GENETIC EFFECTS OF X-RAYS AND CATHODE RAYS ON OOCYTES OF HABROBRACON¹

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DIFFERENCES in sensitivity of oocytes to X-irradiation depending upon stage in meiosis when treatment occurred, have been shown for a number of organisms including *Habrobracon* (WHITING 1938, 1941, 1945a, 1945b; ATWOOD, VON BORSTEL and WHITING 1956). Studies have also shown that death of *Habrobracon* embryos following irradiation of oocytes in late metaphase and in late prophase (diplotene) was attributable to chromosomal and not to cytoplasmic injury except for the highest doses applied to the late prophase oocytes (WHITING 1946, 1948, 1949, 1950, 1955). Differences in sensitivity of metaphase and prophase eggs have been shown to be great. WHITING's data indicate that about 400r is sufficient to kill roughly 50 percent of the embryos when oocytes have been treated in metaphase, but that about 12,000r is necessary if the X-rays are delivered to oocytes during late diplotene. The author's data have essentially confirmed these findings for metaphase oocytes (HEIDENTHAL, CLARK and GOWEN 1955). Both authors have demonstrated that X-rays induce both dominant and recessive lethals in metaphase oocytes (HEIDENTHAL 1952, 1953; WHITING 1954; ATWOOD, VON BORSTEL and WHITING 1956).

In addition to the comparisons of sensitivity with respect to stage, the author's interest has involved comparisons related to differences in *dose rate*. In a previous paper it has been shown that there is no difference in certain genetic effects produced by 124 kv and 50 Mev X-rays (HEIDENTHAL, CLARK and GOWEN 1955). It appears that the different methods by which the ionizations are produced are not important in this biological system. In these experiments the dose rates differed by a factor of about five.

In the present paper this work has been extended to include the effect of 800 kv (peak) electrons. In view of the results with 124 kv and 50 Mev similar effects might be expected (for equal dose and rate of accumulation of dose) if high energy cathode rays were used. However, on account of the higher dose rates associated with electron irradiation, differences in the genetic effect might be expected, provided the chemical reaction (or series of reactions) leading to the genetic change is sensitive to concentrations of radiation-produced active entities, e.g. free radicals.

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MATERIALS AND METHODS

Habrobracon is a small parasitic wasp which lays eggs on the caterpillar of a moth, *Ephestia kuehniella* Zeller. When the females are mated, about two thirds of the eggs are fertilized. These develop into females if the cross is made in such a way as to insure heterozygosity of the sex alleles. The unfertilized eggs develop into males. It has been shown repeatedly that if well-fed virgin females are incubated at 30°C and restrained from oviposition by removing the caterpillars from the vials for overnight, oocytes in first meiotic metaphase accumulate in the four eggs sacs at the distal end of the reproductive tract. If the females in this condition are irradiated and then placed with fresh caterpillars where they can lay eggs, only those eggs treated in first meiotic metaphase are laid, provided the egg-laying period is restricted to six hours. These eggs are examined for hatchability about 36 hours after the egg-laying period. If the aim is to study eggs treated in late prophase, the females which have already laid the metaphase eggs are again restrained from oviposition for overnight. The next day they are allowed to lay eggs for a two-hour period; then they are transferred to fresh caterpillars on which they are allowed to lay eggs for four hours. Eggs laid during the first of these two intervals are a mixture of those treated in late metaphase, diakinesis, and late prophase. Usually they are discarded. But the eggs laid during the four-hour period are, as a group, more uniform. These are the "prophase" eggs. These, too, can be studied for hatchability by examining them 36 hours after the four-hour egg-laying period. The above methods can be used for studies of eggs laid by virgin mothers and, if the surviving larvae are incubated, of adult male progeny. If the purpose is to study diploid eggs or female progeny, the mothers are mated immediately after they have been exposed as virgins to the radiation. Surviving diploid larvae can then be allowed to grow to eclosion as F_1 females. One can secure such virgin F_1 females either from irradiated metaphase or from prophase eggs, at will.

Methods as described above were used to study the hatchability of haploid eggs laid by virgin mothers of a special No. 33 stock which had been inbred shortly before the experiments started, by single brother-sister matings exclusively, for 18 generations. Studies were made of the hatchability of eggs irradiated in late metaphase, others in late prophase. A third series of experiments involved tests of F_1 virgin females for heterozygosity with respect to one or more recessive lethals. The females were reared from eggs irradiated during late prophase and later fertilized by untreated sperm of males from another vigorous wild type stock which had also been bred for 18 generations of brother-sister matings, stock no. 1. All virgin F_1 females were allowed to lay the haploid eggs separately in Stender dishes each of which was supplied with a stung caterpillar free from eggs. Transfers to fresh caterpillars in different dishes were made for each F_1 virgin on each of several successive days. Cultures were incubated at 30°C. Eggs laid by each female were counted each day. About 36 hours later, the cultures were examined for dead embryos and hatched eggs (larvae).

