error is limited to treated females where individual egg counts would not surely tell when a pre-existing lethal was present. In these females the larger number used would greatly reduce the error. The similarity in number of eggs laid per female (45 for the diluted treatment, 47 for the concentrated and 44 for the control group) found in corresponding one-half-day intervals would seem to exclude difference in numbers of eggs laid as the basis for the large change in fecundity already noted in a similar experiment (HER-

TABLE 1

Experiment I. Egg mortalities obtained from irradiating oocytes with 2000r administered in concentrated form (1266r/min) or "diluted" (32r/min) in eight fractions separated by 24 minute intervals: total time three hours 52 minutes

Treatment		Days after irradiation				Total
		1.5-2	2-2.5	2.5-3	3-3.5	
Unirradiated (control)	No. eggs	124	194	351	132	801
	Percent unhatched	8.9	5.2	8.0	6.5	7.1
Concentrated	No. eggs	937	2443	5073	3666	12,119
	Percent unhatched	37.5	44.2	56.2	42.8	47.9
	Percent induced mortality (A)	32.7*	40.0	52.9	38.4	43.9
Diluted	No. eggs	753	2301	5244	3604	11,902
	Percent unhatched	19.4	25.9	38.5	27.0	30.2
	Percent induced mortality (B)	13.3	20.2	33.8	21.4	24.9
Difference in induced percent of mortality (A - B)		19.4	19.8	19.1	17.0	19.0
Intensity effect† (C)		22	25	29	22	25
C/B‡		1.65	1.24	0.86	1.05	1.00

* (37.5 - 7.1) divided by (100 - 7.1), $\times 100 = 32.7$. This method of reckoning and those described below were suggested by DR. H. J. MULLER.

† Percent of survivors of diluted treatment killed by intense treatment = $100(A - B)/(100 - B)$.

‡ Intense effect relative to dilute effect.

SKOWITZ and ABRAHAMSON 1956a). Although there is no marked trend in percent of unhatched eggs in successive groups of eggs laid by control females in both the concentrated and diluted treatment tests the percentage of egg mortality rises successively in the first three groups of eggs and declines in the fourth. In each group of eggs, moreover, the results of the concentrated treatment show a higher incidence of induced egg mortality than do the diluted treatment results. These results, then, prove that a large component of egg mortality is dependent upon the additional X-ray intensity of the more intense series.

On the hypothesis that the fraction of induced egg mortality which shows this intensity effect is due to a multihit event, as in the production of half-translocations, it is of interest to inquire whether, as found for half-translocations (MULLER and HERSKOWITZ 1954; HERSKOWITZ 1954; HERSKOWITZ and ABRAHAMSON 1956a; PARKER 1954; ABRAHAMSON unpublished), the intensity effect is less (in relation to the total induced effect), in eggs oviposited later than it is in those laid earlier. If one supposes that the X-rayed oocytes laid in different periods subsequent to irradiation possess different susceptibilities to egg mortality that are due in part at least to some intensity-independent effect of the irradiation, then the greater this effect is the smaller will be the intensity-dependent difference observed. The last row of table 1 which gives the intensity effect relative to the total induced effect indicates that where the total effect is greatest, in the third half-day, the intensity effect is relatively the least. There is a suggestion that the intensity effect may become greater again after this period. Only one more half-day was studied in Experiment I, however, and one can hardly determine from this experiment whether the relative

intensity effect for egg mortality changes in the course of time as it does for half-translocations.

To obtain more definite information on these matters, Experiment II was performed. In this experiment 3000r were delivered either continuously at about 1266r/min (requiring only two hours and 30 minutes for delivery) or at 32r/min in 16 equal parts with ten minute intervals between successive six minute irradiations (taking four hours, six minutes to deliver). After irradiation, females and Basc males were placed directly in the egg-laying units and the transfer procedure as described was carried out. From 36 females in the diluted treatment and 38 in the concentrated there were 7631 and 8431 eggs oviposited, respectively, in the seven-day period of egg laying studied. The similarity in number of eggs laid per treated female, 212 and 220 respectively, supports a conclusion from Experiment I data that the difference in the way dosage was delivered did not appreciably influence the number of eggs oviposited. However, while the 2000r treatments in Experiment I did not result in any observable decrease in number of eggs laid as compared with the control, there seems to be a considerable decrease in number of eggs laid per female given 3000r treatment in Experiment II as compared with the 297 eggs per female in the control (5657 eggs laid by 19 females).

