RADIOSENSITIVITY OF DEVELOPING REPRODUCTIVE CELLS IN FEMALE COCHLIOMYIA HOMINIVORAX

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THE stage of cell development at the time of irradiation is one of the most important natural conditions that influence radiosensitivity. This relationship has been demonstrated in detail for the various stages of gametogenesis in male *Drosophila*, but corresponding studies of females have been far less extensive. For establishing a relationship between the maturation of the female reproductive cells and their radiosensitivity, an ideal organism is one in which the germ cells develop synchronously; this type of cell development occurs with the screwworm fly, *Cochliomyia hominivorax* (Coquerel) (Diptera, Calliphoridae). The results reported here are an extension of the original radiation experiments with this species (then known as *Callitroga hominivorax*) reported by BUSHLAND and HOPKINS (1951, 1953).

In studies on radiosensitivity of the various meiotic stages, the criterion of damage has been the induction of chromosome aberrations in plants (SPARROW 1951), hatchability of parthenogenetically produced haploid *Habrobracon* eggs (WHITING 1940, 1945a,b), or the analysis of aberrations in the salivary gland chromosomes in *F₁* *Sciara* larvae (BOZEMAN and METZ 1949; REYNOLDS 1941). The criterion of damage in the present study was the induction of dominant lethal changes in the oögonia and oocytes, as measured by the hatchability of eggs fertilized by unirradiated males.

MATERIALS AND METHODS

The details of the process of oögenesis and the effects of gamma radiation on the growth of the reproductive organs in *Cochliomyia hominivorax* will be presented in a separate publication. However, a brief summary of the morphology of the female reproductive system is necessary for an understanding of testing procedures and radiation effects.

Cochliomyia hominivorax is an obligate parasite of warm-blooded animals. The biology of this insect and laboratory rearing procedures have been discussed in several publications (BUSHLAND 1960). The newly eclosed females possess a pair of immature ovaries. An ovary consists of 100–150 ovarioles, each of which will produce one mature egg to be deposited in the first egg mass. At eclosion each ovariole is composed of an anterior germarium, containing oögonial cells, and one egg chamber, containing 15 nurse cells and the oocyte. The nurse cells are constant in number and easily distinguishable from the oocyte nucleus by differ-

ent size, morphology, and deeper staining. The oocyte nucleus is posterior to the group of nurse cells. Maturation of the oocyte occurs synchronously in all ovarioles.

In these experiments the flies were aged at 80°F and fed only water and honey. At this temperature the females mate when two days old, or older, and are ready to deposit an egg mass of 200–250 eggs when 5–6 days old. Virgin females will oviposit readily under laboratory conditions, but their eggs do not hatch. Oviposition can be induced by presenting the females with a small piece of lean meat and keeping them at a temperature of 90°–96°F for a few hours.

For all experiments, gamma radiation treatments were delivered in air in a Co⁶⁰ radiation chamber at a dose rate of approximately 683r/minute. Calibration for the unit was accurate to $\pm 6\%$. The temperature in the radiation room was maintained at 80°F. When pupae were irradiated, both sexes were present and the females were separated from the treated males at eclosion; when adults were to be irradiated, the females were separated from the males shortly after eclosion and kept as virgins until after treatment. In all experiments, matings were not allowed until after the radiation treatments, and all males used were untreated individuals. Random mating was permitted until the females were 5–6 days old. To assure that eggs deposited by unfertilized females were not included in any egg sample used for hatchability studies, each female was allowed to oviposit individually. After producing an egg mass, the female was sacrificed and the spermathecae examined for the presence of sperm.

Egg masses from each group of treated, inseminated females were pooled and separated in one percent NaOH for 8–10 minutes and then rinsed repeatedly in distilled water before being plated and counted. Unless otherwise stated, the eggs used for hatchability studies were selected at random from a pool of many thousands. All hatchability figures were based on counts of from 2,000 to 4,000 eggs for each dose and age group. Hatchability of the eggs was scored after incubation for 24 hours at 30°C. After correction for the small number of natural deaths in the controls, all unhatched eggs were assumed to indicate that a dominant lethal change had been induced in the germ cell at the time of irradiation. Sex-linked recessive lethal changes could also contribute to death in the F₁ male embryos; but insemination by normal, untreated males prevented detection of autosomal recessive lethal changes, and hatchability tests cannot detect dominant lethal changes expressed beyond the egg stage.

