

# RADIATION-INDUCED AND GENETICALLY CONTRIVED DOMINANT LETHALITY IN HABROBRACON AND DROSOPHILA

R. C. VON BORSTEL AND M. L. REKEMEYER

*Biology Division, Oak Ridge National Laboratory,<sup>1</sup> Oak Ridge, Tennessee*

Received March 3, 1959

THE condition of dominant lethality is fulfilled when a haploid nucleus has been altered in such a manner that upon combination with a normal haploid nucleus the resulting heterozygote dies either immediately or eventually. Of the various mutation classes induced by physical or chemical mutagens, dominant lethality occurs with the highest frequency and is the simplest to measure.

Three different types of dominant lethality are recognizable in *Habrobracon* by phenotype (ATWOOD, VON BORSTEL, and A. R. WHITING 1956). Type I dominant lethality, which occurs in oocytes irradiated in the first meiotic metaphase or prophase, is the most predominant and is characterized by a depression of mitotic rate with complete cessation of mitosis after the second or third nuclear division. This is followed by a curious syndrome involving production of Feulgen-negative nuclei that eventually enlarge (VON BORSTEL 1955). In the present investigations we found that most of the dominant lethality induced in sperm is also of this type. Type II dominant lethality, which is inducible in metaphase I oocytes, is characterized by death after blastula formation and before hatching in the haploid (unfertilized) egg, but predominantly after hatching in the irradiated egg fertilized with normal sperm. Type III dominant lethality, which is inducible in prophase I oocytes, is characterized by death after blastoderm formation and before hatching whether or not the egg is fertilized with normal sperm.

Since progeny tests of dominant lethal heterozygotes are impossible, experiments of a reconstruction type were chosen as the simplest analytical procedure for interpreting the nature of radiation-induced dominant lethality. Since chromosome imbalance has been regarded as the main source of dominant lethality (MULLER 1954), the time and characteristics of death of aneuploids from triploids and translocation heterozygotes were compared with the dominant lethal embryos induced by radiation. Both *Habrobracon* and *Drosophila* were used in order that (1) the various types of dominant lethality could be compared from one species to another, and (2) the unique advantages of each genetic material could be used for study of different aspects of the problem.

## MATERIALS AND METHODS

The wasp *Habrobracon juglandis* is parasitic on the larva of *Ephestia kühniella*, the Mediterranean flour moth. For hatchability and adult survival studies, the

<sup>1</sup> Operated by the Union Carbide Corporation for the United States Atomic Energy Commission.

wasps were transferred twice daily to two-inch stender dishes, each with a single new unstung caterpillar. The wasps were kept in incubators maintained at the optimal temperature of 30° C. The egg number was recorded, and dislodged eggs were placed back on the paralyzed host. Thirty hours later, hatchability counts were taken and the stage of death of the dead embryos was recorded. Classification of dead embryos was greatly facilitated by prevention of shriveling through the elevation of humidity with a pan of water in the incubator. After ten days, postembryonic deaths and surviving adults were counted. *Habrobracon* stocks No. 33 virgins and 17  $\sigma^i$  males were used for the radiation experiments.

The hatchability procedure used for *Drosophila melanogaster* embryos was essentially that used by BAKER and VON HALLE (1953). Oregon-R males were irradiated and pair-mated with Oregon-R virgins. After 24 hours, the males were removed. Each female was placed on charcoal-agar medium to facilitate egg counting and, 24 hours later, was transferred to a fresh vial. The dead embryos were fixed and stained for cytological analysis with a whole-mount Feulgen procedure in which the embryos are aligned on cover slips (VON BORSTEL and LINDSLEY 1959). The procedure was used for the translocation heterozygote and triploid studies as well. Care was always taken that dead embryos studied cytologically were from vials in which the hatchability was at least the lowest expected value, to avoid, insofar as possible, selection of unfertilized eggs.

#### *Triploid females*

*Habrobracon*: In *Habrobracon*, hemizygous individuals or individuals homozygous for the same sex alleles become males; heterozygotes become females (P. W. WHITING 1943). Half the fertilized eggs from inbred stocks would normally become diploid males except that most of these individuals die just before or just after hatching. A few do become adults but are usually infertile. Occasional (about one in ten) diploid males are fertile and have diploid sperm. Daughters from the cross of a diploid male with a normal female are always triploids. The offspring from a triploid virgin female would have anywhere from the haploid number of chromosomes (10) to the diploid number (20). Aneuploid embryos from five different triploids were examined.

*Drosophila*: Triploid *Drosophila* were first described by BRIDGES (1921). Their origin is usually unknown. One probable common source is a tetraploid piece of gonial tissue that occurs in the mother and gives rise to diploid ova. Aneuploid embryos from two different spontaneous triploids crossed with Oregon-R males were examined.

#### *Translocations*

*Habrobracon*: Since translocation stocks were unavailable in *Habrobracon*, a method was devised for their detection in genetically unmarked stocks. The translocations were induced with X-rays in males and determined by testing for the resultant inherited partial sterility. Several males from each  $F_1$  female showing reduced egg viability were mated to wild type females, and the  $F_2$  females were tested for reduced hatchability. Those females showing reduced hatch-

ability are considered as being heterozygous for a translocation. (A detailed presentation of this material will be published elsewhere.) Aneuploid females from four different stocks showing inherited partial sterility were examined.

*Drosophila*: Two translocations were used in this study, T(2;3) $S^M$  and T(2;3) $Xa$ . In each case stocks bearing these translocations were crossed to Oregon-R; the daughters bearing the translocations were backcrossed to Oregon-R males, and the dead embryos collected and studied. The specific stocks used were T(2;3) $S^M$  *Cy c3G Sb Ubx/st c3G ca* and *In(3LR) Ubx<sup>130</sup>, Ubx<sup>130</sup> e<sup>s</sup>/T(2;3)Xa*.

#### *Irradiation procedure*

A G.E. Maxitron-250 unit was used to administer the X-radiation. The machine, operated at 250 kvp, 30 ma, had a tungsten target, 0.1 mm of Al inherent and 3 mm of Al added filtration (half-value layer, 0.40 mm of Cu). Dose rates of 330, 675, 1000, and 1885r/minute were used at 39, 31, 23, and 16.5 cm, respectively, in different experiments. Habrobracon eggs in the first meiotic metaphase were irradiated at the lowest rate, 330r/minute; Habrobracon sperm, 675r/minute; *Drosophila* sperm, 1000r/minute; and Habrobracon eggs in the first meiotic prophase, 1885r/minute.

#### METHODS OF ESTIMATING LETHALS

Equations for estimating the frequency of dominant and recessive lethal mutations in Habrobracon were derived by Atwood *et al.* (1956). Since these equations are more generally useful than at first indicated, and since they are used throughout this paper, some of them are listed here and their properties discussed.

The frequency of embryo dominant lethal mutations,  $D$ , that depress hatchability when sperm are irradiated can be estimated by

$$D = 1 - \frac{m - V_u(1 - f)}{f} \quad (1)$$

where  $m$  is the hatchability frequency of eggs from mated females,  $V_u$  is the surviving proportion of unfertilized eggs (estimated from unmated controls), and  $f$  is the proportion fertilized. Adult survival data can be inserted in Equation (1) to estimate  $D'$ , the frequency of total dominant lethal mutations;  $m'$  and  $V'_u$  for adults would correspond to  $m$  and  $V_u$  for embryos.