As was found in Experiment I the induced egg mortality in successive periods of egg laying tended first to rise and then to fall in both the concentrated and diluted treatment series but not in the control (table 2). Although in Experiment I the highest induced mortality was reached 2.5–3 days after irradiation and in Experiment II on 1.5–2 days after irradiation (a difference which could be due to factors varying in the two experiments, like the fact the parents came from different samples, the dose delivered was different, the handling of the females once irradiated differed, and especially the earlier start of intensive laying following irradiation) the concentrated and diluted series within an experiment reached this peak mortality at the same time. Also in Experiment II as in Experiment I the induced egg mortality in different periods of egg laying was always greater in the concentrated treatment group than it was in corresponding periods in the diluted treatment series. Moreover, this peak in both experiments was reached in the third half-day after intensive egg laying had begun.

Unlike Experiment I, in which the absolute difference between the concentrated and diluted treatments in induced percent of mortality in the third egg-laying period was not markedly different from earlier and later periods, in Experiment II (table 2) the third egg-laying period (1.5–2 days) shows a difference of only 7.8 percent whereas several of the periods before and after have values ranging from 18.0 to 28.2 percent. Since this seems to be correlated with the fact that the induced mortality in the diluted treatment is highest in that period, it is suggested that the results reflect a situation like that hypothesized in the preceding paragraph, i.e., that a relatively large portion of the additional killing at this period is not dependent on intensity. Thus, the intensity effect relative to the effect of the diluted treatment is lowest at the same time the diluted treatment gave most effect, namely, in the third period of egg laying in both Experiments I and II (last row of tables 1 and 2). Experiment II even suggests that in this period the intensity-dependent component *may* be ab-

TABLE 2

Experiment II. Egg mortalities obtained following irradiation of females with 3000r administered in concentrated form (1266r/min) or "diluted" (32r/min in 16 fractions separated by ten minute intervals: total time four hours six minutes)

Treatment		Days after irradiation													
		0.5-1	1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5	5-5.5	5.5-6	6-6.5	6.5-7	7-7.5
Unirradiated (control)	No. eggs	256	481	313	526	406	603	353	489	324	257	481	282	483	403
	Percent unhatched*	7.0	6.3	7.7	6.8	5.4	3.0	6.2	5.7	3.7	8.2	9.2	7.8	9.2	9.2
Concentrated	No. eggs	406	487	572	960	770	588	486	995	838	571	744	216	342	456
	Percent unhatched	62.5	67.5	82.3	70.2	55.5	47.0	43.4	37.3	36.2	31.8	30.1	28.2	32.4	29.6
	Percent induced mortality (A)	59.8	65.1	81.9	68.0	52.3	43.1	39.3	32.7	31.6	26.8	25.0	23.0	27.5	24.5
Diluted	No. eggs	58	134	351	783	738	720	503	715	648	404	770	518	699	590
	Percent unhatched	36.2	50.7	75.0	50.5	36.4	26.7	20.6	15.6	18.1	13.1	13.1	9.7	17.9	17.5
	Percent induced mortality (B)	31.6	47.1	73.2	46.9	31.8	21.4	14.8	9.4	12.2	6.8	6.8	3.1	11.9	11.5
Difference in induced percent of mortality (A - B)		28.2	18.0	7.8	21.1	20.5	21.7	24.5	23.3	19.5	20.0	18.2	19.9	15.6	13.0
Intensity effect† (C)		41	34	29	38	30	28	29	26	22	21	20	21	18	15
C/B‡		1.30	0.72	0.40	0.83	0.94	1.33	1.96	2.76	1.80	3.09	2.94	6.78	1.51	1.30

* Average value is 6.8 percent.

† $100(A - B)/(100 - B)$.

‡ Intense effect relative to dilute effect.

solutely, not merely relatively, smaller than in the periods before it and immediately after it (see next to last row of table 2) but this is not borne out by Experiment I.

It is clear, moreover, that in Experiment II less absolute intensity effect was found in the eggs laid in the last seven intervals studied than in the first seven (next to last line of table 2). The earliest period studied has the largest intensity effect while the last one studied has the smallest (and the first seven periods studied have greater intensity effects than the last seven periods in all cases). Thus just as for half-translocations the absolute intensity effect on egg mortality has been found to decline in later laid eggs. It is not evident, however, that the relative intensity-effect declines after the first four half-day periods; it seems instead to be higher.

