EMBRYONIC LETHALS INDUCED BY X-IRRADIATION OF FIRST MEIOTIC METAPHASE OOCYTES OF HABROBRACON¹

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INVESTIGATIONS to be reported in this paper have been concerned chiefly with X-ray-induced embryonic lethal mutations in *Habrobracon*. Some of the data and one of the methods have already been given (HEIDENTHAL 1952, 1953; HEIDENTHAL, CLARK and GOWEN 1955). The purpose of the author's work, as well as that of others, has been in part to study the frequency of different kinds of lethal-bearing genomes, which result from irradiation of oocytes in first meiotic metaphase, a cytologically uniform stage (ATWOOD, VON BORSTEL and WHITING 1956; WHITING 1938, 1941, 1945a,b). Estimates of *genome* frequency have been possible because *Habrobracon* is a naturally parthenogenetic organism. Lethal action can be studied in both haploid and diploid embryos. Evidence that this lethality is attributable to chromosomal (genetic) rather than to cytoplasmic injury, or a combination of the two, has been provided by the extensive work of WHITING on androgenesis (WHITING 1946, 1948, 1949, 1954, 1955).

The purpose of the author's work reported here is to give further data and analysis of embryonic lethals induced by X-irradiation of first meiotic metaphase oocytes.

MATERIALS AND METHODS

First method: Methods for the collection and study of eggs irradiated in first meiotic metaphase were originally developed by WHITING. Details of the method as used by the author have already been given (HEIDENTHAL, CLARK and GOWEN 1955). The main points are as follows. Virgin females can be restrained from oviposition by removing them from their hosts, the caterpillar of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, for 12 or more hours. When this is done, oocytes in first meiotic metaphase collect at the distal end of the reproductive tract, in four egg sacs. They remain in this stage until the wasps are returned to the caterpillars on which eggs can then be laid. After this, later stages of meiosis and embryonic stages progress. If females are irradiated before they are returned to the host, and if they are then allowed to lay eggs for only six hours, all eggs laid are restricted to those irradiated in first meiotic metaphase, those located most distally. The method just described was used in the study of hatchability of eggs laid by irradiated virgin mothers, and also by mothers which

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had been mated to untreated males, during the interval between exposure to radiation and their return to the caterpillars. After the six-hour egg-laying period, the cultures were incubated at 30°C for about 36 hours. Then numbers of dead eggs and larvae were counted. Females were of wild-type stock no. 33, males of another wild-type stock no. 1. When this cross is made, about two thirds of the eggs are fertilized. As diploids the eggs are prospective females. The remaining are haploid and prospective males.

The purpose of studying hatchability of eggs laid by virgin and by mated females was to make certain comparisons. The expectation was that within a sample of X-irradiated eggs there might be some which would be free of dominant lethals but which might require fertilization if they were to live to the stage of hatching of larvae. This should then lead to a higher percentage of hatchability of eggs laid by mated than by virgin mothers. The higher hatchability would simply mean that there were some eggs which had to be fertilized if they were to survive as embryos and hatch as larvae. These then might be thought of as eggs bearing lethals other than embryonic dominants.

Second method: Another method of studying lethals was to test F_1 females which had developed from irradiated first meiotic metaphase eggs for heterozygosity with respect to recessive embryonic lethals (HEIDENTHAL 1952, 1953). The technique was as follows. F_1 females which were not only the daughters of X-rayed mothers, but which had developed exclusively from oocytes irradiated in first meiotic metaphase and fertilized by normal, untreated sperm were collected as virgins. Each F_1 virgin female was then allowed to lay eggs continuously over a period of days with transfer to fresh caterpillars twice during each 24-hour period. Twice a day, at about 12-hour intervals, the F_2 eggs were counted and then incubated for 36 hours after the egg-laying period had ended. At the close of this period of development, counts giving number of larvae and number of dead eggs were made. Since the F_1 mothers were diploid, but the F_2 eggs were haploid, one might expect (1) F_2 eggs carrying one or more embryonic recessive lethals to die, and (2) segregation and independent assortment of such lethals to occur. Thus about one half of the F_2 eggs carrying embryonic lethals would be expected to die if the mother were heterozygous for one lethal; but only one fourth would live if the mother were heterozygous for two independently assorting lethals. One might also foresee greater complexity if greater numbers of lethals, linkage and interaction of lethals were involved. At relatively low dosage, when the incidence of lethals is expected to be rather low, the frequency of such complexities would not be expected to be very high. Dosage, when metaphase eggs are used, has to be kept comparatively low, so as not to exceed about 2000r, because only a very few eggs survive to produce F_1 females at this dose.

The source of the X rays used was a conventional kx-4 roentgen ray machine. Conditions were kept as constant as possible: 124 kv; 10 ma; target-sample distance 33.9 cm; 102r per minute; no filter except that inherent in the tube.