RESULTS AND DISCUSSION OF INDIVIDUAL EXPERIMENTS

Preliminary irradiation studies: A series of four experiments was conducted over a six-month period to determine the general pattern of radiosensitivity in female *Cochliomyia*. Pupae 5–6 days old and adult females one through five days old were treated with 2625r of gamma radiation. With this procedure it was possible to treat oogonia and successively older oocytes. The results of these experiments were essentially the same, but have not been pooled because the females in the earlier experiments were aged at 73° $\pm$ 2°F, and in the later experiments at 80° $\pm$ 2°F. Differences in temperature affect the physiological age of the

females and, consequently, the rate of oocyte development. The results of a typical experiment of this series are shown in Table 1. It is evident that the developing germ cell, as it progresses from an oogonial cell to a mature oocyte, varies considerably in radiosensitivity.

When pupae were irradiated with 2625r the eggs produced had fairly high hatchability. The hatchability figures for adults treated when less than 24 hours old are apt to be misleading because at this age irradiation with 2625r interfered considerably with normal egg production. For example, of four experiments in which females $\frac{1}{2}$ –4 hours old were irradiated, in only one did females produce any eggs. Most females deposited shrunken, yellowish masses that could not be classed as normal eggs and that never hatched. In the group 4–7 hours old when irradiated, one third laid no eggs, and one third laid scattered, irregular, small, yellowish eggs that did not hatch. Only four females deposited eggs of normal appearance; these totaled 229, with low hatchability. When adult females 8–24 hours old were irradiated, most of them produced "normal" eggs of which 34 percent hatched, but some still deposited aborted eggs or shells.

After the females were more than 24 hours old, the radiation treatments had little effect on egg production as such. All females treated when one, two, or three days old produced normal egg masses, and the hatchability of the eggs was relatively high (61–79%). There was a sharp increase in the radiosensitivity of the

TABLE 1

Dominant lethal changes induced in oogonia and oocytes of Cochliomyia hominivorax by 2625r of gamma radiation

Age of female irradiated	Number of eggs scored	Percent hatch
Pupae		
5 Days	1195	54.2
6–6½	2460	65.7
Adults		
$\frac{1}{2}$ –4 Hours	215*	8.8
4–7	229*	7.9
8–26	2599†	34.1
34 ± 9 Hours	2186	61.5
58 ± 9	1960	78.9
72 ± 2	2511	71.3
82 ± 9	1924	61.1
96 ± 2 Hours	2337	3.2
106 ± 9	625	6.08
120 ± 2	2823	17.5
130 ± 9	650	18.5
Controls	3202	95.1

* These egg masses from single female. Other females in group produced no eggs, or abnormal, misshapen, aborted eggs that did not hatch.

† Females in this group also produced some misshapen, aborted eggs. Above hatchability figures represent only normal eggs.

developing oocytes between the third and fourth day of development. Although females irradiated when four or five days old produced large numbers of eggs, the hatchability was low. In addition, within this period of high sensitivity, a difference between four- and five-day-old females was consistently demonstrated in every experiment.

Table 1 also shows the variation in the kind of damage observable after irradiation of the female reproductive tissues. Irradiation at one stage greatly altered egg production, and irradiation at another stage was followed by production of normal numbers of eggs that had high or low hatchability. Since the variation showed no apparent relation to the progress of maturation, these observations suggested a cytological study of the reproductive cells to determine a possible correlation between radiosensitivity and nuclear changes.

Cytological Studies: Whole mounts of ovarioles and oocytes were made from females of various ages and either stained with Feulgen procedure according to the methods of WHITING (1950) or VON BORSTEL and LINDSLEY (1959), or mounted in insect saline and studied with a Zeiss phase-contrast microscope at a magnification of 1600 $\times$. The following account will be limited to observations made on the nuclear components of the oocytes and nurse cells.

The ovarioles of five-day-old pupae contain germaria filled with oogonial cells; the oogonia have not yet differentiated into nurse cells and oocytes. Nurse cells first appear in the ovarioles just prior to eclosion. At 80°F, eclosion usually occurs 7–7½ days after puparium formation. Thus, sometime during the last day of pupal life, one oocyte and 15 nurse cells in each ovariole are differentiated. Each ovariole in a newly eclosed female has a well-defined first egg chamber with the 15 nurse cells and one oocyte, plus an anterior germarium that contains oogonial cells.