In the case of half-translocations (HERSKOWITZ and ABRAHAMSON 1956a), the results indicated a higher relative intensity effect in eggs laid the first day after irradiation than in eggs laid the next three days. The report on half-translocations showed a much reduced absolute frequency of these changes following a continuous protracted as compared with a continuous concentrated treatment given at 1100r/min. Thus these results are similar to those obtained in the study of egg mortality. When, however, in another half-translocation experiment the protracted treatment was given in discontinuous irradiations with the total period of treatment, including

the intervals between exposures, equalling the time (about 30 minutes) required for the continuous protracted treatment in the former experiment, the paradoxical result was found that there was no detectable difference from the continuous concentrated treatment given at 1100r/min! Since the dosage used in the former half-translocation experiment (1000r) was only about one half that in the latter (2200r), the lack of detectable effect of dilution in the latter experiment may have been due either to the higher dose or the higher dose-rate used. However, in the latter experiment, when the intervals of nontreatment were extended there was a significant decrease in half-translocation rate in the most protracted as compared with the concentrated treatments. Whatever the causes of these results may turn out to be, it seemed reasonable to assume that if the intensity-dependent fraction of induced egg mortality results from multihit events, then by using the same intensity as in Experiment I's concentrated treatment (1266r/min) and both raising the dosage above 2000r and delivering the X-rays with varying periods of interruption, one might look for effects (within such an experiment and between it and Experiment I) similar to those found in the two half-translocation experiments mentioned.

Accordingly, in Experiment III (table 3) 2500r were delivered at 1266r/min in eight 15-second irradiations with nontreatment intervals of 0, 4, 16, or 32 minutes between, so that the time required for the respective treatments was two minutes, 30 minutes, one hour 54 minutes, and three hours 46 minutes. In this experiment the females were 14 days old when irradiated (about nine days older than the females

TABLE 3

Experiment III. Egg mortalities obtained after treatment with about 2500r delivered at 1266r/min in eight equal periods with the various time intervals indicated between successive irradiations

Minutes between irradiations	Total treatment time		Days after irradiation							Total
			1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	
Unirradiated (control)	0	No. eggs	73	76	275	306	299	347	113	1489
		Percent unhatched (A)	4.1	3.9	12.7	13.7	12.7	4.6	9.7	9.9
	0	No. eggs	203	166	483	500	533	550	321	2756
		A	54.1	37.2	68.0	69.8	57.2	36.6	28.3	52.5
		Percent induced mortality (B)	49.1	30.3	64.5	66.5	52.5	29.6	20.4	47.3 ± 0.95
4	30 min	No. eggs	216	125	417	456	576	663	301	2754
		A	52.7	39.2	57.4	68.2	58.8	37.7	28.6	50.5
		B	47.5	32.5	52.7	64.7	54.3	30.9	20.8	45.1 ± 0.95
		4B - OB	-1.6	+2.2	-11.8	-1.8	+1.8	-1.3	+0.4	-2.2
		No. eggs	124	100	344	347	410	408	131	1864
16	1 hr 54 min	A	17.7	20.0	56.1	63.4	46.3	30.2	28.2	43.2
		B	8.7	11.2	51.3	59.4	40.4	22.6	20.3	37.0 ± 1.1
		16B - OB	-40.4	-19.1	-13.2	-7.1	-12.1	-7.0	-0.1	-10.3
		Intensity effect* (C)	44	21	27	17	20	9	0	16
		C/16B	5.06	1.88	0.53	0.29	0.50	0.40	0	0.43
32	3 hr 46 min	No. eggs	118	58	236	280	391	402	223	1708
		A	39.8	22.4	62.0	61.0	43.6	26.4	29.6	42.2
		B	33.2	13.9	57.8	56.7	37.4	18.3	21.9	35.9 ± 1.2
		32B - OB	-15.9	-16.4	-6.7	-9.8	-15.1	-11.3	+1.5	-11.4
		Intensity effect† (C)	24	19	16	23	24	14	—	32
		C/32B	0.72	1.37	0.28	0.41	0.64	0.77	—	0.89

* $100(OB - 16B)/(100 - 16B)$.

† $100(OB - 32B)/(100 - 32B)$.

used in other experiments); they were mated to an excess of Oregon-R, wild type males for 24 hours, the males were then discarded and the females were placed in the egg-laying cylinders. For the control, and 0, 4, 16, and 32 minute intervals, 19, 38, 36, 28, and 19 females were tested and an average of 78, 72, 76, 67, and 90 eggs were laid per female, respectively. As in Experiment I, there does not seem to be any clear effect of the irradiation on the number of eggs laid per female.