In irradiated eggs, the frequencies of fertilized eggs dying at any stage of development,  $s_f$ , were computed from the data in Table 1 by

$$s_f = \frac{s_m - s_u(1 - f)}{f} \quad (2)$$

where  $s_m$  is the proportion of embryos from mated females that die at a particular stage of development and  $s_u$  is the proportion of embryos from unmated females that die at the same developmental stage. Equation (2) is of the same form as Equation (1). When the equation is used for all stages of embryonic, larval, and pupal development, it is a step-by-step analysis in which the effect of fertilization

TABLE 1  
*Survival and stages of death of embryos after irradiation of Habrobracon gametes*

Stage irradiated and dose (r)	Viable embryos/total		Number dying at each stage of development*																	
			Embryos			Larvae						Pupae			Adults					
			1	2	3	4	5	6	7	8	9	10	11	12	13	Male	Female			
0 (875 control)	1616/1631	1	1	7	1	5	1	70	9	6	9	10	63	0	1448	0				
Sperm																				
2,000	307/549	165	2	37	20	18	9	6	5	4	2	3	6	3	157	112				
4,000	214/534	247	1	32	31	9	28	11	1	1	8	7	0	1	136	21				
4,000	190/820	467	5	14	103	41	25	10	8	4	2	5	6	2	69	59				
6,000	130/595	417	4	16	21	7	7	3	3	1	0	0	8	2	98	8				
Metaphase I eggs 875																				
Unmated	135/476	190	13	118	15	5	5	6	1	1	6	4	7	0	105	0				
Mated	244/570	209	10	68	18	21	20	15	3	3	6	10	13	0	55	119				
1,100																				
Unmated	111/750	425	22	121	56	15	9	5	3	3	9	3	4	0	75	0				
Mated	122/576	314	21	25	57	37	17	1	3	5	8	3	7	2	25	51				
Prophase I eggs 15,000																				
Unmated	211/537	236	29	25	21	15	33	7	4	4	2	0	10	1	150	0				
Mated	232/531	227	6	12	18	36	22	5	5	6	3	3	14	1	53	120				
18,000																				
Unmated	151/506	221	24	83	16	11	17	12	8	3	3	1	5	0	102	0				
Mated	229/607	250	15	39	50	24	13	21	5	2	9	1	3	0	54	121				

\* The numbers refer to stages of development; for further description, see text.

on the expression of death of irradiated eggs can be compared with that of unfertilized irradiated eggs. In all graphs describing the radiation effects, the frequency of the stages of death of fertilized eggs was computed from the data in Table 1; the negative values obtained in a few instances are caused by random fluctuations in the frequency of death at the various stages of development.

In previous publications (ATWOOD *et al.* 1956; CLARK and MITCHELL 1952; A. R. WHITING and MURPHY 1956), the value of  $f$  has been taken from controls because adults in experiments were usually not counted and  $f$  has been assumed usually to be from 0.60 to 0.67. For an absolute determination from the experiment itself, the proportion fertilized,  $f$ , is given by

$$f = \frac{q + V'_u - m'}{V'_u} \quad (3)$$

where  $q$  is the ratio of surviving adult females to total eggs (ATWOOD *et al.* 1956). Equation (3) can be restated simply as

$$f = 1 - \frac{p}{V'_u} \quad (4)$$

where  $p$  is the ratio of surviving adult males from mated females to total eggs from mated females. This formulation is valid under any condition of lethality, and is used in the data analyses throughout except for the triploid females, where a value of  $f$  of 0.67 was used because no adults survived. Equation (3) also was used to estimate  $f$  values for the irradiated sperm given in Table 1 for computing Figures 2 and 5. Except where data from unmated control females indicated a considerable discrepancy,  $V_u$  was taken to be 0.95 and  $V'_u$  was taken to be 0.90; variations from these values as much as ten percent will not have more than a four percent effect on  $f$  or  $D$ .

#### CLASSIFICATION

*Habrobracon embryos:* The stage of death can be determined with reasonable accuracy on the unfixed unstained *Habrobracon* embryo. The egg chorion is two microns thick and is transparent. The embryos are classified in five different categories, Stages 1, 2, 3, 4, and 5 (Figure 1). Stages 3 and 4 can be further subdivided, although the stages are not separated in the data reported here. The stages of death are sequential in embryogenesis, but the absolute times of death during embryogenesis have not been accurately determined.

Stage 1 is the embryos in which death occurs during karyokinesis before the blastoderm forms; the embryos have a mottled white appearance and one end, or both, is filled with a clear fluid. Stage 2 is the blastula stage up to the beginnings of tissue differentiation and is characterized by two homogeneous white bands. Embryos in Stage 3 are those in which the yolk mass is changing from white to yellow and the yolk is beginning to become more centrally located. Stage 4 is a nearly fully developed embryo and can be readily distinguished by the presence

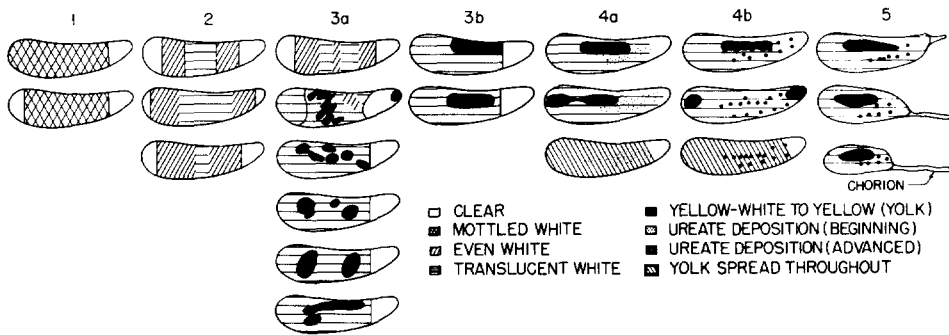


FIGURE 1.—Stages at which radiation death is expressed during the development of Habrobracon embryos.

of tracheal elements and ureate cells. Stage 5 is made up of fully developed embryos that die at any time during the hatching process. Stage 3a is distinguished from 3b by the heterogeneous distribution of the yolk in the former. Stage 4a embryos are those in which tracheal elements are just beginning to form and 4b embryos have fully formed tracheal elements and ureate cells. The gut occasionally ruptures in Stage 4 embryos, and the yolk in these may be homogeneously distributed throughout or be somewhat concentrated in the posterior end of the embryo. The embryos in Figures 3, 4, and 5 were classified in this manner.

At random intervals, the embryos were fixed and stained. For the most part, this was done for microscopic examination of embryos that had died in Stage 1 (preblastula). Five different stages of death in fixed and stained Habrobracon embryos have been described (A. R. WHITING, CASPARI, KOUKIDES, and KAO, 1958) that correspond roughly to the stages determined on the unstained embryos. Table 2 shows the same batch of irradiated dead embryos staged unstained, then fixed, stained, and staged by cytological methods. No attempt was made to

TABLE 2

*Comparison of methods for determining time of death of Habrobracon embryos irradiated as Metaphase I eggs with 1100r*

Stage of embryonic development	Eggs from virgins				Eggs from mated females			
	Unstained		Stained		Unstained		Stained	
	Number analyzed	Percent of total	Number analyzed	Percent of total	Number analyzed	Percent of total	Number analyzed	Percent of total
1	362	69.4	136	73.5	377	65.9	113	67.2
2	15	2.9	8	4.3	28	4.9	9	5.4
3	90	17.2	30	16.2	56	9.8	18	10.7
4	45	8.6	8	4.3	69	12.1	15	8.9
5	10	1.9	3	1.6	42	7.3	13	7.7
Total	522	100.0	185	99.9	572	100.0	168	99.9

keep track of the individual embryos from the unstained through the stained states, and the two analyses were done by different investigators. The good agreement demonstrates that the much faster analysis of unfixed, unstained material is reliable. In every other case in which microscopic analysis was performed, the embryos were first classified with respect to stage of death and then arranged on cover slips in a manner that allowed direct individual comparison with the stage of death as determined on the unfixed, unstained embryos. Microscopic analysis at intervals provided a control for the much faster analysis on the unfixed, unstained embryos.

*Habrobracon larvae and pupae:* Although *Habrobracon* larvae go through five instars, it is convenient to assign only four stages (6, 7, 8, and 9) to the larval developmental period. Stage 6 is composed of larvae that die after hatching but that never feed. Stage 7 larvae feed but die at the end of the first larval instar or at the beginning of the second. Stage 8 larvae die in the intermediate stages of growth. Stage 9 larvae have finished feeding and die before spinning a cocoon.

Stage 10 is composed of larvae that have spun a cocoon, and pupae in stages too early for the sexes to be discriminated. Stage 11 pupae are white or just beginning to darken; in these, sex can be determined. Stage 12 pupae are well developed in body coloration but the wings have not expanded; the antennae may or may not be still attached to the thorax. Stage 13 pupae have expanded wings and appear ready to eclose.

*Drosophila embryos:* Analysis of dead *Drosophila* embryos was done on fixed and stained whole-mount preparations only. The embryos were classified in three categories: Stage 1 is made up of embryos that died during karyokinesis, before enough nuclei were present to form a blastoderm. Stage 2 consists of embryos that died at or just before blastulation. Stage 3 is comprised of embryos that died after tissue formation began but before hatching. Even though the last category is a broad one, our interest is particularly centered on Stage 1 since it is most predominant after irradiation of the gametes.