During the first day of adult life, major changes take place in the nurse cells. In older pupae and newly eclosed adults the individual chromosomes in the nurse cells are greatly thickened and stain deeply. The formation of polytene chromosomes is characteristic of an endomitotic process, in which the chromosome material is reduplicated without cell or nuclear division and the individual chromosomes become vastly thickened. The endomitotic process is completed during the first day of adult life, and is followed by complete dissociation of the enlarged chromosomes, so that after 24 hours all the nurse cells are completely filled with loosely associated, Feulgen-positive chromatin threads, which exist in great multiplicity. This endomitotic process has been described in detail by PAINTER and REINDORP (1939) and KING *et al.* (1956) for *Drosophila melanogaster*, and the process in *Cochliomyia* is very similar. After the endomitotic process is completed, growth of the egg chamber begins. Vitellogenesis reaches a peak between the second and third day after eclosion; the oocyte grows rapidly in size, and during this period the volume of the ovary as a whole increases five-fold.

The oocyte nucleus in the meantime undergoes the early stages of the first meiotic division. Close examination of more than 50 whole-mount preparations from females three days old ± 2 hours showed that the oocyte nucleus was in pro-

phase of the first meiotic division (see Figures 1 and 4). The chromatin material is arranged in a spherical mass of Feulgen-positive, elongated, fine threads. Figure 1 shows only a portion of the chromatin material, taken at a single focal level; Figure 4 shows a greatly enlarged, composite, diagrammatic representation of the chromatin material with several focal levels superimposed. Because of the very small size of the chromosomes, it was difficult to identify the stages within prophase I as precisely as can be done with plant chromosomes. The present studies indicate that perhaps the stage of prophase present in three-day-old oocytes corresponds more closely to late pachytene and diplotene than to any other of the prophase stages, but further studies of the age period of $2\frac{1}{2}$ – $3\frac{1}{2}$ days will be necessary to divide prophase I into its various components. The present results can, however, be closely compared with those of WHITING (1945a), who treated diplotene of prophase I and metaphase I in *Habrobracon*. Within any age investigated, there was very little variation in the appearance of the oocyte nuclei in any of the preparations, which indicates that the oocytes progress through the first meiotic division very slowly.

Between the third and the fourth day, the oocyte nucleus undergoes great changes in chromosome condensation, spiralization, size, and stainability. In females four days old ± 2 hours the chromosomes are extremely condensed and stain deeply (Figures 2 and 5). The chromosomes are extremely small, but a clear metaphase plate is distinguishable. In more than 75 nuclei observed, all were in the metaphase I condition described above. Figures 2 and 5 represent the normal condition of the oocyte chromosomes in a four-day-old female. The centrioles are clearly visible; the centromere region of the chromosomes is separating but the ends of the dyads are still in contact. Movement through metaphase I is very slow. Between the fourth and fifth days the chromosomes separate more noticeably. In preparations of five-day-old oocytes the centrioles are not visible; chromosome movement has progressed so that it is apparent that the centromeres have reached the centriole region. However, because of the small size of the entire complex, it was sometimes difficult to tell whether or not the dyads were completely separated. Nevertheless, the anaphase I condition was always observed in oocytes from five-day-old females (Figures 3 and 6). The first meiotic anaphase is arrested in this "early" stage until the mature oocyte is oviposited. Feulgen preparations of freshly laid eggs showed that anaphase I of the first meiotic division is completed after oviposition, and is followed by the second meiotic division.

Comparison of these cytological data with the preliminary radiation experiments (Table 1) indicated a relationship between nuclear morphology and radiosensitivity that could be studied quantitatively, and a further series of experiments was initiated.

Dosage-response studies for oocytes treated in the first meiotic division: The following experiments were conducted to study the induction of dominant lethals in oocytes in prophase, metaphase, and anaphase of the first meiotic division. Adult females were irradiated with graded doses of gamma radiation when three, four, and five days old, ± 2 hours. Thirty-one separate radiation treatments were

FIGURES 1-6.—Meiotic nuclei in *Cochliomyia hominivorax* oocytes.

FIGURES 1-3: Photomicrographs, 1000 $\times$. FIGURE 1.—Prophase I from oocyte of three-day-old female, taken at a single focal level and showing only a portion of chromatin material. Portion of nurse cell nucleus upper left, follicle cell nuclei right border. FIGURE 2.—Metaphase I from oocyte of four-day-old female. FIGURE 3.—Anaphase I from oocyte of five-day-old female.