The induced egg mortalities in different groups of eggs for the 32-minute intervals were lower than the corresponding groups for 0-minute intervals in seven of eight comparisons, and for the 16-minute intervals induced rates were lower than the corresponding 0-minute intervals in all eight comparisons. The overall induced egg mortalities for the 32-minute and the 16-minute intervals were also significantly different from the overall rate for the 0-minute intervals. The results for the 32-minute and 16-minute intervals were not significantly different from each other, although the overall intensity effect was greater in the former than the latter protracted treatment. Similar to the results with half-translocations is the fact that the intensity-dependent difference of 11.8 percent between the 0-minute and 32-minute intervals (in the period 1-3.5 days) was less than the 19 percent obtained in Experiment I, even though the duration of the protracted treatments was nearly the same in the two experiments and the latter involved a lower dosage of X-rays. The doses in Experiment III were delivered in the same way as in the half-translocation work (Experiment IV of HERSKOWITZ and ABRAHAMSON 1956a), the only difference being that the dosimeter reading was 1266r/min whereas it earlier had read 1100r/min when placed the same distance from the target with the machine's controls set the same way. In the comparable half-translocation experiment there was no detectable difference between the treatments with 0-minute intervals and 4-minute intervals. Similarly, for Experiment III, in three of eight comparable periods the induced egg mortality values for the 4-minute treatment group were higher than for the zero. Also, the overall rate for the four minute intervals is not significantly less than the zero minute. It may be concluded, then, that as with half-translocations, there is an intensity-dependent fraction of induced egg mortality which becomes smaller when irradiation treatment is both increased and delivered more intensely, even though the time for the total irradiation is kept the same, and which is even smaller when irradiation is given in several short intensive parts spaced over 30 minutes instead of being delivered intensively in two minutes.

Experiment IV (table 4) was similar in type to Experiment III. The dose was 3800r delivered at 1266r/min in twelve 15-second irradiations separated by 0, 4, 16, or 32 minutes (requiring three minutes, 47 minutes, two hours 59 minutes, and five hours 55 minutes, respectively, to complete the treatments). The number of females studied was 29, 35, 68, 47, respectively, and nine in the control, and the procedure for mating and egg laying were the same as in Experiment II. The number of eggs laid per female was, for the respective groups, 58, 113, 110, 122, and 101. In Experiment IV as in Experiment II, the average number of eggs laid by females receiving the most concentrated irradiation was less than the average produced by the control females. Following both the treatments given with 32-minute and 16-minute intervals each half-day egg mortality rate was lower than the corresponding rate

TABLE 4

Experiment IV. Egg mortalities obtained after treatment with 3800r delivered at 1266r/min in 12 equal periods with the various time intervals indicated between successive irradiations

Minutes between irradiations	Total treatment time		Days after irradiation								Total
			1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5	
Unirradiated (control)	0	No. eggs	72	130	91	130	95	133	97	130	912
		Percent unhatched (A)	6.9	17.7	4.4	11.5	5.3	11.3	5.2	10.0	9.3
0	3 min	No. eggs	71	245	102	178	330	283	195	266	1670
		A	64.8	70.7	82.3	86.5	73.0	56.2	50.7	54.0	65.7
		Percent induced mortality (B)	61.3	67.8	80.5	85.1	70.3	51.8	45.7	49.3	62.2 ± 1.2
4	47 min	No. eggs	220	512	389	719	622	513	313	685	3973
		A	52.3	75.0	91.8	75.4	58.5	47.6	39.9	39.1	60.4
		B	47.4	72.5	91.0	72.8	54.3	42.2	33.8	31.8	56.4 ± 0.8
		4B - OB	-13.9	+4.7	+10.5	-12.3	-16.0	-9.6	-11.9	-17.5	-5.8
16	2 hr	No. eggs	454	712	1062	1401	1328	848	663	1045	7513
	59 min	A	41.0	57.0	76.6	61.8	42.9	33.8	28.1	29.7	48.3
		B	34.9	52.6	74.3	57.9	37.1	27.0	20.7	22.5	43.0 ± 0.6
		16B - OB	-26.4	-15.2	-6.2	-27.2	-33.2	-24.8	-25.0	-26.8	-19.2
		Intensity effect* (C)	41	32	24	65	53	34	32	35	34
32	5 hr	C/16B	1.18	0.61	0.32	1.14	1.43	1.26	1.60	1.55	0.79
	55 min	No. eggs	470	582	813	924	859	595	482	1000	5725
		A	37.9	53.6	75.4	58.4	42.3	31.4	28.2	25.8	45.3
		B	31.6	48.9	72.9	54.2	36.4	24.4	20.8	18.2	39.7 ± 0.6
		32B - OB	-29.7	-18.9	-7.6	-30.9	-33.9	-27.4	-24.9	-31.1	-22.5
		Intensity effect† (C)	43	37	28	68	53	36	31	38	37
		C/32B	1.36	0.71	0.38	1.25	1.45	1.48	1.49	2.09	0.93

* $100(OB - 16B)/(100 - 16B)$.

† $100(OB - 32B)/(100 - 32B)$.

after treatment with 0-minute intervals (table 4). Although the 4-minute intervals had two of eight half-day values higher than those for the corresponding 0-minute intervals, nevertheless the overall rate for the former is significantly lower than for the latter ($P < .01$). Again, there was no significant difference between the results for 16-minute and 32-minute intervals, although the intensity effect of the latter was larger. While it is permissible to compare those results of Experiments III and I for which the duration of protracted treatments was almost identical, it is somewhat misleading to compare Experiment IV with either I or II in this manner, since the duration of the diluted treatments in IV is quite different from those in I and II.