## RESULTS

### *Radiation-induced dominant lethality*

Type I lethality, that expressed before blastoderm formation, is predominant when *Habrobracon* or *Drosophila* gametes are irradiated with doses that are lethal to the majority of embryos. This is shown in Figure 2, where dose-action curves compare the proportion of embryos dying before blastoderm formation with the embryo hatchability. With *Habrobracon* sperm (2c) or metaphase I eggs (2b) or *Drosophila* sperm (2d), the dose-hatchability responses are approximately exponential; to a large extent, this is a manifestation of the simple exponential nature of the Type I lethality in response to dose. The *Habrobracon* eggs irradiated in prophase I exhibit two-hit kinetics (Figure 2a). *Drosophila* eggs irradiated in prophase I also show that two hits are requisite for lethality (PARKER 1955; KING, DARROW, and KAYE 1956; KING 1957). The *Habrobracon*

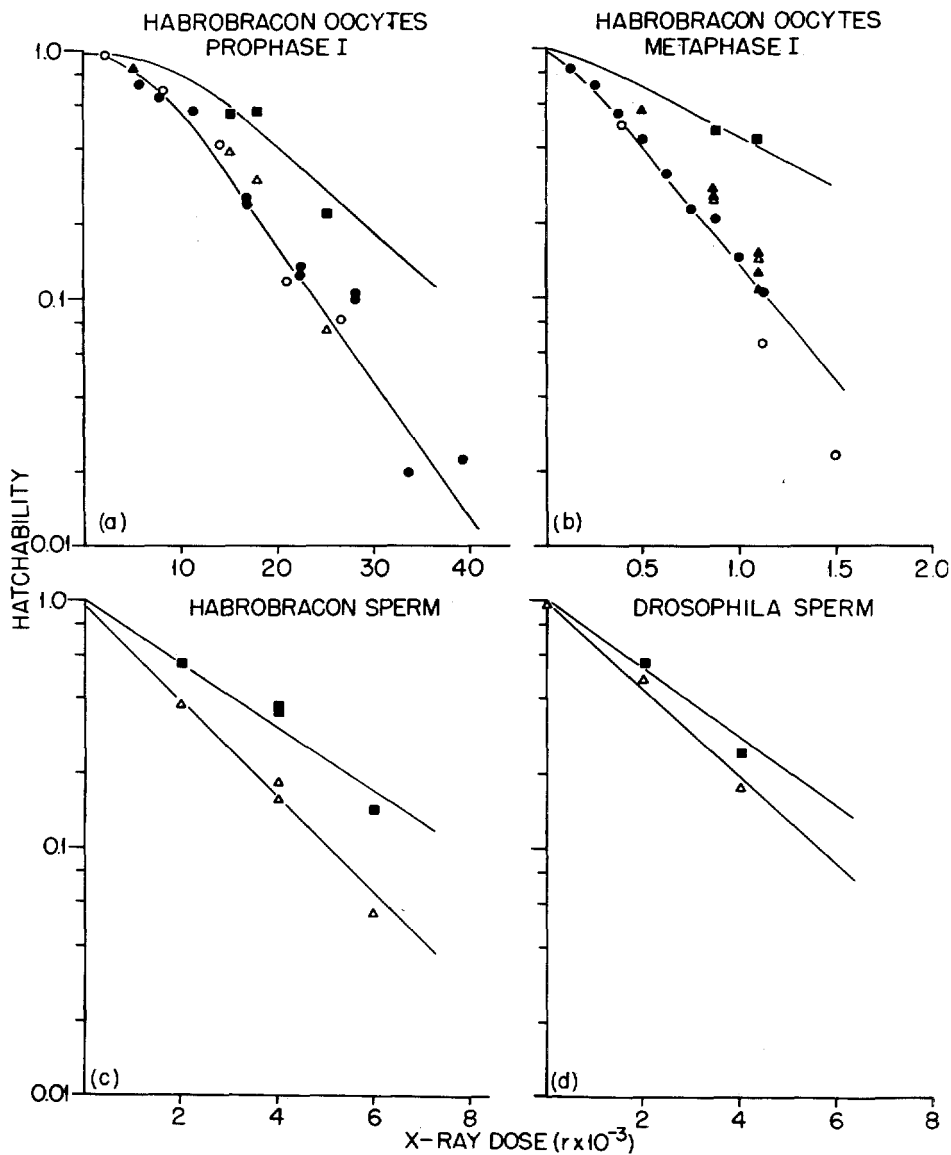


FIGURE 2.—Dose-action curves for Habrobracon prophase I (a) and metaphase I (b) oocytes and Habrobracon (c) and Drosophila (d) sperm. Hatchability data are from A. R. WHITING, 1945 b ●; KENWORTHY, 1956 ○; ATWOOD *et al.*, 1956 ▲; and these experiments △. ■ 1.0 — proportion dying before blastoderm formation.

oocyte data are from unfertilized eggs; induced recessive lethals contribute about 0.10 of the deaths to both metaphase I eggs and prophase I eggs at the 0.37 survival level.



Cytologically, the Type I lethality in *Habrobracon* is characterized by meiosis at the normal rate and then by an abrupt depression in the rate of mitosis, with complete cessation at the second or third division. A few hours later, anomalously Feulgen-negative nuclei arise, which eventually enlarge (VON BORSTEL 1955). In *Drosophila*, the Type I syndrome is similar in that no or few Feulgen-positive nuclei are seen; however, Feulgen-negative nuclei seem not to be produced after the cessation of mitosis. Absolute criteria for distinguishing dominant lethal embryos from unfertilized eggs have not been established in *Drosophila*.

That Type I lethality is truly dominant is demonstrated in Figures 3 and 4, where it can be seen that the time of expression of the early death induced in a *Habrobracon* egg nucleus is affected only slightly by fertilization with a normal sperm. The early expression of death is represented by the first peak in the graph.

Type II dominant lethality, which is induced at only the first meiotic metaphase and whose expression is delayed by fertilization, is illustrated in Figure 3. There is a consistent but slight depression in the frequency of Stage 1 deaths after fertilization but the most marked effect is on the Stage 3 lethals. This group is depressed dramatically, and the expression of death then occurs as a long slow wave that reaches a maximum during late embryogenesis and the early larval period. The frequency of death then declines but rises again slightly during the midpupal period. At the higher dose level of 1100r (Figure 3), where there is an average of about two inactivating events per nucleus, Stage 3 in the unfertilized group is depressed by absorption into the large number of Stage 1 deaths; and in the fertilized class, most of the expression of delayed death occurs at the earlier stages of development. Delay is therefore inversely correlated with dose. It can be seen, however, that a conditional delay is still expressed during the early larval period (Stage 6 in Figure 3b).

An obvious shift of time of death during embryogenesis after fertilization occurs with both the Type II dominant lethality and the Type III dominant lethality, which is lethal after blastulation and before hatching whether or not the eggs are fertilized (VON BORSTEL, ATWOOD, and A. R. WHITING 1955). The different terminology is retained, however, since (1) the delay does not extend into the posthatching stages in the eggs fertilized subsequent to irradiation at prophase I, where Type III lethals are typically induced, (2) the stages of meiosis that were irradiated to induce Type II and Type III lethality are vastly different in both morphology and radiation sensitivity, (3) the patterns of frequency of postblastulation death differs among eggs irradiated in prophase I and metaphase I, (4) it is conceivable that the basis of Type II dominant lethality may be different from Type III lethality, and (5) though there still remains a very real possibility that Type II and Type III are a continuum (see DISCUSSION), the less marked expression of delay in prophase I irradiated eggs has the heuristic value of indicating that a more severe damage must take place in prophase.