FIGURES 4-6: Diagrammatic representations of the three stages drawn from magnifications of 1600 $\times$ and greatly enlarged. FIGURE 4.—Prophase I, with several focal levels superimposed. FIGURE 5.—Metaphase I. FIGURE 6.—Anaphase I.

performed, with ten sets of controls. Each calculation of hatchability was corrected for its respective control by Abbott's formula. Altogether, 82,517 eggs were scored for hatchability following different doses of radiation. The data are summarized in Figure 7. Each point on the graph represents the average number of cells with at least one dominant lethal. A linear regression line has been fitted to the points for each age group.

When dominant lethals are used as a criterion of radiosensitivity, three-day-old females with oocytes containing nuclei in prophase I are much less sensitive than four-day-old females (nuclei in metaphase I) and five-day-old females (nuclei in anaphase I). The slopes of the lines (b value) for the three-day-old oocytes compared with the four- and five-day-old oocytes are obviously different: three-day-old, $+0.005895$; four-day-old, $+0.02170$; and five-day-old, $+0.02263$. The slopes of these lines express the amount of increase in dominant lethals per roentgen of radiation. Oocytes in prophase I condition respond in a different manner from those in the other two stages. Oocytes in metaphase I and anaphase I show very similar kinds of response to radiation, and the slopes are of the same order. Although the cytological variation between these two stages is apparently very slight, radiation experiments demonstrated that metaphase I oocytes are consistently more radiosensitive than anaphase I oocytes.

For all stages irradiated, the response to an increase in dose was approximately linear, although the wide difference in the slopes of the lines suggests a large quantitative difference in response. SPARROW (1951) has stated that "probably the most reliable method of determining the sensitivity would be to determine

FIGURE 7.—Percentage of dominant lethals induced by gamma radiation in the oocytes of *Cochliomyia hominivorax*. Oocytes in females treated at three days old in prophase I (O), four days old, metaphase I (●), and five days old, anaphase I (X).

what dosage given at the various stages of the nuclear cycle would be required to induce a constant percentage of cell lethals." From the data represented in Figure 7, and the calculated slopes of the lines, calculations of the LD_{50} dose for the three stages of oocytes gave the following results: three-day-old, $LD_{50} = 7,939r$; four-day-old, $LD_{50} = 1,309r$; five-day-old, $LD_{50} = 1,639r$. Thus, the radiosensitivity of the metaphase I stage is six times higher than that of prophase I.

A comparison of the percent hatchability of the three stages at two doses of radiation is as follows:

Age of female	Treated with	
	4,000r	10,000r
Three days	58%	36%
Four days	0.85%	0%
Five days	6%	0%

Studies of dominant lethals induced in oogonial cells: The cytological studies showed that only oogonial cells were present in five-day-old pupae. According to the data in Table 1, 46 percent of the mature eggs produced after treatment of oogonial cells with 2625r contained at least one dominant lethal. This result is rather striking, since the oogonial cells have had ample time to recover from the adverse effects of radiation in the seven or eight days intervening between pupal irradiation and oviposition by the adult female. Furthermore, oogonial cells must undergo the processes of differentiation and meiosis up to anaphase I before they are oviposited. Thus, the type of dominant lethal encountered in these cells, which were oogonia at the time of treatment, must represent a type of chromosomal damage not serious enough to cause complete degeneration and resorption during maturation.

A series of experiments was conducted to estimate to what extent oogonial dominant lethals are eliminated from the germ line, and to determine the comparative radiosensitivity of this stage. Pupae were treated with graded doses of radiation when $4\frac{1}{4}$ – $5\frac{1}{4}$ days old, and in one experiment, five days old ± 2 hours. As an added precaution, only flies emerging $2\frac{1}{2}$ days after the radiation treatment were used in the experiments, thus insuring that the females studied were those treated as five-day-old pupae. The results are summarized in Table 2 and Figure 8.

When oogonial cells were irradiated, the number of eggs deposited by each female decreased as the radiation dose increased. This result indicates that the damage in some of the cells was great enough to eliminate them from the germ line, and that eliminated cells were not rapidly replaced by undamaged ones. Thus, the amount of dominant lethality observed is based on the selected portion of the cells that survive to be oviposited as mature eggs. The decrease in hatchability is related to an increase in the radiation dose in a nonlinear manner.

