DEVELOPMENTAL EFFECTS OF X-RAY INDUCED EUPLOID AND
NEAR-EUPLOID MUTANTS IN HETEROZYGOUS CONDITION
IN *DROSOPHILA MELANOGASTER*. I. DELAY IN EGG
HATCHING AND LARVAL DELAY AND DEATH
PRIOR TO PUPATION¹

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TYPICALLY, mutant genes in homozygous condition are deleterious. The usual effect of mutant genes in heterozygous condition, however, is still a matter of controversy. The most convincing evidence for the position which holds that heterozygosity is usually harmful, is derived from work on lethals in heterozygous condition in *Drosophila* (STERN, CARSON, KINST, NOVITSKI and UPHOFF 1952; MULLER and CAMPBELL 1950 unpublished communication; others, and more recently, HIRAIZUMI and CROW 1960). These studies were concerned with lethality before eclosion, longevity and fertility. Further evidence for this view is also available from studies with yeast (JAMES 1960).

To date, the largest detrimental effect of heterozygous mutants of any kind, excluding egg lethals, was reported in studies on length of larval period (HERSKOWITZ and BAUMILLER 1959). Other investigations have shown a detrimental effect of such mutants during the egg stage (for preliminary results see BAUMILLER and HERSKOWITZ 1959, 1960).

The present paper reports extensive experiments undertaken to determine the effect on time of egg hatching of individuals carrying X-ray induced euploid and near-euploid mutants in heterozygous condition. In addition, those individuals hatching from the egg at the most normal time in both the treated and control groups were compared with regard to their time of pupation and ability to pupate. Late hatching individuals were also studied on the same basis.

MATERIALS AND METHODS

An Oregon-R, wild-type strain of *Drosophila melanogaster*, freed of spontaneously occurring, typical X-linked lethals by selecting as progenitors only females whose progeny were in approximately a 1:1 ratio as male:female, was

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used in these experiments. Four-day-old, virgin females were mated to 2-4 day-old males who had or had not received 3000r of X rays (almost all, if not all, of the males in each experiment were also virgins). After a two-day mating interval the flies were transferred to net-enclosed glass cylinders, about 2,000 females per cylinder. Cylinders were then placed on petri dishes containing a yeasted, sugar-carragar, tegosept-M medium (one percent sodium propionate was substituted for tegosept-M in later experiments). Eggs were collected over half-hour periods.

In the earlier experiments (Table 1) which tested time of hatching, the egg collecting dishes were incubated for 20 hours and then all larvae were removed and scored. This procedure was continued each hour until hour 30, and again on the following day, when about 45 hours had elapsed since egg collection. Once a regular curve of egg hatching in the control (C) and treated (I) group became evident, this method was modified. In the new procedure, used in later experiments, larval counts were made each hour from hour 20 until one hour after the greatest number had hatched in the control group. (The hour during which the greatest number hatch in the control is termed hour "R".) Those hatching more than one hour after R were not recorded until the following day, i.e., about 45 hours after egg collection. (The interval from hour R + 1 until hour 45 = "S".)

An experiment was designed to test time of pupation and ability to pupate of individuals of the C or I group which had hatched during either the R or S interval (Table 4). Eggs laid during one-half hour periods were collected for ten hours, about 200 plates, 100 C and 100 I, being accumulated. Six dishes (3 C and 3 I) to be counted by the method already described were selected from a four-hour period, located about midway in the ten-hour collecting period. C and I larvae (male and female, at random) were collected during R hour (rather than being discarded as in the previous experiments) and were separately placed in

TABLE 1

*Hourly egg hatching rates of F_1 larvae arising from P_1 males who had (I) or had not (C) been treated with 3000r of X rays**

Hour	No. hatching/total hatching Percent C Percent I		P
21	1.15	2.30	0.10
22	2.75	2.43	0.53
23	17.87	17.03	0.85
24†	43.68	26.89	<0.0001
25	23.83	27.70	0.08
26‡	5.50	11.35	<0.0001
27	3.21	6.22	0.006
28	1.72	4.19	0.0045
29	0.23	1.62	0.006
30	0.11	0.27	0.99
Total larvae	873	740	...

* Data used in Figure 1.

† Hour R.