Type III dominant lethality is shown in the data from irradiation of eggs in the first meiotic prophase (Figure 4). Again, fertilization with normal sperm causes a slight depression of the Stage 1 lethals. The frequency distribution pattern of

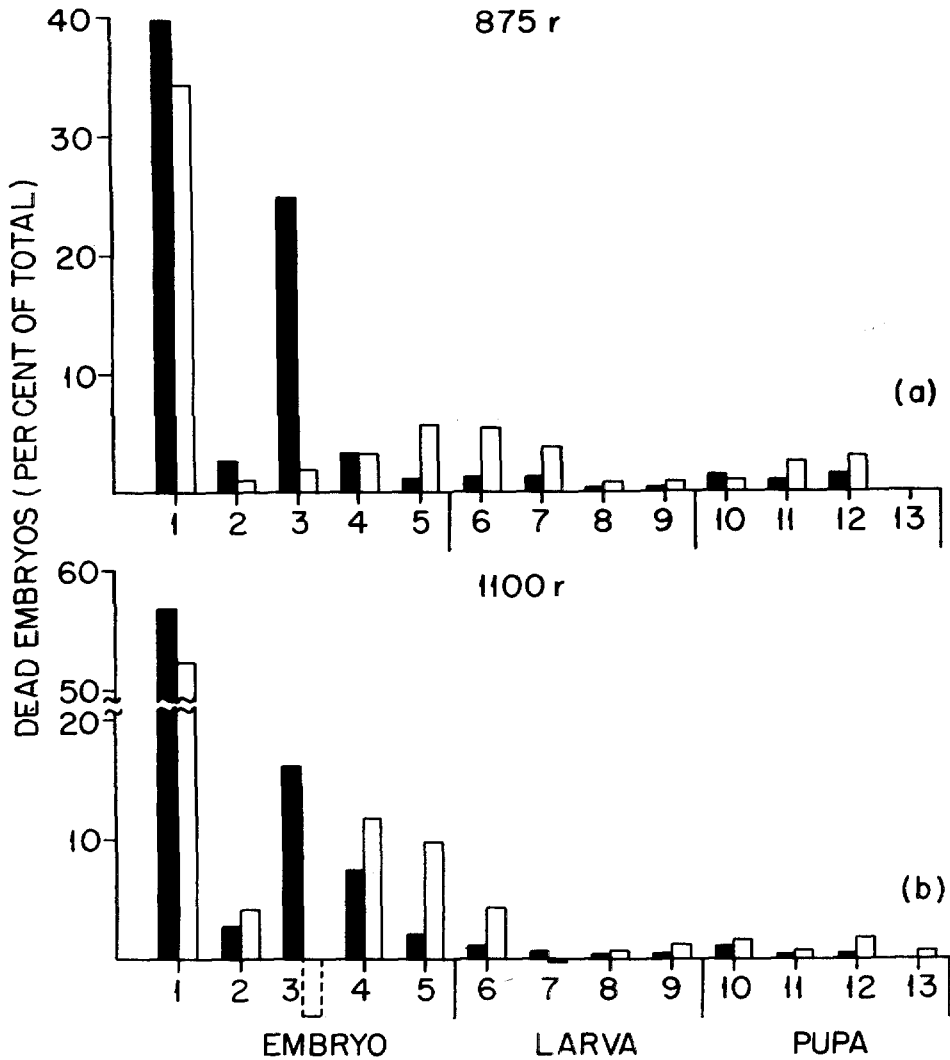


FIGURE 3.—Frequencies of death at different stages of development of embryos irradiated with 875r (a) and 1100r (b) as eggs at the first meiotic metaphase. Solid columns, unfertilized; open columns, fertilized with normal sperm.

postblastulation death (Stage 2 on), stage by stage, among the unfertilized eggs is modified from that characteristic of the deaths from irradiation of metaphase I eggs (Figure 3a, b). It is interesting that the increased frequency in death at Stage 6 not only was increased over those found in irradiated metaphase I eggs but is as high as or higher than the frequency of fertilized prophase I eggs dying at the same stage. The frequency of death at Stage 6 of the unfertilized and fertilized embryos irradiated at prophase I possibly accounts in part for the absence of Type II dominant lethality induced in irradiated prophase I eggs.

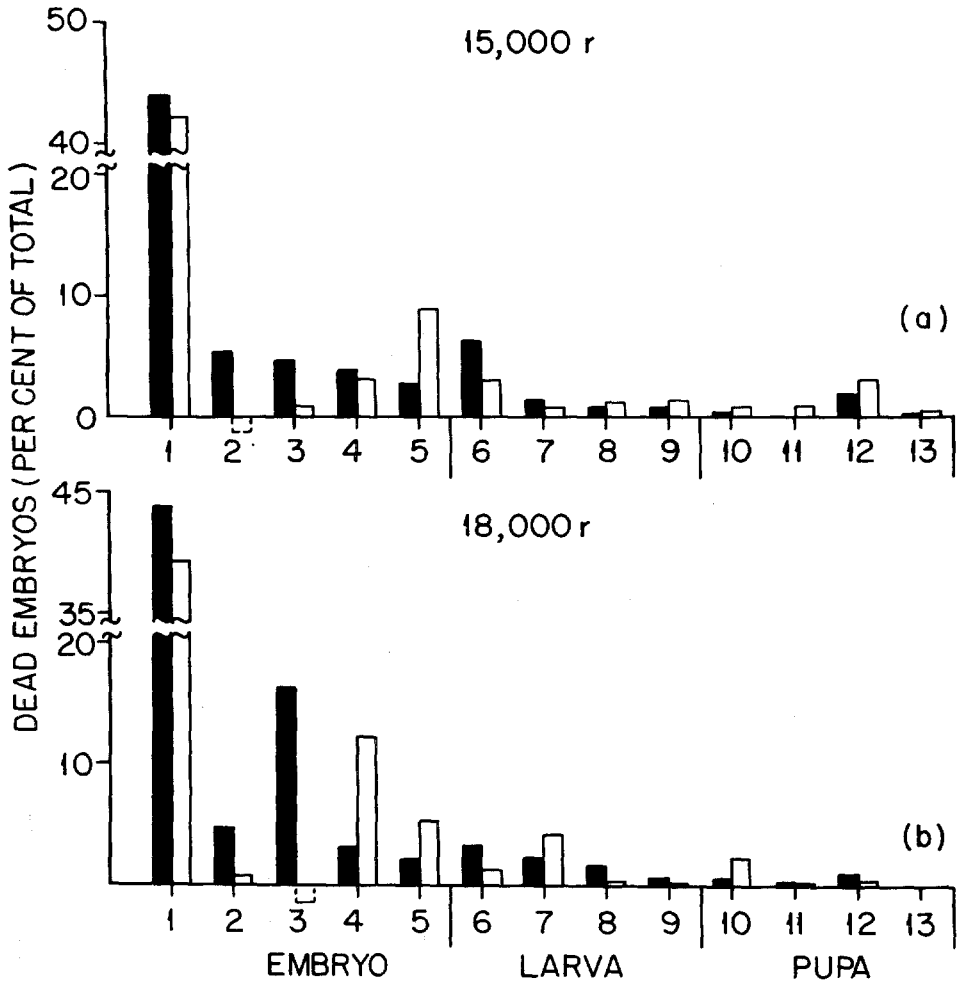


FIGURE 4.—Frequencies of death at different stages of development of embryos irradiated with 15,000r (a) and 18,000r (b) as eggs at the first meiotic prophase. Solid columns, unfertilized; open columns, fertilized with normal sperm.

When sperm are irradiated, many deaths occur during the larval stages, particularly at 4000r (Figure 5). This indicates that a certain amount of Type II dominant lethality is probably induced in sperm. Two experiments at 4000r (Figure 5b, c) are shown to illustrate the variability that can occur from experiment to experiment. At 6000r (Figure 5d), the frequency of deaths during all larval and pupal stages is depressed, presumably because the deaths at Stage 1 account for nearly 90 percent of all deaths.