DISCUSSION

Any discussion of radiosensitivity involves comparisons of "sensitive" with "insensitive" stages. These terms are purely relative, for no stage in these studies

TABLE 2

Radiation-induced dominant lethal changes in oögonia of Cochliomyia hominivorax; pooled data from three experiments. (Five-day-old pupae irradiated; females separated at eclosion and mated to normal males.)

Radiation dose (r)	Number of females egged	Number of females that oviposited*	Number of females fertilized	Number of eggs scored	Average number of eggs/female	Percent hatch
1,000	60	34	33	6647	201.4	84.9
2,000	60	39	36	6150	170.8	74.7
2,311	30	21	21	2985	142.1	69.3
3,000	58	26	24	1578	65.8	48.2
4,000	96	18	18	301	16.7	27.2
5,000	40	0	...	...	...	...
Controls	62	46	42	9584	228.2	90.8

* During 2-3 hours allowed for oviposition.

FIGURE 8.—Egg production and egg hatchability after treatment of oögonial cells of *Cochliomyia hominivorax* with gamma radiation.

was completely insensitive to radiation. A clear pattern of radiosensitivity emerges, however, when one considers the radiation data in conjunction with the cytological data.

In the earliest stages of oögenesis the growth of the oocyte is seriously hampered

by irradiation, presumably because of the effects on the nurse cells. It has been demonstrated that egg formation can be hindered by damage to the nurse cells before they become fully differentiated. GROSCH and SULLIVAN (1954), KING and SANG (1959), and KING (1960) have presented evidence for the participation of nurse cell nuclei in vitellogenesis. Differentiation of the nurse cells often involves a series of chromosomal multiplications without spindle formation, disappearance of the nuclear membrane, or cell division; consequently, these cells become highly polyploid. The results of the present studies substantiate the conclusion of GROSCH and SULLIVAN (1954) that nurse cells are more sensitive before they become polyploid. In our unpublished studies, irradiation of the reproductive tissues of *Cochliomyia* after endomitosis was completed (at 12–24 hours of age) had no effect on the growth of the ovaries at doses of up to 8,000r.

After the nurse cells become polyploid and enlarged, egg formation as such is not affected by even large doses of radiation. However, the oocyte nucleus is progressing into the first meiotic division, and the chromosomal changes involved in this process greatly influence radiosensitivity and the viability of the egg produced. The present results show that metaphase of the first meiotic division is the most sensitive stage, anaphase slightly less so, and that prophase is very resistant to radiation damage.

There is remarkable agreement between the results of these experiments and others that utilized different indices of radiation damage. WHITING (1940, 1945a,b) studied hatchability of haploid eggs in *Habrobracon* and found that metaphase I was the stage of highest sensitivity and prophase I the least. These two stages differed by a factor of 32 in the *Habrobracon* studies, as compared with a factor of six in the present studies; but in hatchability tests of haploid eggs, autosomal recessive as well as dominant lethal changes are detected. In studies of chromosome aberrations in *F₁* *Sciara* larvae from irradiated parents, BOZEMAN and METZ (1949) found anaphase I to be the period of maximum sensitivity and prophase I to be comparatively insensitive. The oocytes of *Sciara*, like those of *Cochliomyia*, develop synchronously, and *Habrobracon* females can be induced to store mature eggs, so that in all these studies oocytes could be treated in known stages of development. The slight discrepancy between the results with *Sciara* and those with *Habrobracon* and *Cochliomyia* probably results from differences in terminology. The variation between what is here called metaphase I and early, or even mid-, anaphase I is very slight. Very likely, different authors have given different names to the same stages, since demarcation is seldom definite.

In studies of *Mormoniella* oocytes, FLUKE (1957) found certain stages to be more radiosensitive than others, but observed that egg hatchability never reached high levels of recovery as in prophase I for *Habrobracon* and *Cochliomyia*. PARKER (1955, 1959), KING (1955), and KING *et al.* (1956) found that *Drosophila* females also differ greatly in the radiosensitivity of oocytes treated at various stages of development. For *Drosophila* oocytes in stages 7 and 14, PARKER (1959) presented survival curves remarkably like the curves in Figure 7, and which he interpreted to represent “one-hit” and “two-hit” curves. However,

when the oocytes do not develop synchronously, treatment of many cells in the same stage of development is clearly more difficult. Furthermore, in *Drosophila*, diakinesis and metaphase I occur after the egg leaves the ovariole (SONNEBLICK 1950). These factors make direct comparisons of *Mormoniella* and *Drosophila* with *Cochliomyia*, *Habrobracon*, and *Sciara* somewhat difficult.