The results for III and IV agree in that the amount of intensity-dependent egg mortality increases with the protraction of the treatments up to that of 16 minute intervals, and is comparatively small when the treatment extends over less than an hour. It should also be noted that, as in Experiment II, the two most protracted irradiations of both Experiments III and IV have peak periods of egg mortality with a somewhat smaller absolute intensity effect than do the periods just before and after the period of highest egg mortality, and they have (if the last period studied in III is discounted), as in Experiments I and II, by far the smallest intensity effect relative to the dilute effect of any of the periods studied.

TABLE 5

Experiment V. Egg mortalities obtained after treatment with 600r and 2400r delivered continuously at 1266r/min or interruptedly with 36 minutes between each irradiation at 32r/min in 18 one-minute and 18 four-minute irradiations, respectively

Treatment		Days after irradiation						Total
		1-1.5	1.5-2	2-2.5	2.5-3	3-3.5	3.5-4	
Unirradiated (control)	No. eggs	195	180	696	370	814	464	2719
	Percent unhatched	3.6	4.4	7.8	5.1	4.2	5.8	5.5
600r Intense (I)	No. eggs	539	675	1780	1502	2255	1407	8158
	Percent unhatched (A)	28.0	12.6	16.8	11.1	9.5	10.0	13.0
	Percent induced mortality (B)	23.5	7.5	12.0	5.9	4.2	4.8	7.9 $\pm$ 0.3
Dilute (D)	No. eggs	1004	765	2076	1610	2147	1548	9150
	A	9.9	10.5	14.6	10.4	7.9	8.4	10.3
	B	4.7	5.3	9.6	5.2	2.5	3.1	5.1 $\pm$ 0.2
	600 DB - 600 IB	-18.8	-2.2	-2.4	-0.7	-1.7	-1.7	-2.8
	Intensity effect* (C)	19.7	2.3	2.7	0.7	1.7	1.8	3.0
	C/600 DB	4.20	0.43	0.28	0.13	0.68	0.58	0.59
2400r Intense (I)	No. eggs	1018	564	2744	2141	3686	1596	11,749
	A	51.2	45.4	73.8	66.1	44.4	36.2	55.3
	B	48.4	42.2	72.3	64.3	41.2	32.5	52.7 $\pm$ 0.2
Dilute (D)	No. eggs	884	434	2561	1646	2463	1233	9221
	A	21.9	31.8	64.6	38.2	21.1	20.2	36.8
	B	17.5	28.0	62.5	34.6	16.5	15.6	33.2 $\pm$ 0.5
	2400 DB - 2400 IB	-30.9	-14.2	-9.8	-30.8	-24.7	-16.9	-19.5
	Intensity effect† (C)	38	20	25	47	30	20	29
	C/2400 DB	2.16	0.71	0.40	1.36	1.82	1.28	0.87

* $100(600 \text{ IB} - 600 \text{ DB}) / (100 - 600 \text{ DB})$.

† $100(2400 \text{ IB} - 2400 \text{ DB}) / (100 - 2400 \text{ DB})$.

If the part of egg mortality which is intensity-dependent depends on multihit events, then, as was previously found for half-translocations, one might expect this fraction to increase with dose at a rate faster than linearity. To test this, Experiment V (table 5) was performed in which 600r and 2400r were delivered. A continuous treatment at 1266r/min was given to furnish a lower and a higher dose given intensely and a discontinuous treatment with 36 minutes between each irradiation was given at 32r/min in 18 one-minute and 18 four-minute irradiations, respectively, in order to furnish a lower and a higher dose given slowly with interruptions. The lower intense dose took about 29.5 seconds to deliver, and the higher intense one minute 58 seconds; the lower dilute dose was delivered over a period of six hours 30 minutes, the higher dilute over seven hours 24 minutes. In both dilute and intense treatments the flies were X-rayed four times as long at the higher dose than they were at the lower. The intervals between irradiations in the dilute series are the same length of time for both doses. Consequently both groups of treated females had the same length of time for "repair" of "damage". The numbers of females studied were 38 in the control, 132 in 600r intense, 133 in 2400r intense, 133 in 600r dilute, and 124 in 2400r dilute treatment series. The numbers of eggs laid per female were 71, 62, 88, 69, and 74, respectively; these values do not suggest any significant effect of a dosage of 2400r on the number of eggs laid.

In every case in table 5 the egg mortality for a given dose was found to be lower in the dilute than in the intense treatment series. It is further to be noted that although the absolute intensity effect (next to last line of table 5) was not strikingly

low at the period of peak mortality which occurred the third half-day, the relative effect is at a decided minimum then. Similar observations are found in earlier experiments.