RESULTS AND DISCUSSION

First method: A summary of the data secured by the first method, which involved studies of hatchability of F_1 eggs laid by irradiated mothers, is given in Table 1. A decline in hatchability with increasing dose, as well as variability within dose, is clearly apparent. Since the hatchability percentage given for a particular dose represents more than one experiment for that dose, variability among experiments would be expected. The method of variance analysis was applied to these data in an effort to estimate components of variance pertaining to such variability. Did females lay eggs which were equally susceptible to radiation effects as measured? Were the results reasonably consistent within dose? The body of these analyses is not given, but it did indicate that the comparisons, on the whole, were not significant. It seems safe to conclude, therefore, that variability of the kinds indicated was within reasonable limits of error.

The primary purpose of collecting F_1 data was, as already indicated, to discover whether or not a significant difference in hatchability of eggs laid by virgins and by mated mothers would be found. Since each of the two sets of data looked as though it would fit a simple exponential function fairly well, simple exponential equations have been calculated for both groups of data:

For eggs laid

$$\text{by virgin females} \quad \log \text{ percent survival} = 1.987586 - .000787D \quad (1)$$

For eggs laid

$$\text{by mated mothers} \quad \log \text{ percent survival} = 1.942784 - .000583D \quad (2)$$

D indicates the dose in roentgens. A test for the significance in difference between slopes of these lines is given as follows (not weighted).

Deviations from individual slopes		Deviations between slopes	
df	M.S.	df	M.S.
14	.006346	1	.066479

TABLE 1

Summary of data on hatchability of eggs X-rayed in first meiotic metaphase

Dose	Virgin females			Mated females		
	Eggs laid	Larvae	Percent eggs hatched	Eggs laid	Larvae	Percent eggs hatched
None	1735	1606	92.6 $\pm$ 0.6	1079	995	92.2 $\pm$ 0.9
100r	1810	1407	77.7 $\pm$ 1.0	184	147	79.9 $\pm$ 2.9
200r	183	132	72.1 $\pm$ 3.3	...	...	...
250r	1417	922	65.1 $\pm$ 1.3	774	479	61.9 $\pm$ 1.8
400r	2077	1041	50.1 $\pm$ 1.1	1345	722	53.7 $\pm$ 1.4
500r	823	356	43.3 $\pm$ 1.7	452	180	39.8 $\pm$ 2.3
750r	821	211	25.7 $\pm$ 1.5	...	...	...
1000r	952	129	13.6 $\pm$ 1.2	...	...	...
1250r	429	29	6.8 $\pm$ 1.2	...	...	...
1500r	2648	156	5.9 $\pm$ 0.5	2826	261	9.2 $\pm$ 0.5
1750r	1102	64	5.8 $\pm$ 0.7	1285	135	10.5 $\pm$ 0.9

The *F* value for the comparisons is 10.5. Therefore, a highly significant difference between the slopes is indicated. The divergence appears to start at about 600r. Such a difference is consistent with the thought that there is a group of eggs within the sample of irradiated metaphase oocytes which requires fertilization, if survival through embryonic development to hatching of larvae is to occur.

The two equations given above, can be used to calculate a number of estimates. (1) The percentage of eggs which survive (*s*), because they are free of all lethals, can be calculated for any given dose by using equation (1). (2) The percentage of all irradiated eggs which require fertilization if survival to hatching of larvae were to occur (*pr*) can be expressed as $pr = 1.5 (s_1 - s)$, where *s*₁ has been calculated by using equation (2). Here it is assumed that only two thirds of the eggs were fertilized. (3) The percentage of eggs in which at least one embryonic lethal has been induced can be expressed as $d = 100\% - pr - s$, or $d = 100\% - 1.5s_1 + .5s$. Calculations for *d*, *pr*, and *s* for a number of doses, are shown in Table 2. Here one can see that, in a general way, the percentages of eggs which survive because they have no lethals (*s*) decline with rising dose and that the percentages of eggs which have at least one embryonic dominant lethal increase with dose. However, the percentages indicated by *pr* rise from 400r, and then decline at doses above 1000r. Such a decrease would be expected because at dose 2500r at which all eggs have at least one dominant lethal, there can be no class of eggs which can survive if fertilized. Values of *pr* follow a curve which rises and then falls to zero.