#### *Genetically contrived dominant lethality*

None of the embryos from the five mated or unmated triploid *Habrobracon* females reported here survived past the larval stage of development (Table 3),

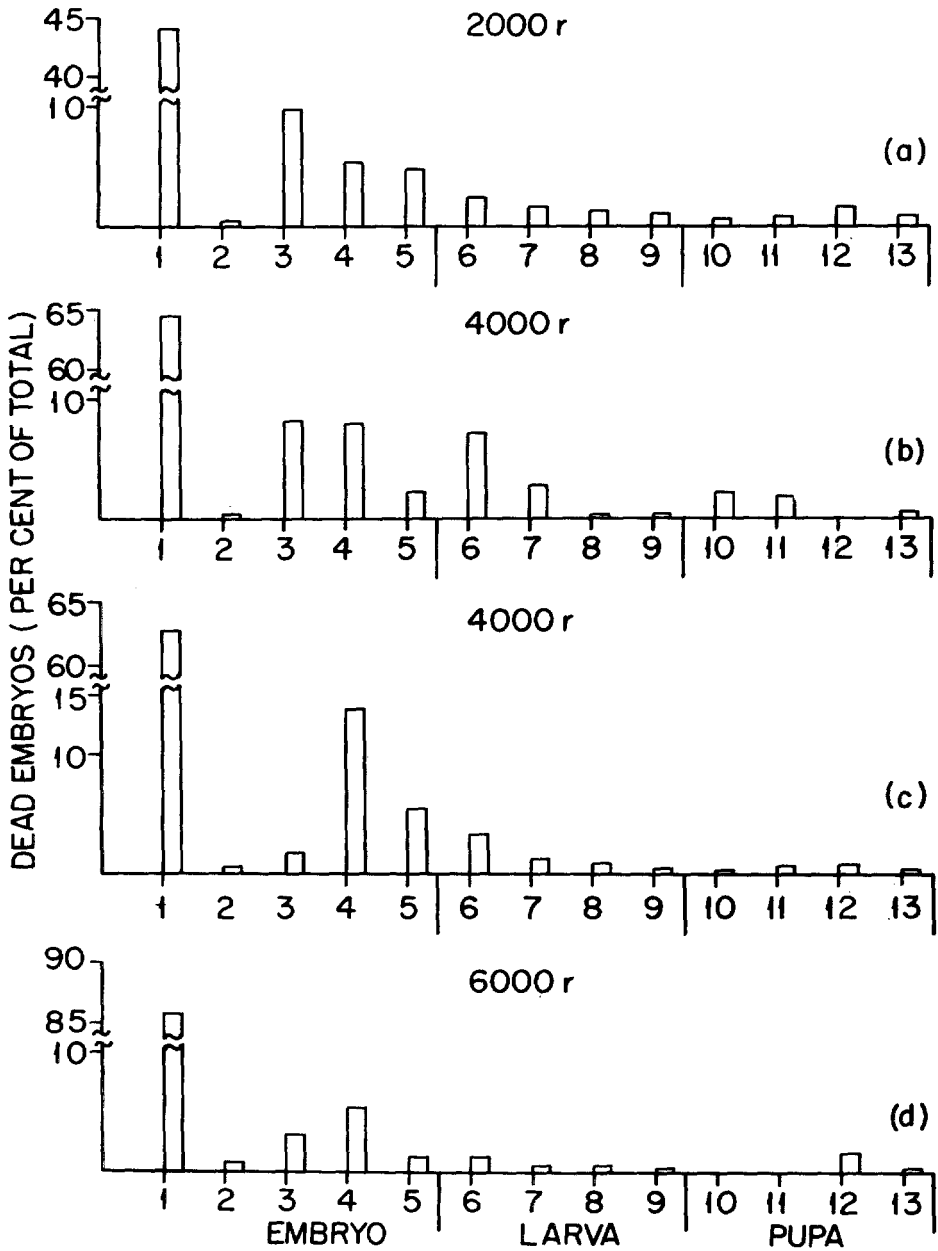


FIGURE 5.—Frequencies of death at different stages of development of embryos from normal eggs fertilized with irradiated sperm. Replicate 4000r experiments (b and c) indicate inherent variability.

TABLE 3  
*Stage of death of gametes from triploid Habrobracon females*

	Viable embryos/total	Stage of development at death								
		Embryos					Larvae			
		1	2	3	4	5	6	7	8	9
Unmated										
1	0/17	..	..	7	10	..	..	..	..	..
2	0/50	6	1	31	12	..	..	..	..	..
Mated										
3	0/39	2	..	8	21	8	..	..	..	..
4	2/37	3	..	1	20	11	..	1	..	1
5	1/41	4	1	4	23	8	..	1	..	..

but haploid male survivors have occasionally been found (A. R. WHITING, personal communication). All embryos from the two triploid *Habrobracon* virgins died before hatching and, predominantly, after blastoderm formation (Figure 6). The few embryos that died before blastoderm formation (in both the fertilized and unfertilized groups) were stained and examined. All had gone through at least eight cleavages and all had Feulgen-positive nuclei and were therefore not comparable to radiation-induced Type I lethal embryos. When the females were mated with normal males (in this case, haploid brothers), the embryos died slightly later in development, but the majority died before hatching (Figure 6).

A mated *Drosophila* triploid female has a high likelihood of producing some viable offspring since the haploid number is four. Also segregation in a *Drosophila* triploid is not random, and a balanced chromosome complement is obtained less often than expected (BEADLE 1935). SANDLER and NOVITSKI (1956) called this nonrandom segregation in triploids the "crowding effect" and they argue that an assumed general homology shared by all the chromosomes provides a consistent explanation for the phenomenon. Furthermore, embryos trisomic for chromosome 4 are viable, and the X chromosome as a trisomic allows enough viability for about two thirds of the animals of this constitution to hatch and some of those hatching to survive larval and pupal life and become superfemales. Of the embryos from triploid females that did not hatch, over 90 percent died at the blastoderm stage or later (Table 4). Those dying earlier could have been unfertilized eggs.

TABLE 4  
*Stage of death of gametes from triploid Drosophila females*

Viable embryos/total	Stage of development at death			
	Preblastoderm	Blastoderm	Postblastoderm	Hatching
59/365	13	16	124	3

Dead embryos were sampled for frequency reaching each stage.

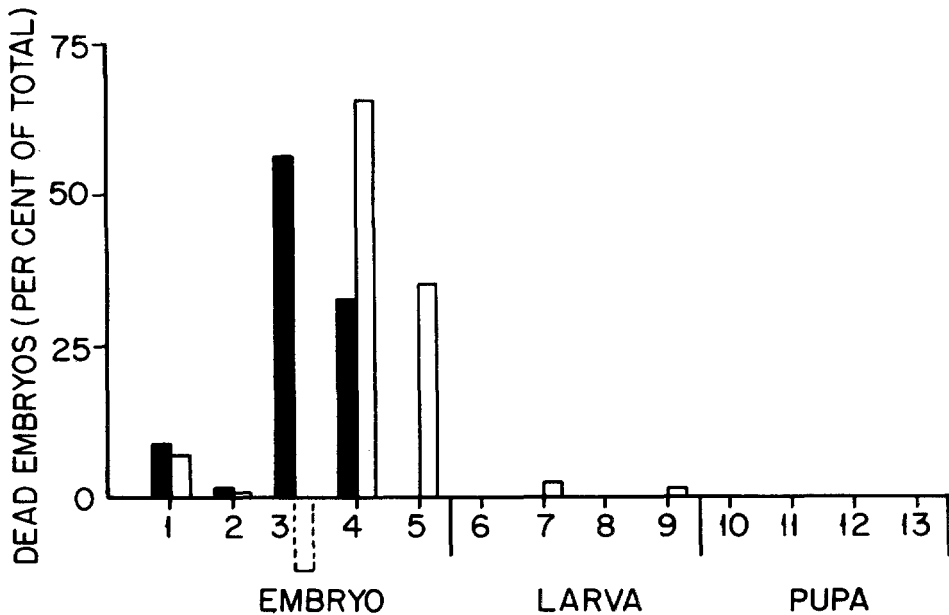


FIGURE 6.—Frequencies of death at different stages of development of aneuploid eggs from triploid *Habrobracon* females. Solid columns, unfertilized; open columns, fertilized with normal sperm.

During meiosis in a translocation heterozygote, the chromosome segregation pattern is such that 50 percent or more of the gametes have a deficiency for a chromosome segment and a duplication of another chromosome segment. When aneuploid eggs were oviposited by virgin *Habrobracon* translocation heterozygotes, the embryos died before hatching (Figure 7). Wasps from aneuploid eggs fertilized with normal sperm died predominantly after hatching, during the larval or pupal stages. These closely resembled in action the Type II dominant lethals induced by radiation in metaphase I eggs: the embryos from unfertilized eggs died with reasonable specificity during mid-embryogenesis, whereas the death pattern of all embryos from fertilized eggs tended to rise with a long, slow wave that had its highest peak just before, during, and after hatching and another small peak at mid-pupation. With all five translocations, most of the embryos died during embryogenesis after blastoderm formation; most of them had many cells (50,000 or more) with well-differentiated cell membranes. This is especially remarkable since, presumably, a large piece of chromosome is missing from the genome in each instance. These genes were therefore not necessary for development up to that time. The embryos from translocation heterozygotes that did die in Stage 1 were sampled. The few that were fixed and stained did not appear to have died in the manner characteristic of the radiation-induced Stage 1 dominant lethals; that is, more than seven nuclear divisions had occurred and the nuclei were Feulgen positive and did not swell.