Use of the method just described depends upon the following considerations.

All eggs laid by F_1 virgins are haploid. This means that any eggs bearing one or more recessive lethals, effective during embryonic development, fail to hatch. Segregation involving one lethal should give a hatchability of about 50 percent. Independent assortment of two lethals should yield roughly 25 percent hatchability. The F_1 mothers have ten pairs of chromosomes. One might expect, then, that at sufficiently high doses, various combinations of independently assorted lethals, linked lethals, and semilethals would yield varying percentages of hatchability, depending upon the conditions.

In experiments in which metaphase eggs were studied, the low voltage X-rays were delivered by 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. Later the machine was modified to give higher doses needed for the more resistant prophase work. Distance was shortened to 9.9 cm; amperage was increased to 20 ma; and voltage was increased to 157 kv. Under these conditions the machine delivered about 1200r per minute.

The source of the high energy electrons was a resonant transformer type electron beam generator (KNOWLTON, MAHN and RANFTL 1951; LAWTON, BELLAMY, HUNGATE, BRYANT and HALL 1951) that produced electrons of 800 kv (peak) energy. Dose was accumulated at a rate of about 200 to 5000 roentgens per *second*. Irradiation dose was measured by using a specially constructed air ionization chamber. Dose is expressed in roentgens, where one r represents an energy absorption of 5.34×10^{12} ev/gm of air.

During the irradiation the wasps were held in a thin walled, flat cylinder type of enclosure, which was made from 0.003 inch thick sheet polyethylene and was $2\frac{1}{2}$ inches in diameter and $\frac{1}{8}$ inch in height. The reason for restricting the height of the enclosure was to insure that the wasps would always be in the same part of the electron beam pattern. As many as 75 wasps were irradiated at a time. The absorbing thickness, i.e., wasp plus polyethylene container wall, was estimated to be less than 1 mm (0.1 gr/cm^2), which is well within the total penetration range of the electron (0.3 gr/cm^2) and in the range where the dose would be nearly uniform throughout the thickness.

RESULTS AND DISCUSSION

The data on hatchability of eggs irradiated in first meiotic metaphase and in prophase are given in Table 1. At a dosage of 1000r, what appears to be a significant difference in viability of embryos is apparent; 13.6 percent for X-rays and 20.0 percent for cathode rays. Note, however, that at 1500r no such significant difference appears. Below 1000r, the doses are less certain. Studies of doses above 1500r are not feasible because the hatchability percentages are so low. The data for prophase eggs treated with 12,000r show no difference in hatchability: 40.5 percent for X-rays and 41.1 percent for cathode rays. At a dose of 28,000r, the data show hatchability percentages of 5.5 (author's data) and 9.5 (WHITING 1945) for X-rays; 9.7 for cathode rays. If within these three values, comparisons

TABLE 1

Hatchability of first meiotic prophase and metaphase eggs treated with low voltage X-rays and with cathode rays

Dose	Stage treated	Kind of radiation	Number of experiments	Number of eggs	Number of larvae	Percent eggs hatched
Control			3	1,735	1,606	92.6 $\pm$ 0.6
1,000r	First meiotic	X-rays	4	952	129	13.6 $\pm$ 1.2
	metaphase	cathode	9	1,598	320	20.0 $\pm$ 1.2
1,500r	First meiotic	X-rays	15	2,648	156	5.9 $\pm$ 0.5
	metaphase	cathode	5	881	37	4.2 $\pm$ 0.7
Control			2	355	328	92.4 $\pm$ 0.6
12,000r	First meiotic	X-rays	3	629	255	40.5 $\pm$ 2.0
	prophase	cathode	3	479	197	41.1 $\pm$ 2.5
28,000r	First meiotic	X-rays	3	495	27	5.5 $\pm$ 1.1
	prophase	X-rays*	3	772	73	9.5 $\pm$ 1.1
		cathode	8	1,629	157	9.7 $\pm$ 0.7

* Compiled from data of WHITING 1945.

are made involving dose rate as between X-rays and cathode rays, a small discrepancy is shown (5.5 and 9.7 compared to 9.5 and 9.7). If the first of the two values is regarded as representing a significant difference, then a small difference in hatchability has to be related to a large difference in dose rate since for the prophase work the cathode rate exceeded the X-ray rate by a factor as large as $250 \times$ (5000r per second for cathode rays and 20r per second for X-rays). Furthermore, there is obviously no difference between X-ray data of WHITING and the cathode ray data. It should also be noted that the data for prophase eggs treated with 12,000r rather than 28,000r, show no significant difference (40.5 percent for X-rays and 41.1 percent for cathode rays).