The overall intensity-dependent difference in induced egg mortality between the two 2400r treatments was 19.5 percent and between the two 600r treatments only 2.8 percent, the former being seven times the latter. On the basis of a linear increase with dose for the intensity-dependent fraction of egg mortality one would have expected a four-fold increase. Actually the increase might be expected to be less than four-fold if there is an intensity-independent fraction which at the higher dose is so large that it reduces the observed difference between the intense and dilute treatments by operating in the manner already described. The fact that the observed increase was seven-fold definitely places it above expectation on the linearity hypothesis. This is in agreement with the inference that much if not all of the intensity effect noted in Experiment V results from multiple X-ray "hits".

GENERAL DISCUSSION

The experiments performed furnish information concerning the effect of intensity and dosage of X-rays on rate of oviposition (table 6). In none of the tests in which irradiations of 2500r or less were delivered was there any sure change in the oviposition rate regardless of the intensity with which the dosage was delivered. The females laid between 84 percent and 124 percent as many eggs as did the control females. When 3000r and 3800r were given, however, three of the six groups of irradiated females gave only between 55 percent and 74 percent of the number of eggs produced by their respective control siblings, the other three groups having between 113

TABLE 6

Average number of eggs laid per female throughout the entire period studied and, as given in parentheses, in the period extending through the fourth day after irradiation (included to permit comparison with earlier work on half-translocations). C = Concentrated or intense treatments, D = Diluted or dispersed treatments

Experiment	Manner of treatment	Dose in r						
		0 (Control)	600	2000	2400	2500	3000	3800
I	C	44 (44)		47 (47)				
	D			45 (45)				
II	C	297 (154)					220 (112)	
	D						212 (91)	
III	D ₀ minutes = C	78 (72)				72 (64)		
	D ₄ minutes					76 (68)		
	D ₁₆ minutes					67 (62)		
	D ₃₂ minutes					90 (78)		
IV	D ₀ minutes = C	101 (75)						58 (42)
	D ₄ minutes							113 (85)
	D ₁₆ minutes							110 (86)
	D ₃₂ minutes							122 (90)
V	C	71 (71)	62 (62)		88 (88)			
	D		69 (69)		74 (74)			

percent and 120 percent the control oviposition rate. These data show that in *D. melanogaster* it is very likely that dosages lower than 2500r have a relatively small effect on oviposition and that dosages higher than 3000r decrease the number of eggs laid. Our results agree with those obtained by GOWEN and STADLER (1952) who found no effect on oviposition rate in *D. melanogaster* females treated with dosages up to 2500r but a decreased rate with higher dosages. Our results differ from those reported by ANNAN (1955) who found a decrease in oviposition rate of *D. robusta* females treated with a dose as low as 2500r. While differences between species or in dosimetry may account for this difference in results, it is likely that 2500r in *melanogaster* causes a decreased oviposition by an amount which is usually masked by the high variability in oviposition of control females.

Although there was a decrease in oviposition rate of irradiated as compared with unirradiated females in both Experiments II and IV, the results do not justify an alteration in the conclusions drawn from these experiments and those already reached from Experiments I and III which were similar in design but used lower dosages having no detectable effect on oviposition rate. Since from Experiment II it was concluded that the intensity effect was larger in eggs oviposited soon after treatment than those laid later, it became desirable to examine the data in terms of successive numbers of eggs oviposited rather than in terms of time of oviposition. This was done by rearranging the data from each female according to successive numbers of eggs oviposited as follows: The first group of eggs (labelled "1-15") contained all the data from the first egg-laying period throughout which the female was fertile up to but not including the period in which the 16th egg was laid, the second group (labelled "16-30") contained the data from the egg-laying period in which the 16th egg was laid up to but not including the one in which the 31st egg was laid, etc.; if any egg count contained so many eggs that it overlapped two groups, the data were placed in the later-oviposited group. The data for individual females used in Experiment II are summarized in table 7. (We are indebted to MISS SARAH CLEVINGER for executing these calculations.) Since in this experiment there were on the average 21 eggs laid per female per half-day in the seven days studied, the groups correspond approximately with the half-day periods, being about 25 percent smaller, so that the fourth group about corresponds with the third half-day.