Values in Table 2 can also be used to calculate the percentage of diploid larvae heterozygous for lethals other than embryonic dominant lethals among all surviving diploid larvae. The percentage of such heterozygous larvae (*PR*) for a given dose is $PR = \frac{pr}{pr + s}$, or $\frac{3(s_1 - s)}{3s_1 - s}$. Estimates similar to these have been made by others (ATWOOD, VON BORSTEL and WHITING 1956). Equation (5) of their paper gives $r = \frac{m - V_u}{m - V_u(1 - f)}$, where *r* is comparable to the author's *PR*,

TABLE 2

Estimated percentages of lethal and nonlethal bearing oocytes in total sample irradiated

Dose	400r	500r	1000r	1500r	2000r
<i>s</i> *	47.1	39.3	15.9	6.4	2.6
<i>pr</i> [1.5 (<i>s</i> ₁ - <i>s</i>)]	6.2	8.3	10.5	7.9	5.1
<i>d</i> [100% - <i>pr</i> - <i>s</i>]	43.7	52.4	73.6	85.6	92.3
<i>PR</i> $\frac{3 (s_1 - s)}{3 s_1 - s}$	11.7	17.4	39.9	55.5	66.2

* *s*=percent carrying no lethals; *pr*=percent carrying lethals other than dominants; *d*=percent dominant lethals; *PR*=percentage of diploid larvae heterozygous for other than dominant lethals among all hatched diploid larvae.

m is the observed hatchability of eggs laid by mated females, V_u is the viable proportion of unfertilized eggs, and f is the proportion of eggs fertilized (assumed to be .66). In this work m and V_u represent percentages calculated directly from the observed data; but in the author's work, s_1 and s which are comparable to m and V_u , respectively, have been calculated from the two equations. However, for purposes of comparison one can substitute the author's values for s_1 and s for m and V_u in the equation $r = \frac{m - V_u}{m - V_u(1 - f)}$. Then $r = \frac{3(s_1 - s)}{3s_1 - s}$. This is the author's value for PR . It is thus apparent that the method of estimations used by the author and by ATWOOD, VON BORSTEL and WHITING are essentially the same, except for the author's use of the equations for any given dose as compared to the direct calculations for doses for which there have to be direct data. The author has no data for doses of 875r and 1100r as do ATWOOD, VON BORSTEL and WHITING. Nevertheless comparisons can be made as follows:

Dose	PR	r
500r	17.4	16.4
875r	35.1	30.0
1100r	43.4	49.4

Such comparisons seem to be well enough within the range of expected variability to warrant a conclusion of generally good confirmation.

Second method: A summary of the data secured by the second method, that of studying hatchability of F_2 eggs laid by F_1 virgin mothers, is given in Table 3 and Figure 1. These data are based on the assumption that a given mother which had developed from an irradiated oocyte might be free of lethals or heterozygous for one or more recessive lethals; or she might be heterozygous for one or more sublethals or combinations of sublethals and recessives. The main purpose was to separate the F_1 mothers which were free from recessive lethals from all others. A very high hatchability of F_2 eggs should be expected if an F_1 female were free from lethals, because both stocks used in these experiments had been strictly inbred by brother-sister matings and selected for a high hatchability of eggs, 90 or more percent. Lower hatchability would, of course, be expected if the mothers

TABLE 3
Percentages of F_1 virgin mothers heterozygous for recessive lethals

Dose	F_1 virgins tested	Author's data	Percent heterozygotes	Arwoon's data
		F_1 virgin heterozygotes	(R)	r (F_2 method)
0	468	3	0.6	...
500r	178	13	7.3	5.8
871r	...	..	...	16.2
875r	...	..	...	19.7
1000r	181	26	14.4	...
1100r	...	..	..	15.2
1100r	...	..	..	16.8
1500r	179	33	18.4	...

FIGURE 1.—Incidence of recessive lethal heterozygotes among F_1 females which developed from oocytes X-irradiated during first meiotic metaphase and fertilized soon afterward by untreated sperm.

were heterozygous for any kind of lethal(s). If, for example, only 50 percent of the eggs were to hatch, the F_1 mother would be classified as heterozygous for one recessive lethal or possibly the equivalent in sublethals. If, though, a mother were heterozygous for two independently assorting lethals, only about 25 percent of the F_2 eggs should hatch, etc.

The control data given in the histogram of Figure 1 show that 95 percent of all F_2 counts yielded a hatchability of 85 percent or more. Comparable percentages for 500r, 1000r, and 1500r are all lower: 86 percent, 78 percent, and 75 percent, respectively. Such data suggest confirmation of a rather generally accepted conclusion that ionizing radiation produces, apart from striking mutations, many other genetic changes which are slightly deleterious in their effects.

The control data also show evidence of heterozygosity for lethals in three F_1 females. In thinking about the origin of such "spontaneous" lethals in *Habrobracon*, one has always to remember that in the kind of cross used for these experiments all males are haploid. Thus there can be no males within stocks which would be heterozygous for recessive lethals. The only way a father could contribute a recessive lethal to a daughter would be by way of a mutation directly induced in the sperm which would take part in the development of the daughter. However, the mother of the F_1 female would be able to contribute lethals not only directly by mutation in the oocyte, but by inheritance from *her* mother. One consequence of this type of inheritance is that recessive lethals tend to be relatively rapidly eliminated from stocks.