Data on the incidence of recessive lethal heterozygotes among all F_1 females are summarized in the histograms of Figure 1. It will be recalled that the F_1 virgins which were tested by counts of F_2 eggs, developed from eggs irradiated by electrons, in the late prophase stage. The dose was 12,000r. The percentage of females carrying at least one recessive lethal is 7.4 (14/189). Somewhat comparable data, for X-rays, is found in the work of others (ATWOOD, VON BORSTEL and WHITING 1956). Table 1 of this paper shows that for one experiment, with dosage of 15,000r, the percentage of F_1 females carrying recessive lethals is 10.5 (14 heterozygotes/134 females tested); for a second experiment at the same dose the percentage is 14.4 (17/118). These two experiments taken together give 12.3 percent (31/252). The difference between 7.4 percent for cathode rays when dose is 12,000r and 12.3 percent for X-rays when dose is 15,000r, suggests that if difference in dose rate delivered by the two sources of radiation produces a difference in effect, this difference would have to be very small. Whichever type of radiation would be used, a difference of 3000r in dose would be expected to yield a corresponding difference in hatchability. Since the percentages yielded by 12,000r and 15,000r are relatively low, the difference due to $\pm 3000r$ would be

FIGURE 1.—Incidence of recessive lethal heterozygotes among F_1 females which developed from oocytes treated with 12,000 roentgens of cathode radiation during late, first meiotic prophase.

expected to be only a few percent. If one takes errors of variability into consideration, a difference of 4.9 percent seems to be reasonable.

Reference to Figure 1 will show that among controls, there were three females which tested as though they were heterozygous for one or more recessive lethals. These are thought of as "spontaneous" lethals. Such a rate can be regarded as a kind of composite for both eggs and sperm from which the F_1 mothers developed, since in a given case a lethal could have occurred in either an egg or a sperm. Conceivably the mother of an F_1 could transmit a lethal which she had inherited from her mother since females are diploid. A mother would not have received a lethal from her father except as it would have originated in his germ cells, because males are haploid and cannot exist as bearers of lethals. It is probable then that most "spontaneous" lethals should be thought of as having been transmitted to F_1 daughters from their mothers, although spontaneous rate in the gametes of the fathers cannot be neglected. It should also be remembered that stocks had been

closely inbred by 18 generations of single brother-sister mating and that during this process efforts were made to select for viability. Furthermore, if one deals with a natural host-parasite relationship, one cannot invariably be sure that eggs die because of the genetic constitution. This means that only very occasionally a count might be somewhat reduced by such environmental conditions.

Further inspection of Figure 1 will show that of the 189 F_1 females tested, a total of 14 were heterozygous for one or more lethals, that of these 14, eight can be regarded as heterozygous for one lethal, five for two lethals, and one for three or more. However, it is possible that this assumption is too simple to explain the realities fully. On the basis of a Poisson distribution, the incidence of heterozygotes with two and with three lethals is too high. A decisive reason for this discrepancy is not readily available. However, it seems possible that recombination and assortment of sublethals might occasionally modify the hatchability counts in such a way as to make it *seem* as though the counts were reflecting the simpler condition of a single or double recessive. A comparison of control and experimental data as given in Figure 1 is of some interest in this connection. Here one can see that there were fewer F_1 females among experimentals than among controls, which showed a hatchability of F_2 eggs as great, or greater than 90 percent. Among controls there were 89 percent of the females which laid F_2 eggs yielding this high hatchability. This value compares with only 68 percent among F_1 experimental females. Such observations suggest confirmation of a widely held conclusion, that ionizing radiation produces, apart from striking mutations, many other genetic changes which are slightly deleterious in their effects. Conceivably such sublethals may have had some effect, possibly not very great, on the hatchability counts of F_2 eggs laid by F_1 mothers listed as heterozygotes for one or more lethals. At the present time this is a technically difficult matter to settle decisively.

SUMMARY

This paper is concerned with the problem of difference in *dose rate*. Are genetic effects the same, or different, when dose rates are widely different, but total dose accumulated the same? A conventional 124 kv X-ray machine was used for the low dose rate work; an electron beam generator which yielded a rate as high as 5000r per *second* was the source of high dose rates. The materials irradiated were *Habrobracon* oocytes in first meiotic prophase and metaphase stages. These were studied for hatchability (presence of dominant and recessive lethals). F_1 virgin females were reared from prophase oocytes treated with 12,000r. These were tested for heterozygosity with respect to recessive lethals. The data so accumulated indicate that with these materials, when total doses of X-rays and cathode rays are the same, the wide difference in dose rate has no significant effect.

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