We find no distinct rise in mortality after the first batch of eggs had been laid, either in the concentrated or dilute treatment series. Instead, all of the first three batches show a similarly high mortality and the mortality in succeeding batches in both series gradually falls. The absolute intensity effect (next to last line of table 7) follows a rather similar declining curve. As shown in tables 1-5, the relative intensity effect, taking the total effect at the dilute dose as the base of reference, does not show evidence of declining in the later batches but remains high in them (last line table 7). In the earlier batches the relative intensity effect is at a minimum, at the same stages as the total mortality is at a maximum, as shown in the other tables in which the data were arranged according to time. However, in table 7 where the data show that this mortality maximum is spread out over the first three batches rather than being concentrated in the third one the minimum of the relative intensity effect is similarly spread out and shows a slight tendency to be more extreme at only one

TABLE 7

Data from Experiment II, table 2, arranged in successive groups of eggs laid by individual females. See text for meaning of the groups and how these were determined

Treatment			Successive egg groups											
			1-15	16-30	31-60	61-90	91-120	121-150	151-180	181-210	211-240	241-270	271-300	>300
Unirradiated (control)	No. eggs	un-	19	296	601	464	683	503	527	540	404	506	392	722
	Percent hatched		10.3	9.5	5.7	6.7	4.0	7.2	5.1	6.5	8.7	8.5	5.2	4.6
Concentrated	No. eggs	un-	204	536	1146	1129	1150	968	1221	767	629	372	154	155
	Percent hatched		70.6	69.6	69.1	61.8	51.7	41.9	34.0	33.5	28.9	26.6	18.2	17.9
	Percent induced mortality (A)		69.0	67.0	67.0	59.0	48.2	37.7	29.2	28.7	23.7	21.2	12.2	11.9
Diluted	No. eggs	un-	224	483	1012	979	1172	834	847	654	415	466	243	302
	Percent hatched		52.2	54.2	48.5	32.6	23.5	15.7	14.5	13.9	12.5	14.4	6.6	9.5
	Percent induced mortality (B)		48.7	50.9	44.7	27.7	17.9	9.6	8.3	7.6	6.1	8.2	0.0	2.9
Difference in induced percent of mortality (A - B)			20.3	16.1	22.3	31.3	30.3	28.1	20.9	21.1	17.6	13.0	12.2	9.0
Intensity effect* (C)			40	33	40	43	37	31	23	23	19	14	12	9
C/B			0.82	0.65	0.91	1.55	2.06	3.23	2.77	3.02	3.12	1.71	>3.	3.10

* $100(A - B)/(100 - B)$.

point. This point lies in the second batch of eggs, corresponding in this case more nearly to the third half-day because of the very small number of eggs laid at first. It therefore becomes less certain that there is a sharp maximum of susceptibility to the production of intensity-independent damage to egg viability, lying at some stage separated from that of the first eggs laid. But it becomes more certain that the eggs of the earlier laid groups have, in general, a much higher susceptibility to damage of this kind than do those laid later, and that much of the excess of egg mortality at these more sensitive stages is caused by these intensity-independent events. It is the remainder—the intensity-dependent events—which correspond more nearly with the half-translocations that we have studied previously. That there is a maximum susceptibility to these events also in the earlier stages has been shown by the higher absolute intensity effect (next to last line in table 7) occurring in the earlier batches.

The present results and others mentioned for *D. melanogaster* make it clear that the investigations on the effect of X-ray delivery rate on the frequency of half-translocations (HERSKOWITZ and ABRAHAMSON 1956a) were uncomplicated by induced variations in oviposition rate, since the highest dose used in that work was 2200r. Since the study of the relation between half-translocation frequency and dosage (HERSKOWITZ 1954) used 4000r as the higher dose it is likely that the oviposition rate was lower for this treatment than for the lower dose treatment of 1000r. Since the frequency of half-translocations decreased in successive groups of eggs laid, if at the higher dose the same eggs were oviposited as at the lower one but at a slower rate, then the frequency observed at the higher dose may have tended to be biased as an

overestimate (see also HERSKOWITZ and ABRAHAMSON 1956a). However, other factors which tended to make the higher dose value an underestimate have been discussed (HERSKOWITZ 1954). These are in our opinion sufficient to counterbalance the effect just discussed and thus to allow the conclusions to be valid. Finally, it has been shown (ABRAHAMSON 1956) that for the doses 500r and 2000r (covering almost the same dose range as in the present Experiment V) in which the effect of radiation on egg deposition is negligible, half-translocations rose in frequency as the $3/2$ power of the dose.

The experiments presented all agree that a large fraction of eggs which will survive diluted treatments with X-rays will perish if the same dosage is given in a concentrated manner. This supports the view that much egg mortality is caused by multiple X-ray "hits". While there is no basis for concluding that *different* types of intensity-dependent events have been studied in the different experiments, there is also no proof that the *same* fraction was always studied, although economy of hypothesis requires this last view to be regarded as reasonable.