‡ Beginning of time S.

groups of 100 into incubation chambers. These chambers consisted of one-half pint bottles which were covered on all sides with yeasted, sugar-carragar, sodium proprionate culture medium. As each incubation chamber received its complement of 100 larvae, an excess of yeast was added. Slow-hatching larvae (those which hatched during interval S) from either the C or I groups were similarly collected into incubation chambers. In order to assure a large sample of larvae of all four types (RC, RI, SC and SI), the remaining egg collecting dishes (ca. 194) were washed of hatched larvae at the proper time, i.e., before the beginning of the R or S interval, by flooding the dishes with distilled water, loosening the larvae by shaking and decanting the water and suspended larvae. These dishes were then blotted with filter paper and inspected. All larvae remaining on the plates were removed by picking, so that the dishes were larvae-free at the beginning of the R hour or S interval; such dishes could then be utilized for collecting R or S larvae, respectively. Only about ten plates in the C and I group were needed to collect R individuals; the remainder were used in the collection of S larvae. The number of pupae appearing in each incubation chamber was recorded daily from day 6 through day 11 in the experiment. On day 11 there was no evidence of viable larvae in either experiment.

The washing technique described was not used on any petri dish more than once, i.e., a plate washed to furnish R larvae was never utilized in the collection of S larvae. Other tests showed that washing eggs with distilled water at room temperature failed to alter the normal pattern of hatching as recorded at one-hour intervals.

X-radiation treatments were administered at Firmin Desloge Hospital, Department of Radiology, at room temperature, utilizing a Westinghouse Thermax tube with a tungsten target at 13.9 cm, 130 KVP, 5 MA with a 1 mm aluminum filter, the rate of delivery being 215r per minute.

The cultures were grown and the experiments carried out at a temperature of $25 \pm 2^{\circ}\text{C}$.

RESULTS

Figure 1 (data in Table 1) shows a typical example of control and experimental curves of egg hatching. Such curves can be compared in two ways. First, if the later part of the curve (for period S, which starts one hour after the hour when the greatest percentage have hatched in the control) are compared, it is found that those eggs which carry X-ray induced mutants in heterozygous condition always contain a greater percentage of late individuals than the control. This can be seen in Table 2 which has been drawn up from the results of six different experiments each containing two or more sets of plates, a set being made up of one control and one irradiated plate. The control group shows an average of 3.29 ± 0.40 percent of individuals hatching late while, on the average, the radiation heterozygotes show 9.17 ± 0.49 percent hatching late; an induced difference of 6.08 ± 0.63 percent. This is a minimal estimate of the percentage of eggs delayed in time of hatching by the presence of radiation-induced mutants in heterozygous condition.

FIGURE 1.—Hatching curve for F_1 eggs from irradiated and unirradiated P_1 males (3000r). Hour R is 24. Hour 26 and following represent time S. Differences at hours 24, 26, 27 and 29 are significant, with $P = 0.006$ to 0.0001 .

It is desirable to obtain an estimate of the total mutant effect on egg hatching. This can be done by dividing the control curve into A and B regions, the division being made in the middle of the hour when the greatest number hatch. The treated individuals are then divided into two groups C and D at the same hour as used for the control division (see Figure 1). If the treated series showed no effect, then $X = 0 : A/B = C/D$, or $D = (B/A)C = X$. However, if the effect of delay is to take a percentage of treated individuals and move them into period S (late hatching) then X will equal 5.9 percent. On the other hand, if the effect of heterozygous mutants is to move the entire irradiated population toward later hatching than normal, then $X > 5.9$ percent. It is calculated from Table 3 that, on the average, 20.09 ± 3.55 percent of the treated individuals have a demonstrably later hatching time than have individuals in the control.