The histogram of Figure 1 also shows data for 500r, 1000r, and 1500r. At the two highest doses, where the frequencies of lethals would be expected to be the highest, one can see evidence of clustering near the 50 and 25 percent values on the abscissa. If such percentages are assumed to be due exclusively either to presence of one or of two independently assorting lethals, such values should fit a Poisson distribution. On this basis the frequency of two lethals is too high when compared to that for one. No really adequate explanation for such a discrepancy is currently available. Possibly, as previously indicated, such mechanisms as interaction of sublethals with each other, or with recessives, have played a part. Such thoughts are offered only tentatively.

Table 3 shows the percentages of lethal heterozygotes (R) among F_1 adult females for controls and for the three doses used. In making the calculations for values of R , all counts which showed hatchability of 60 percent or less were somewhat arbitrarily scored as though they represented F_1 virgins heterozygous for one or more recessive lethals or the equivalent. Obviously values of R rise approximately linearly with dose. To some extent, these results can be compared to those of ATWOOD, VON BORSTEL and WHITING (1956). Their results are given in Table 3 under r . Variability obviously exists, but agreement seems to be reasonably satisfactory.

Values of R in Table 3, as already indicated, give percentages of recessive lethal bearing heterozygotes among *adult* females (diploids); values of PR , as given in Table 2, show the percentages of diploid larvae, *prospective* females, heterozygous for lethals other than dominant lethals. If the lethals among the first instar diploid larvae and among adult females were the same in *kind* as, for example, recessive lethals exclusively, then values for R and PR ought to be about the same for a given dose. This would imply that death in later larval and pupal stages, if it were to occur, would be accidental, due to environmental conditions acting at random among all larvae and pupae. Death during such stages, prior to eclosion,

would simply reduce the number of females which would hatch, regardless of the lethal bearing condition. This would mean that there would have to be no class of lethals which could act postembryonically. Comparisons of R and PR are given in Table 4. Here one can see that the values of R and PR rise approximately linearly with dose, but that for each dose there is a rather large difference (P), between R and PR . This kind of observation can be explained on the assumption that the embryonic heterozygotes are a mixed group. For a given dose, some would have to be heterozygous for the lethals which could later be detected in surviving adult females: others might be heterozygous for lethals sufficiently severe to kill diploids prior to eclosion but not before first larval instar. Thus there would be a class of lethals acting in postembryonic stages. Estimates of this latter group, comparable to P but based on direct observations of percentages of dead larvae and pupae and called D_c , have been made by Atwood, von Borstel and Whiting (1956). Values for D_c and for P are given in Table 4. The authors have called the lethals represented by D_c "conditionally delayed dominant lethals." They have concluded "that fertilization does not change the frequency of dominant and recessive lethals, but postpones the expression of some of the dominant lethals until postembryonic stages."

SUMMARY

Two methods have been used to study the frequency of various kinds of lethals induced by X-irradiation of *Habrobracon* oocytes in first meiotic metaphase. The first involved the study of hatchability of eggs laid by X-irradiated virgin females and by others which were X-rayed as virgins and then mated to untreated males prior to the egg-laying period. Doses ranged from 100r to 1750r. Simple exponential equations were calculated for both groups of data. The slopes were tested for significance of difference, and the difference was found to be highly significant ($F = 10.5$). This was interpreted to mean that within a sample of X-irradiated eggs there are some which require fertilization in order to survive to first larval instar.

TABLE 4

Frequencies of lethal bearing larvae (PR), of lethal bearing adults (R), of "postembryonic dominant lethals" (P), and of "conditionally delayed dominant lethals" (D_c) as given by Atwood, von Borstel and Whiting (1956)

Dose	PR	Author's data R	P	Atwood's data D_c
500r	17.4	7.3	10.1	11.3
871	..	..	..	19.3
875	..	..	..	12.9
1000	39.9	14.4	25.5	..
1100	..	..	..	40.3
1100	..	..	..	27.3
1500	55.5	18.4	37.1	..

The second method involved tests of F_1 virgins which had developed from X-irradiated oocytes which were later fertilized by untreated sperm. Doses used were 500r, 1000r, 1500r. The main purpose was to separate all F_1 virgins which were heterozygous for one or more recessive lethals from those which were free of lethals. This was done by securing hatchability percentages of F_2 eggs laid separately by each F_1 virgin.

Comparison and analysis of the two methods indicate that the lethals detected by the first method are a mixed group. It includes the recessive lethals which persist in heterozygous condition among the adult F_1 females; and another group which ATWOOD, VON BORSTEL and WHITING have called "conditionally delayed dominant lethal." These are lethals which express themselves when heterozygous, in larval and pupal stages. To the extent that comparisons of the work of these authors and that of this paper is possible, it has been shown that the agreement is reasonably good.

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