It had been the plan almost from the start of these investigations to see how much the results obtained in studies of the intensity-dependent fraction of egg mortality are in parallel with the results obtained with half-translocations. In all of the tests made there has been some similarity: 1. Intensity effects were obtained for both egg mortality and half-translocations when similar intensity changes were used over similar dose ranges. 2. The absolute intensity effect for both was greater in eggs laid the first four days after treatment than in eggs laid later. 3. The absolute intensity effect obtained for both events was greater when at the same time the dose was lowered and delivered less intensely, even though the time to complete the protracted irradiation was kept the same. 4. In experiments performed in the same way, in which the intervals between successive partial intensely-administered irradiations were varied but the total dose delivered remained constant, both types of effect varied according to the same pattern; namely, while 30-minute long treatments gave results which were not statistically different from results obtained after use of more concentrated treatments, the frequencies from two other still more protracted treatments, of the order of two to four hours or three to six hours, were significantly lower yet not significantly different from each other. 5. A similarity in the dependence of half-translocation frequency and the intensity-dependent fraction of egg mortality on X-ray dose was also found, in that both increased with dose at a rate greater than expected on a one-event hypothesis for their origin.

Because of these parallelisms it is concluded that almost all, if not all, of the intensity-dependent egg mortality represented results of multiple "hits" by X-rays. But however likely it may seem, there is no direct proof in the present results that any of the intensity-dependent egg mortality effect has a genetic basis. It might be assumed, without refutation being possible, that what has been studied is some nongenetic multihit event (such as one producing cytoplasmic damage) the behavior of which happens to parallel the multihit genetic event of half-translocation. It is nevertheless permissible to propose the hypothesis that part of the intensity effect studied in the present work has a genetic basis in multiple chromosome breaks, as does half-translocation. This is supported by the fact that there is very good reason

for believing, from the effect of ploidy on mortality (WHITING 1955) and the lower LD₅₀ for X-rays when the nucleus rather than the cytosome is irradiated (ULRICH 1955), that genetic rather than cytoplasmic events are responsible for the great majority of egg deaths after oocytes are irradiated. Also supporting this opinion is SONNENBLICK's (1940) finding that in some *Drosophila* eggs which fail to develop after X-radiation there are chromosomal abnormalities which would be likely to cause death and WHITING's (1948, 1955) similar observations following irradiation of *Habrobracon* eggs at metaphase I.

Since it is extremely likely that egg mortality produced by X-raying sperm is largely due to genetic change in sperm-contributed chromosomes (MULLER 1940), it would seem reasonable to assume that such changes are to some extent capable of being induced by X-rays, with the same effect, in the chromosomes of oocytes. Such dominant lethal mutations would undoubtedly be composed not only of genetic changes requiring two or more but also of those produced by single "hits". Since, in the present studies, single-hit dominant lethal mutations would not be expected to show an intensity effect (unless additional special assumptions were made), deaths from this origin would be therefore included among the induced egg mortalities, especially with the more dilute treatments.

One can state that the intensity effect can kill 22.5 percent of all eggs laid and comprises 36 percent (22.5/62.2, in Experiment IV, table 4) of the induced egg mortality at a dose of 3800r delivered at 1266r/min. These are minimal values since even in the most diluted treatments some multihit events would occur.

Finally, it should be mentioned that if the intensity effects are due to multibreak events it would be desirable to obtain evidence concerning how much egg mortality could be due to eucentric half-translocations which killed because of aneuploidy, aneucentric full or half-translocations, or some multibreak event within a chromosome or tetrad. Experiments to test some of these possibilities have been completed and preliminary reports have been published (HERSKOWITZ and SCHALET 1956; HERSKOWITZ and ABRAHAMSON 1956b).

SUMMARY

A series of experiments similar to those performed to study the effect of X-ray intensity and dose on the frequency of half-translocations induced in oocytes was carried out with the purpose of studying egg mortality, as determined by the failure of eggs to hatch into larvae, and oviposition rate. As previously reported for *D. melanogaster*, it was found that doses up to 2500r produced no grossly observable reduction in the number of eggs laid, while higher doses did. The results also prove that there is an intensity-dependent fraction of induced egg mortality which can, under certain conditions of irradiation, result in the death of at least 22.5 percent of all eggs oviposited during the first five days after treatment. Parallelism was found between half-translocations and the intensity-dependent component of egg mortality in all comparisons of behavior which were made, comprising evidence that the latter is largely if not entirely the result of multiple X-ray-induced events. The hypothesis is advanced that part, at least, of the intensity-dependent fraction of egg mortality represents dominant lethal mutations which have their basis in two or more independently produced breaks in the maternal chromosomes.

In addition to the intensity-dependent fraction of egg mortality there is an intensity-independent (or relatively independent) fraction. This intensity-independent fraction is relatively very large in the eggs laid during the first two egg-laying days after irradiation (when the intensity-dependent fraction also is at a maximum but at a lower one) and it may have a rather sharp peak at a stage slightly separated from that of the eggs that were most mature at the time of irradiation.

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