Although the standard error of X is large, this is probably due principally to the method used to acquire data. Hatched larvae are counted each hour and there is no accommodation made for the fact that the actual peak of hatching may on some occasions straddle a two-hour period. Thus, at about 23.5°C the peak usually falls during the 24th hour. However, due to slight temperature, humidity or other changes, the peak may move to the latter half of the 23rd through the

TABLE 2

Delay to the S period in hatching of F₁ eggs from irradiated (I) (3000r) or unirradiated (C) P₁ males*

Experiment number	C		I		Difference (percent SI-SC)
	Total larvae hatching	Percent S*	Total larvae hatching	Percent S*	
1	1200	6.4	230	11.3	4.9
	1554	2.1	302	12.6	10.5
2	1459	1.3	279	9.2	7.9
	897	1.8	217	8.3	6.5
3	923	3.6	249	8.8	5.2
	761	3.2	357	6.2	3.0
	709	1.6	416	6.7	5.1
4	880	2.5	309	11.3	8.8
	985	3.2	352	7.4	4.2
	1085	2.9	396	8.1	5.2
5	182	4.4	190	10.0	5.6
	413	5.3	223	8.1	2.8
	582	2.0	395	8.4	6.4
6	601	3.2	302	11.6	8.4
	1359	5.8	491	9.6	3.8
	13,590	$\bar{x} = 3.29 \pm .40\dagger$	4,708	$\bar{x} = 9.17 \pm .49\dagger$	$5.88 \pm .63§$
		Induced rate‡ = $6.08 \pm .63§$			

* S = larvae hatching from two through 45 hours after the greatest quantity of C larvae had hatched.

$$\dagger \sigma_x^2 = \sqrt{\frac{\sigma_x^2}{N}}$$

$$§ \sigma_{(\text{difference})} = \sqrt{\sigma_2^2 + \sigma_1^2}$$

‡ Induced rate = (irradiated - control) / (1.0 - control)

first half of the 24th hour. The first method of interpreting data avoids this difficulty to a large degree but results in the wide variation in effect (from +40.8 to -3.7 percent) in individual experiments.

Table 4 summarizes the results of an experiment designed to test the fate of larvae, both in the control and treated series which hatched during period R, as well as the fate of larvae from late hatching eggs (period S); delay in pupation as well as larval death were scored.

The delay of egg hatching due to X-ray induced, heterozygous mutants in the experiment (Table 4) does not differ significantly from the value reported in Table 2. The induced delay in hatching was 6.1 ± 0.9 percent.

When C (control) larvae taken from R hour (the hour when the greatest percentage hatch in the control) are allowed to develop, it is found that about six percent are delayed in pupation, of which 5/6, or 82 percent died before reaching the pupal stage. On the other hand, about 15 percent of the RI larvae (irradiateds that were not delayed in the egg stage) were delayed in pupation, and of these about 77 percent failed to pupate. Thus, the induced rate of delay and of death in the larval stage, in the R individuals demonstrating no effect in the egg stage, was 9.7 ± 0.82 percent and 6.7 ± 0.7 percent, respectively. Since

TABLE 3

Delay in hatching of F_1 eggs from irradiated (I) (3000r) or unirradiated (C) P_1 males

Experiment number	C			I			$\bar{x} = \frac{x}{D-(B/A)C}$
	Total larvae hatching	A	B	Total larvae hatching	C	D	
1	1200	32.7	67.3	230	22.8	77.2	29.3
	1554	39.5	60.5	302	24.6	75.4	38.5
2	1459	56.4	43.6	279	42.9	57.2	24.0
	897	62.4	37.6	217	46.9	53.1	24.8
3	923	51.4	48.6	249	33.8	66.2	34.3
	761	54.4	45.6	357	52.3	47.7	3.9
4	709	71.4	28.6	416	56.4	44.0	21.6
	880	61.0	39.0	309	38.0	62.0	40.8
5	985	51.3	48.7	352	53.2	46.8	-3.7
	1085	57.3	42.7	396	43.2	56.8	24.7
6	413	51.5	48.5	223	50.2	49.8	2.6
	582	62.5	36.5	395	58.3	41.7	7.7
6	182	56.0	44.0	190	52.1	47.9	7.0
	601	55.1	44.9	302	39.5	60.5	28.3
	1359	51.9	48.1	491	45.2	54.8	17.5
	13,590			4,708			
							$\bar{x} = 20.09 \pm 3.55^*$