The results obtained by SPARROW (1944), SPARROW, MOSES and DUBOW (1952) and SPARROW, MOSES and STEELE (1952) in studies of radiosensitivity of meiotic stages in the plant *Trillium* are generally in close agreement with those with insect tissues. In *Trillium*, metaphase I is a highly sensitive stage, but some prophase stages such as diplotene are also very sensitive. It should be noted, however, that in *Trillium*, chromosome contraction reaches a maximum during diplotene, after which the chromosomes elongate (SPARROW, MOSES and DUBOW 1952). In *Cochliomyia* and *Habrobracon*, maximum contraction is achieved at metaphase I, and this is the stage of maximum sensitivity. As noted by SPARROW, MOSES and DUBOW (1952), peak sensitivity corresponds approximately with the most contracted stage. On this basis, no real discrepancy exists between the work with insects and that with *Trillium*.

The basis of differential radiosensitivity between meiotic stages can be sought in demonstrable differences in the nucleus, or in possible changes in the cytoplasm as they relate to the presence or absence of the nuclear membrane.

The disappearance of the nuclear membrane as a factor in increased radiosensitivity was discussed by BOZEMAN and METZ (1949) for *Sciara*, but it is probably not of major importance. Oogonial cells, in which the nuclear membrane is still present, are quite radiosensitive; and sperm and spermatids, both of which have an intact nuclear membrane, have repeatedly been demonstrated to differ in radiosensitivity.

On the other hand, numerous studies have indicated that dominant lethality observed after irradiation is chromosomal in nature (MULLER 1954; WHITING 1955; PARKER 1959). Radiation-induced dominant lethals have been attributed to those aberrations that result in chromosome bridges formed at anaphase I and anaphase II (WHITING 1945a,b; PARKER 1955, 1959; KING 1957) or during cleavage (PONTECORVO 1941; PONTECORVO and MULLER 1941; MULLER and PONTECORVO 1942). GOODSPEED (1929) suggested that differences in chromosomal tension could account for differences in radiosensitivity. This hypothesis was recognized and expanded by WHITING (1945a,b), who suggested that varying ability of chromosomes to rejoin might account for differences in radiosensitivity. The ability to rejoin would be facilitated by the greater movement of chromosomes in prophase I and hampered by the tension exerted on them in metaphase and anaphase I. SPARROW and MALDAWER (1950) presented data indicating that rejoining of broken chromosome ends affects the detectable radiosensitivity of chromosomes. Differences in rejoining ability between meiotic stages were demonstrated by PARKER (1959). In his studies, oocytes irradiated in the most resistant stage showed increased survival after fractionation of the radiation dose or centrifugation, but survival of oocytes in the most sensitive stage did not increase when the dose was fractionated. Very likely, then, rejoining does

play a role in the greater sensitivity of metaphase I, as originally suggested by WHITING. However, a difference between stages in the number of actual or potential breaks produced may also exist (SPARROW and MALDAWER 1950; KING 1955).

HOENIGSBERG, GALLUCCI and GIAVELLI (1961) suggested that the amount of water present in or around the chromosomes in the condensed *vs.* the uncoiled state may affect the mutational response. Differences in respiration or in oxygen availability between cells in various stages may also influence radiosensitivity (OSTER 1958). Numerous other factors that could be involved in the differential radiosensitivity of cells in different meiotic stages have been aptly discussed by WHITING (1945a,b), BOZEMAN and METZ (1949), SPARROW (1951), and SPARROW and FORRO (1953).

Dominant lethal changes induced in oogonia must be considered apart from those in oocytes, since oogonia represent a selected group. KING *et al.* (1956) found that in *Drosophila melanogaster*, dominant lethals are not detectable after irradiation of oogonia. They reasoned that oogonial cells containing dominant lethals cannot produce a 16-cell egg chamber, and that cells bearing dominant lethals are probably resorbed at an early stage and replaced by undamaged, viable cells. In *Cochliomyia*, some of the dominant lethal changes induced in oogonial cells were severe enough to eliminate the cells from the germ line and significantly reduce the number of oocytes produced, but some types of dominant lethal changes persisted through differentiation and maturation to be detectable in the zygotes. Dominant lethals induced in oogonia were similarly detectable by egg-hatchability tests in *Habrobracon* (LACHANCE 1958), an organism uniquely suited to detection of dominant lethals. Furthermore, viable chromosome rearrangements can be induced in *Drosophila* oogonial cells (ABRAHAMSON 1961).