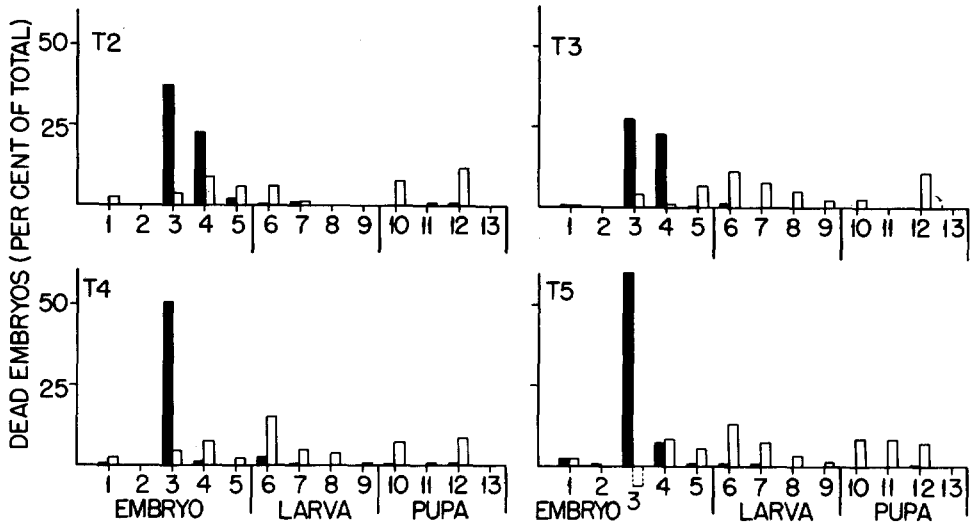


FIGURE 7.—Frequencies of death at different stages of development of aneuploid eggs from four different *Habrobracon* translocation heterozygotes. Solid columns, unfertilized; open columns, fertilized with normal sperm.

Of the 297 dead embryos from two different *Drosophila* translocation heterozygotes, 93.1 percent died at the blastula stage or later (Table 5). Those that died before blastula formation (corrected for those lost) represent only 5.4 percent of the total embryos and possibly were unfertilized eggs.

DISCUSSION

The aneuploid embryos from triploid virgin *Habrobracon* females provide a unique opportunity for testing the effect of extreme genetic imbalance on embryogenesis. Both the haploid and diploid embryos develop normally; embryos from a triploid virgin will contain anywhere from the haploid to the diploid number of chromosomes. If anything as drastic as radiation-induced loss of several chromosomes should bring about Type I dominant lethality, it should be mimicked precisely by aneuploids from triploid virgins. Not one of the embryos

TABLE 5

*Stage of death of gametes from Drosophila female translocation heterozygotes*

Translocation	Viable embryos/total	Dead embryos			Lost during fixation and staining procedures
		Preblastoderm (little or no chromatin)	Blastoderm	Postblastoderm	
Xasta	192/371	10	3	146	20
Star of Muller	285/470	10	2	126	47

resembled a Type I lethal; therefore, it follows that Type I dominant lethality is not caused by the loss of one or more chromosomes. The same argument can be advanced for the genetic imbalance provided by the segregation of translocation heterozygotes, except that the loss is less extreme. But with the translocations, the effect of a hypohaploid genome on development can be assayed as well as the hypodiploid genome, and there again the Type I lethality is not mimicked.

The aneuploid embryos from the triploids can be regarded as resembling Type III lethals since, having the hypodiploid set (between 10 and 20 chromosomes), most of them died after blastula formation and before hatching. When the eggs were fertilized, the chromosome number was between 20 and 30, and though time of death shifted to later embryonic stages, most of the embryos still died before hatching. The translocation heterozygotes produced offspring that died in a manner directly analogous to the Type II dominant lethals induced with radiation at metaphase I.

A class of radiation-induced lethality comparable to the Type II dominant lethality has not been demonstrated in *Drosophila* and is not demonstrable except for the sex chromosomes. Therefore, all postblastulation-prehatching deaths described here are by definition analogous to the *Habrobracon* Type III class. This class is seen in the dose-action curve of *Drosophila* sperm (Figure 2d). The postblastulation-prehatching dominant lethals comprise the area between the two curves, and, interestingly enough, occur at a lower frequency than postblastulation lethals in *Habrobracon*. Among possible explanations are that, with the lower chromosome number in *Drosophila*, chromosome aberrations are less likely if the target for Type I lethality is of a nearly constant size from *Habrobracon* to *Drosophila*, or that sperm are inactivated at low doses in *Drosophila* and that some of the Type I deaths are actually unfertilized eggs (see KAPLAN 1958; D. L. LINDSLEY, C. W. EDINGTON, and E. S. VON HALLE, unpublished). The difference between the curves when the *Habrobracon* eggs are irradiated (Figure 2a, b) is exaggerated by induction of any recessive lethals, since recessive lethal mutations cause death predominantly after blastulation (A. R. WHITING *et al.* 1958) and the metaphase I and prophase I egg data used for Figure 2a, b are from unfertilized eggs.

In addition to the genetically contrived embryo dominant lethals from triploids and translocation heterozygotes, *Drosophila* embryos were constructed that lacked the X and Y chromosomes with their associated nucleoli (VON BORSTEL and REKEMEYER 1958). These embryos came from the cross of attached-X females (XX/0) with attached-XY males (XY/0). Approximately 25 percent of the offspring have neither the X nor the Y chromosome but do retain the complete autosomal complement. These embryos die after the tenth to twelfth mitosis, many divisions after and under entirely different circumstances from the radiation-induced Type I dominant lethal *Drosophila* embryos. This is an important consideration since GAULDEN and PERRY (1958) have shown that ultraviolet microbeam irradiation of the nucleolus of the grasshopper neuroblast permanently inhibits mitosis. In *Drosophila*, lack of the nucleolus does not mimic



the primary radiation lesion. This does not exclude the nucleolus or nucleolus-organizer region as the radiosensitive target in *Drosophila* but does impose the restriction of nucleolar alteration rather than nucleolar inactivation. In eggs irradiated in the first meiotic prophase, the nucleolus is present, but only the nucleolar organizer is present during the first meiotic metaphase and in sperm. Type I dominant lethality is identical for eggs irradiated in metaphase I or prophase I or for irradiated sperm. This indicates that, should the nucleolus be involved, which we believe unlikely, the nucleolar organizer rather than the nucleolus would have to be considered as the primary target.

The classification of radiation-induced dominant lethals presented in this paper is phenotypic rather than causal. Since a relatively clear-cut separation of the phenotypes exists and since certain of the induced dominant lethal types can be mimicked, provisional explanations for the three types can be presented. These are regarded as nuclear effects since radiation damage to the cytoplasm is considered to be without influence; numerous reasons for this have been listed elsewhere (ATWOOD *et al.* 1956). Furthermore, separate inactivation of the *Habrobracon* nucleus and cytoplasm by ionizing radiation has shown that one  $\alpha$  particle striking the nucleus can bring about death of the embryo (ROGERS and VON BORSTEL 1957) whereas  $21 \times 10^6$   $\alpha$  particles must strike the cytoplasm of individual eggs to inactivate 90 percent of a batch of eggs (VON BORSTEL and ROGERS 1958). Since the egg cytoplasm is tremendously resistant to the radiation and since cytoplasmic damage yields a recognizable syndrome, cytoplasmic injury can be considered as contributing nothing to the three classes of dominant lethality discussed here. For *Drosophila*, ULRICH (1955, 1956) has shown that the egg nucleus is far more sensitive to X-radiation than the cytoplasm.