$$* \sigma_x = \sqrt{\frac{\sigma_x^2}{N}}$$

this larval effect of mutants in heterozygous condition is additional to the minimal effect on egg hatching, we may add to the minimal six percent of individuals delayed in the egg stage an additional 9.7 percent of individuals delayed in the larval stage, to give a minimal total of about 16 percent of hatching individuals demonstrating a radiation-induced effect. About 68 percent of those individuals which showed an induced delay in the larval stage, but no effect in the egg stage, die before pupation $(.067/.097) \times 100$. Whether the additional mutants detected by the larval experiments represent a separate mutant class or actually are mutants previously detected in the egg hatching experiments as analyzed by the second method (Table 3) cannot, as yet, be determined. Because of this coincidence in the size of effect (16 percent versus 20 percent) it is not at this time possible to distinguish mutants which have their effect in the egg stage from those which have their effect in the larval stage.

S larvae showed a further induced delay of 23.6 ± 0.7 percent, of which about 82 percent die prepupally $(.236/.194) \times 100$. Of all hatching individuals, only 1.3 percent show an effect in both the egg and the larval stage $(.236 \times .054 \times 100)$; 7.0 percent of all hatching individuals tested die owing to induced mutation in heterozygous condition $(6.7 + [.194 \times .054 \times 100])$.

DISCUSSION

The 20 percent of individuals in which the effect of mutants in heterozygous condition has been detected in the present experiments is the highest proportion

TABLE 4

Delay in the egg stage and delay and death in the larval stage of F_1 progeny of males who had (I) or had not (C) received 3000r of X rays and who were (S) or were not (R) delayed in hatching

Egg stage	Total larvae hatching	Percent S* $\pm \sigma_1^\dagger$	Induced rate ‡ $\pm \sigma_{(I-C)}^\S$
C	2,950	2.9 $\pm$ 0.2	6.1 $\pm$ 0.9
I	1,057	8.9 $\pm$ 0.9	
Larval stage		No. of tests	Percent pupated $\pm \sigma_{ }$
Day 6	RC	22	93.8 $\pm$ 0.7
	RI	23	84.7 $\pm$ 0.4
	SC	25	83.0 $\pm$ 0.4
	SI	24	63.4 $\pm$ 0.6
Final day	RC	22	94.9 $\pm$ 0.6
	RI	23	88.2 $\pm$ 0.4
	SC	25	88.1 $\pm$ 0.5
	SI	24	71.0 $\pm$ 0.6

* S = larvae hatching from two through 45 hours after the greatest quantity of C larvae had hatched.

$$^\dagger \sigma = \sqrt{\frac{P \times Q}{N}}$$

‡ Induced rate = (irradiated — control)/(1.0 — control)

$$^\S \sigma_{(I-C)} = \sqrt{\sigma_I^2 + \sigma_C^2}$$

$$^{||} \sigma_x^2 = \sqrt{\frac{\sigma_x^2}{N}}$$

thus far found, egg lethals excluded. This effect is attributed to euploid and near-euploid mutants, that is, to mutants unassociated with any gross change in gene quantity. This conclusion is based on the fact that gross aneuploidy for the X, second, or third chromosomes, or a major portion of any one of these, causes dominant lethality usually in the egg stage (see HERSKOWITZ and SCHALET 1957). So, the mutants which survive the egg stage are usually eutelomeric, eucentric and euploid or near-euploid. Cytologically detectable mutants of this type produced by 3000r of X rays delivered to mature sperm are sufficiently frequent to explain all the induced deleterious effect reported here (BAUER, DEMEREC and KAUFMANN 1938). In the event that the present effects result from eucentric rearrangements, then point mutation at the site of breakage and position effects would probably be the main causative factors. It may be noted, however, that effects of the same magnitude, attributable primarily to point or near-point mutants and not to gross rearrangements have been obtained in other work (BAUMILLER and HERSKOWITZ, in preparation).