Direct comparison of oogonial radiosensitivity with that of the other meiotic stages is difficult. The hatchability data suggested that this stage was more resistant than metaphase I and anaphase I, but less resistant than prophase I. When a dose of 3,000r was administered to oogonial cells, subsequent egg production was 0.310 of the controls, and hatchability of the eggs produced was 0.507 of the controls. However, if the loss of a cell before oviposition and dominant lethality in a mature ovum are considered as independent events, the estimated number of undamaged cells in the original sample irradiated with this dose would have been 0.16. This figure compares very closely with the radiosensitivity of oocytes irradiated with 3,000r in metaphase I. At a dose of 5,000r the oogonial cells were practically all destroyed, since mature ova were not produced. Oocytes treated with 5,000r in metaphase I had nearly 1.00 dominant lethal per cell, but those treated in prophase I had only 0.33. These comparisons show that oogonial cells are quite radiosensitive, although the chromosomes are not in a contracted state and differ vastly from metaphase I chromosomes.

The probable sequence of events leading from radiation treatment to death of the embryo can, then, be summarized as follows:

- (1) Irradiation causes various types of breaks in the chromosomes (not ex-

cluding point mutations), which can then either reconstitute in the original configuration or produce aberrations. The type of chromosome aberration formed and the number persisting until oviposition will vary with the stage of the cell irradiated. Possibly the number of breaks formed in various stages may also differ.

(2) When oocytes are irradiated, dominant lethal changes persist until the fertilized egg is laid. When oögonia are irradiated, the more severe types of damage are eliminated during maturation, but some dominant lethals persist to be detected in the zygotes. In *Cochliomyia* the oögonial cells eliminated from the germ line are not replaced immediately, and the number of mature oocytes produced decreases with increasing dose.

(3) Embryonic death may occur any time after the egg is deposited. Time of death will vary with the chromatin condition of the oocytes at irradiation (WHITING 1940). The time of death of embryos bearing dominant lethals induced in oocytes has been investigated by VON BORSTEL (1959) and VON BORSTEL and REKEMEYER (1959). Their results indicated that, in general dominant lethality could be attributed to chromosome loss or severe chromosome imbalance in the embryo; but similar studies of irradiated oögonia are lacking.

SUMMARY

In female *Cochliomyia hominivorax*, radiation-induced dominant lethal changes in the reproductive cells (which develop synchronously) were measured by egg-hatchability tests and correlated with cytological observations of the stage of nuclear development.

(1) In pupae 4–6 days old, only oögonial cells are present. Irradiation at this age reduced the number of mature oocytes produced as the dose increased, but the lowered hatchability of eggs that were produced indicated that some dominant lethals persisted through maturation to be detectable in the embryo.

(2) In old pupae and newly eclosed adults, differentiation of the oocytes and nurse cells takes place. Irradiation of adults less than 24 hours old interfered considerably with egg production; the younger the female at irradiation, the fewer normal eggs were produced.

(3) Irradiation of females older than 24 hours resulted in production of normal numbers of eggs. In three-day-old females the oocyte nucleus is in prophase I, in four-day-old females metaphase I, and in five-day-old females anaphase I. Dosage-response studies indicated that the LD₅₀ radiation dose for oocytes in metaphase I is 1,309r, anaphase I, 1,639r, and prophase I, 7,939r. For oocytes irradiated in the first meiotic division, the relation between dose and dominant lethals induced was linear for the range investigated.