*Type I dominant lethality:* This results in death before the blastoderm is formed and, both in *Drosophila* and *Habrobracon*, is distinctive in appearance. In neither organism can the Type I dominant lethality be mimicked by loss of chromosomes or chromosome parts, nor, in *Drosophila*, by the loss of the sex chromosomes with their associated nucleoli. It has been suggested, for *Habrobracon*, that this kind of dominant lethal, in which mitosis is permanently inhibited at the second or third nuclear division, is associated with a defect in deoxyribonucleic acid (DNA) synthesis since the Type I syndrome is associated with the production of Feulgen-negative nuclei that eventually enlarge (VON BORSTEL 1955). Other than inferred evidence from irradiation of other organisms that DNA synthesis is blocked by radiation or mustards while synthesis of ribonucleic acid and protein are not (BODENSTEIN and KONDRITZER 1948; HERRIOTT 1951; KANAZIR and ERRERA 1954; HAROLD and ZIPORIN 1958a,b), the data to support such a hypothesis are limited. Furthermore, KELLY (1957) has compiled an impressive amount of evidence indicating that inhibition of DNA synthesis by radiation is secondary to an inhibition of mitosis. CAVE and BROWN (1957) suggest that the most likely explanation for the dominant lethality that occurs very early in *Lilium* is genetic imbalance in the embryo that slows the rate of division; nuclear division would not be synchronized with growth-controlling

substances, and the nuclei are then subject to further deleterious changes. If a mechanism other than genetic imbalance is substituted in this scheme, such as a drastic decrease in division rate from radiation damage to centromeres (or even centrioles in animals) or to a nuclear system not necessarily involving chromosomal aberration, then CAVE and BROWN's hypothesis could explain Type I dominant lethality. A mutant has been discovered recently that, when homozygous in a female *Habrobracon*, mimics the Type I lethal syndrome in each laid egg (VON BORSTEL, unpublished). Feulgen-negative nuclei that eventually enlarge are produced. It appears that, in the mutant, normal activation of the egg is prevented and the syndrome resembling the Type I lethal embryo is caused by an imbalance of nuclear and cytoplasmic differentiation. This further indicates that the permanent inhibition of mitosis caused by radiation need not be achieved through chromosomal aberration.

*Type II dominant lethality:* This class of radiation-induced lethality is mimicked in character by the aneuploid proportion of the offspring from *Habrobracon* translocation heterozygotes. It seems reasonable to conclude that the deaths in each instance have a common origin—chromosomal imbalance. Irradiation of eggs in metaphase I produces chromosome fragments, but no bridges, in anaphase I (A. R. WHITING 1945a,b). Bridges are seen in subsequent divisions, indicating that sister union has occurred and the breakage-fusion-bridge cycle is generated (McCLINTOCK 1938). Hatchability is lower than would be expected from chromosomal aberrations alone (A. R. WHITING 1945b).

Besides those Type II lethals resulting from the breakage-fusion-bridge cycle, some of the radiation-induced Type II dominant lethals may be caused by formation of a translocation with the subsequent incorporation into the pronucleus of the aneuploid segregant from the resulting heterozygote (MULLER and HERSKOWITZ 1954; PARKER 1954). These are the so-called "half translocations" (ABRAHAMSON, HERSKOWITZ, and MULLER 1956). Other lethals could be caused by formation of translocation dicentrics and still others through production of gross interstitial deletions. It is reasonable to assume that all these could be mimicked in action by the aneuploids from translocation heterozygotes. The proportion of Type II lethals of each kind would, of course, vary markedly with the dose since some would be one hit and others would be two hit. Data presented by HERSKOWITZ and SCHALET (1957) and by PARKER and HAMMOND (1958) indicate that only a small proportion of the dominant lethals induced in *Drosophila* oocytes could come from translocation formation and aneuploid segregation during the ensuing meiotic divisions.

As the radiation dose to metaphase I eggs increases from 875 to 1100r (Figure 3), the mean of the stage of death of the fertilized eggs is pushed back toward the mean of the stage of death of the unfertilized eggs. This indicates that injury is more severe and that probably several chromosomes become involved. At the higher dose to metaphase I eggs, most of the conditionally delayed dominant lethals are expressed before hatching, but enough still occur after hatching to yield a measurable frequency of conditionally delayed dominant lethality when hatch-

ability is the criterion and the frequency is computed algebraically, whereas none are found at any dose when prophase I eggs are irradiated (Table 5, Arwood *et al.* 1956).

*Type III dominant lethality:* This type of dominant lethality remains somewhat of an enigma. In some respects it would appear to be a more marked form of the Type II dominant lethal—the delayed expression brought about by fertilization of the egg with a normal sperm is still extant but is contracted into the pre-hatching period of embryogeny (Figure 4). If this is the case, then more chromosomes would have to be involved in bridge-breakage in each embryo than those postulated as occurring in eggs irradiated at 1100r at metaphase I (Figure 3b). However, even at the 1100r dose to metaphase I eggs in which an average of two or more inactivating events per nucleus have occurred, the conditional delay still protrudes into the postembryonic period. Still, if chromatid breakage has a much lower prophase/metaphase sensitivity ratio than Type I lethality or recessive lethal or visible mutations (A. R. WHITING and MURPHY 1955), then Type III lethality could be merely a severe manifestation of Type II lethality. Type III dominant lethality is best mimicked by the aneuploidy resulting from segregation in triploid *Habrobracon* virgins. This would suggest that loss of one or more chromosomes is brought about by the radiation. It would seem that nothing could mimic chromosome loss better than chromosome loss, but based on chromosome mechanics alone, it is difficult to visualize the origin of such an aneuploid from irradiation of diplotene chromosomes. Mitigating against the hypothesis of chromosome loss accounting for all the Type III lethals is the substantially high proportion of deaths that occur during the larval and pupal stages in unfertilized eggs, which, curiously enough, do not seem to be covered by a normal sperm (Figure 4b). They could be recessive lethals in the haploid that are balanced by conditionally delayed dominant lethals in the diploid. The exact role of recessive lethals in total mortality of unfertilized eggs remains a problem for the future.

The beautiful simplicity inherent in the chromosome imbalance theory of dominant lethality as the primary source of all dominant lethals makes this theory difficult to discard. The theory enjoys an extraordinary advantage in that it is easy to find evidence to support it and nearly impossible to find direct evidence against it. The breakage-fusion-bridge cycle can still be invoked as the source of all the three classes of radiation-induced dominant lethals described here, but restrictions must be included for any postulated type of bridge-breakage that could cause the very early deaths. The principal restriction is that Type I dominant lethals must arise from a specific kind of break that does not get involved in normal chromosomal rearrangement, or that a chromosome breaking during the course of a special type of breakage-fusion-bridge cycle has properties that cause it to block mitosis. Of course, for the mitotic inhibition hypothesis, a mechanism for the mitotic block must be evolved that is compatible with exponential kinetics for sperm or eggs in metaphase I or two-hit kinetics for eggs in prophase I.

## CONCLUSION

Although chromosome imbalance phenomena can mimic some of the dominant lethality induced by radiation, the majority of radiation-induced dominant lethals in *Habrobracon* and *Drosophila* are not mimicked by genetically contrived loss of chromosomes or loss of chromosome parts.

## SUMMARY

1. Three kinds of dominant embryo lethals, distinguished phenotypically, are induced by radiation in *Habrobracon*; a similar situation exists in *Drosophila* but only two kinds are distinguished by our criteria.

2. The first type (Type I), and most predominant kind, of dominant lethal is expressed before blastoderm formation in both *Habrobracon* and *Drosophila*.

3. The second type (Type II) occurs in *Habrobracon* and results in death of the haploid embryo after blastoderm formation and before hatching, and predominantly after hatching if the embryo is fertilized with normal sperm. An analogue of this type has not been sought in *Drosophila*.

4. The third type (Type III) brings about death of the *Habrobracon* embryo after blastoderm formation and before hatching in either the haploid or diploid embryo; the type of dominant lethal in *Drosophila* that dies during later embryogeny is analogous since death occurs in the diploid embryo after blastoderm formation but before hatching.

5. Aneuploid eggs from *Habrobracon* females heterozygous for a translocation mimic the Type II radiation-induced dominant lethals.

6. Aneuploid eggs from *Habrobracon* triploid females and from *Drosophila* translocation heterozygotes and triploids mimic Type III dominant lethality.

7. *Drosophila* zygotes deprived by genetic means of the X and Y chromosomes and lacking the associated nucleoli develop further than radiation-induced Type I dominant lethal embryos.