A dose of 3000r of X rays to mature sperm is expected to produce about 8.7 percent recessive lethals on the X chromosome (see HERSKOWITZ 1946). Since the X chromosome makes up about one-fifth of the treated euchromatin in F_1 females, the total percentage of classical induced recessive lethals in the whole genome would be about 43.5 percent (5×8.7). On the other hand, about 34.8 percent of the F_1 males would be expected to carry such lethals (4×8.7). Of all the progeny, therefore, assuming male and female offspring to be in a 1:1 ratio, an average of 39.2 percent recessive lethals may be expected. However, recessive lethals alone cannot cause the greater portion of the effect reported here. Delay in egg hatching due to mutants in heterozygous condition was shown to be at least 6.1 percent. Unpublished experiments by Miss MARIE COYLE, testing the effect of single X-ray induced lethals on the X chromosome in heterozygous condition, show that, on the average, such a lethal causes an average of about five percent increase in individuals hatching during the S period (personal communication). Assuming that this would also be true of autosomal lethals, the total contribution of recessive lethals to delay in the egg stage would be about two percent ($.392 \times .05 \times 100$) for the experiments reported here, or about one-third of the minimal egg-hatching result obtained.

YAMADA and PUCK 1961, using human cells in culture, have shown a mitotic lag due to radiation. Although they are unable to conclude that the lag is due to chromosomal damage, nevertheless, they consider such damage to be the most likely explanation. If, as they suggest, the damage is due to some direct interference with the make up of the chromosome, and the mitotic lag is caused by the extra time needed for repair of such interference, then the egg-hatching effects reported here might have a similar basis, for such an effect would probably involve only early mitosis in the egg. However, the fact that larvae still show delay, whereas the damage in tissue culture is overcome after several cell divisions, leads one to postulate a genic rather than chromosomal explanation of the present data.

There is, as yet, no estimate of the effect of heterozygous lethals on delay in the larval stage. However, induced larval deaths which total seven percent of all larvae cannot be completely explained by their presence. STERN *et al.* 1952, estimate that the preadult mortality due to recessive lethals in heterozygous condition is on the average 4.5 percent. Even if all preadult mortality caused by recessive lethals occurred during the larval stage, only 1.8 percent of individuals would die in the present experiment ($.392 \times .045 \times 100$). Thus, about 74 percent of the mortality found must be explained by other factors.

Although the present experiments were not designed to detect mutants which speed up hatching or pupation, i.e., which are presumably heterotic, they would have been detectable had they occurred with a frequency which approached that of mutants having detrimental heterozygous effects. The highly inbred Oregon-R stock employed is in many respects homozygous (a view supported by the narrow distribution of egg-hatching times in the control, as shown in Figure 1) and in some respects heterozygous (due to spontaneous mutation). On the view that the stock is to be considered principally homozygous, the present experiments sup-

port the view that the introduction of heterozygous mutants causes a detrimental rather than a heterotic effect, the former being the expectation of the classical hypothesis while the latter is the expectation of the balance hypothesis (DOBZHANSKY 1959). On the other hand, it may be argued that the Oregon-R stock is one which normally contains appreciable, balanced heterozygosity. In this case, the present experiments demonstrate that heterozygosity for alleles of more-or-less random type, which would be the kind produced by radiation, is deleterious, that is, the results indicate that heterosis is not due to heterozygosity *per se*. It is still possible that heterosis occurs only when there is heterozygosity for particular alleles.

SUMMARY AND CONCLUSIONS

D. melanogaster males were or were not treated with 3000r of X rays, and the first released sperm were used to produce the F_1 . The F_1 that hatched from the egg comprised the population studied with respect to time of egg hatching and delay and death prior to pupation.

Results of the egg-hatching experiments were analyzed by two methods. By the first method the curves were compared with respect to only one extreme. This method showed a radiation-induced effect of about 6.1 percent more individuals delayed in time of egg hatching. The second method of analysis compared the total curves. By this analysis about 20 percent of the larvae showed a radiation-induced delay in time of egg hatching.

Larvae which by the first method were undelayed egg hatching, demonstrated approximately a 9.7 percent induced effect of delay in reaching pupation. The fact that this larval effect when added to the egg-hatching effect measured by the first method equals the total effect as determined by the second method, may or may not be accidental. Of the 9.7 percent of larvae which were late in pupation, about 69 percent fail to pupate. Among the larvae which were delayed in time of hatching, about 23.6 percent were again delayed in reaching pupation, and of these approximately 82 percent failed to pupate.

The effects of delay and death studied here are attributed to euploid and near-euploid mutants in heterozygous condition. The basis for this conclusion is discussed briefly, as is the relation of these results to the detection of a heterotic effect of X-ray induced mutants.

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