8. None of the forms of aneuploidy contrived genetically in these experiments mimic the radiation-induced Type I dominant lethal, but a mimicking situation not involving aneuploidy has recently been discovered.

## ACKNOWLEDGMENTS

We are grateful to DR. K. C. ATWOOD, DR. D. L. LINDSLEY, DR. R. F. KIMBALL, DR. S. WOLFF, and DR. M. E. GAULDEN for having critically read the manuscript. We are indebted to DR. A. R. WHITING for the microscopical analysis of the stained embryos listed in Table 2 and to MISS PATRICIA A. SMITH for her helpful assistance with some of the hatchability experiments. DR. LINDSLEY provided the *Drosophila* stocks.

## LITERATURE CITED

- ABRAHAMSON, S., I. H. HERSKOWITZ, and H. J. MULLER, 1956 Identification of half-translocations produced by X-rays in detaching attached-X chromosomes of *Drosophila melanogaster*. *Genetics* 41: 410-419.

- ATWOOD, K. C., R. C. VON BORSTEL, and A. R. WHITING, 1956 An influence of ploidy on the time of expression of dominant lethal mutations in *Habrobracon*. *Genetics* **41**: 804-813.
- BAKER, W. K., and E. S. VON HALLE, 1953 The basis of the oxygen effect on X-irradiated *Drosophila* sperm. *Proc. Natl. Acad. Sci. U.S.* **39**: 152-161.
- BEADLE, G. W., 1935 Crossing over near the spindle attachment of the X chromosomes in attached-X triploids of *Drosophila melanogaster*. *Genetics* **20**: 179-191.
- BODENSTEIN, D., and A. A. KONDRITZER, 1948 The effect of nitrogen mustard on nucleic acids during embryonic amphibian development. *J. Exptl. Zool.* **107**: 109-122.
- BRIDGES, C. B., 1921 Triploid intersexes in *Drosophila melanogaster*. *Science* **54**: 252-254.
- CAVE, M. S., and S. W. BROWN, 1957 The detection and nature of dominant lethals in *Lilium*. III. Rates of early embryogeny in normal and lethal ovules. *Am. J. Botany* **44**: 1-8.
- CLARK, A. M., and C. J. MITCHELL, 1952 Effects of X-rays upon haploid and diploid embryos of *Habrobracon*. *Biol. Bull.* **103**: 170-177.
- GAULDEN, M. E., and R. P. PERRY, 1958 Influence of the nucleolus on mitosis as revealed by ultraviolet microbeam irradiation. *Proc. Natl. Acad. Sci. U.S.* **44**: 553-559.
- HAROLD, F. M., and Z. Z. ZIPORIN, 1958a Effect of nitrogen and sulfur mustard on nucleic acid synthesis in *Escherichia coli*. *Biochim. et Biophys. Acta* **28**: 482-491.
- 1958b Synthesis of protein and DNA in *Escherichia coli* irradiated with ultraviolet light. *Biochim. et Biophys. Acta* **29**: 439-440.
- HERRIOTT, R. M., 1951 Nucleic acid synthesis in mustard gas-treated *E. coli* B. *J. Gen. Physiol.* **34**: 761-764.
- HERSKOWITZ, I. H., and A. SCHALET, 1957 Induced changes in female germ cells of *Drosophila*. V. The contribution of half-translocation and nondisjunction to the dominant lethality induced by X-raying oocytes. *Genetics* **42**: 649-660.
- KAPLAN, W. D., 1958 The sterility component of X-ray induced dominant lethals in *Drosophila melanogaster*. Pt. 2, *Proc. 10th Intern. Congr. Genet.* p. 142.
- KANAZIR, D., and M. ERRERA, 1954 Métabolisme des acides nucléiques chez *E. coli* B après irradiation ultraviolette. *Biochim. et Biophys. Acta* **14**: 62-66.
- KELLY, L. S., 1957 Effect of radiation on DNA synthesis in mammalian cells. pp. 143-163. *Progress in Biophysics* Vol. 8. Edited by J. A. V. BUTLER and B. KATZ. Pergamon Press, New York.
- KENWORTHY, W., 1956 The effect of oxygen concentration on the dose-action survival curves for irradiated *Habrobracon* eggs. *Am. Naturalist* **90**: 119-126.
- KING, R. C., 1957 The problem of dominant lethals in *Drosophila melanogaster* females. *Proc. Natl. Acad. Sci. U.S.* **43**: 282-285.
- KING, R. C., J. B. DARROW, and N. W. KAYE, 1956 Studies on different classes of mutations induced by radiation of *Drosophila melanogaster* females. *Genetics* **41**: 890-900.
- McCLINTOCK, B., 1938 The fusion of broken ends of sister half-chromatids following chromosome breakage at meiotic anaphases. *Missouri Agr. Exptl. Sta. Bull.* **290**: 1-48.
- MULLER, H. J., 1954 The nature of genetic effects produced by radiation. pp. 351-473. *Radiation Biology*, Vol. I. Edited by A. HOLLAENDER. McGraw-Hill, New York.
- MULLER, H. J., and I. H. HERSKOWITZ, 1954 Concerning the healing of chromosome ends produced by breakage in *Drosophila melanogaster*. *Am. Naturalist* **88**: 177-208.
- PARKER, D. R., 1954 Radiation-induced exchanges in *Drosophila* females. *Proc. Natl. Acad. Sci. U.S.* **40**: 795-800.
- 1955 The origin of dominant lethals in irradiated oocytes of *Drosophila*. *Genetics* **40**: 589 (Abstr.).

- PARKER, D. R., and A. E. HAMMOND, 1958 The production of translocations in *Drosophila* oocytes. *Genetics* **43**: 92-100.
- ROGERS, R. W., and R. C. VON BORSTEL, 1957 Alpha-particle bombardment of the *Habrobracon* egg. I. Sensitivity of the nucleus. *Radiation Res.* **7**: 484-490.
- SANDLER, L., and E. NOVITSKI, 1956 Evidence for genetic homology between chromosomes I and IV in *Drosophila melanogaster* with a proposed explanation for the crowding effect in triploids. *Genetics* **41**: 189-193.
- ULRICH, H., 1955 Die Bedeutung von Kern und Plasma bei der Abtötung des *Drosophila*-Eies durch Röntgenstrahlen. *Naturwissenschaften* **42**: 468.
- 1956 Die Strahlenempfindlichkeit von Zellkern und Plasma und die indirekte mutagene Wirkung der Strahlen. *Verhandl. deut. zool. Ges. Hamburg 1956*, pp. 150-182.
- VON BORSTEL, R. C., 1955 Feulgen-negative nuclear division in *Habrobracon* eggs after exposure to X-rays or nitrogen mustard. *Nature* **175**: 342.
- VON BORSTEL, R. C., and D. L. LINDSLEY, 1959 Insect embryo chromosome techniques. *Stain Technol.* **34**: 23-26.
- VON BORSTEL, R. C., and M. L. REKEMEYER, 1958 Division of a nucleus lacking a nucleolus. *Nature* **181**: 1597-1598.
- VON BORSTEL, R. C., and R. W. ROGERS, 1958 Alpha-particle bombardment of the *Habrobracon* egg. II. Response of the cytoplasm. *Radiation Res.* **8**: 248-253.
- VON BORSTEL, R. C., K. C. ATWOOD, and A. R. WHITING, 1955 Delayed expression of induced dominant lethals in diploid *Habrobracon*. *Genetics*. **40**: 564 (Abstr.).
- WHITING, A. R., 1945a Dominant lethality and correlated chromosome effects in *Habrobracon* eggs X-rayed in diplotene and in late metaphase. I. *Biol. Bull.* **89**: 61-71.
- 1945b Effects of X-rays on hatchability and on chromosomes of *Habrobracon* eggs treated in first meiotic prophase and metaphase. *Am. Naturalist* **79**: 193-227.
- WHITING, A. R., and W. E. MURPHY, 1956 Differences in response of irradiated eggs and spermatozoa of *Habrobracon* to anoxia. *J. Genet.* **54**: 297-303.
- WHITING, A. R., S. CASPARI, M. KOUKIDES, and P. KAO, 1958 Stages at death of X-ray-induced embryo lethals in haploids and heterozygotes of *Habrobracon*. *Radiation Res.* **8**: 195-202.
- WHITING, P. W., 1943 Multiple alleles in complementary sex determination. *Genetics* **28**: 365-382.