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LITERATURE CITED

- ABRAHAMSON, S., 1961 Chromosome rearrangements induced by X-rays in immature germ cells of *Drosophila*. *Nature* **191**: 523-524.
- VON BORSTEL, R. C., 1959 On the nature of dominant lethality induced by radiation. *Atti Assoc. Genet. Ital.* **5**: 35-50.
- 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, 1959 Radiation-induced and genetically contrived dominant lethality in *Habrobracon* and *Drosophila*. *Genetics* **44**: 1053-1074.
- BOZEMAN, M. L., and C. W. METZ, 1949 Further studies on sensitivity of chromosomes to irradiation at different meiotic stages in oocytes of *Sciara*. *Genetics* **34**: 285-314.
- BUSHLAND, R. C., 1960 Screwworm research and eradication. *Advances in Vet. Sci.* **6**: 1-18.
- BUSHLAND, R. C., and D. E. HOPKINS, 1951 Experiments with screwworm flies sterilized by X-rays. *J. Econ. Entomol.* **44**: 725-731.
- 1953 Sterilization of screwworm flies with X-rays and gamma rays. *J. Econ. Entomol.* **46**: 648-656.
- FLUKE, D. J., 1957 The effect of X-rays on egg hatch and egg laying in *Mormoniella*. *Radiation Research* **7**: 315.
- GOODSPEED, T. H., 1929 The effects of X-rays and radium on species of the genus *Nicotiana*. *J. Heredity* **20**: 245-259.
- GROSCH, D. S., and R. L. SULLIVAN, 1954 The quantitative aspects of permanent and temporary sterility induced in female *Habrobracon* by X-rays and beta radiation. *Radiation Research* **1**: 294-320.
- HOENIGSBERG, H. F., E. GALLUCCI, and A. GIAVELLI, 1961 The oxygen effect in irradiated mature and meiotic germ cells of *Drosophila melanogaster*. *Experientia* **17**: 1-8.
- KING, R. C., 1955 Dominant lethal mutation and X-chromosome elimination after X-irradiation of female *Drosophila melanogaster*. *Radiation Research* **3**: 143-152.
- 1957 The problem of dominant lethals in *Drosophila melanogaster* females. *Proc. Natl. Acad. Sci. U.S.* **43**: 282-285.
- 1960 Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. *Growth* **24**: 265-323.
- 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.
- KING, R. C., A. C. RUBINSON, and R. F. SMITH, 1956 Oogenesis in adult *Drosophila melanogaster*. *Growth* **20**: 121-157.
- KING, R. C., and J. H. SANG, 1959 Oogenesis in adult *Drosophila melanogaster*. VIII. The role of folic acid in oogenesis. *Growth* **23**: 37-53.
- LACHANCE, L. E., 1958 The effect of chelation and X-rays on fecundity and induced dominant lethals in *Habrobracon*. *Radiation Research* **11**: 218-228.
- MULLER, H. J., 1954 The manner of production of mutations by radiation. Vol. I, Part 1, pp. 475-626. *Radiation Biology*. Edited by A. HOLLAENDER. McGraw-Hill Book Co., Inc. New York.
- MULLER, H. J., and G. PONTECORVO, 1942 The surprisingly high frequency of spontaneous and induced chromosome breakage in *Drosophila*, and its expression through dominant lethals. *Genetics* **27**: 157-158.
- OSTER, I. I., 1958 Radiosensitivity. *Genen en Phaenen* **3**: 53-66.
- PAINTER, T. S., and E. C. REINDORP, 1939 Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. *Chromosoma* **1**: 276-283.

- PARKER, D. R., 1955 The origin of dominant lethals in irradiated oocytes of *Drosophila*. *Genetics* **40**: 589.
- 1959 Dominant lethal mutations in irradiated oocytes. *Biological Contributions*. Edited by M. R. WHEELER. Univ. Texas Publ. **5914**: 113-127.
- PONTECORVO, G., 1941 The induction of chromosome losses in *Drosophila* sperm and their linear dependence on dosage of irradiation. *J. Genet.* **41**: 195-215.
- PONTECORVO, G., and H. J. MULLER, 1941 The lethality of dicentric chromosomes in *Drosophila*. *Genetics* **26**: 165.
- REYNOLDS, J. P., 1941 X-ray induced chromosome rearrangements in the females of *Sciara*. *Proc. Natl. Acad. Sci. U.S.* **27**: 204-208.
- SONNEBLICK, B. P., 1950 The early embryology of *Drosophila melanogaster*. Chpt. 2. pp. 62-167. *Biology of Drosophila*. Edited by M. DEMEREC. John Wiley and Sons, Inc. New York.
- SPARROW, A. H., 1944 X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. *Proc. Natl. Acad. Sci. U.S.* **30**: 147-155.
- 1951 Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. *Ann. N. Y. Acad. Sci.* **51**: 1508-1540.
- SPARROW, A. H., and F. FORRO, JR., 1953 Cellular radiobiology. *Ann. Rev. Nuclear Sci.* **3**: 339-368.
- SPARROW, A. H., and M. MALDAWER, 1950 Differential rejoining as a factor in apparent sensitivity of chromosomes to X-ray breakage. *Proc. Natl. Acad. Sci. U.S.* **36**: 636-643.
- SPARROW, A. H., M. J. MOSES, and R. J. DUBOW, 1952 Relationships between ionizing radiation, chromosome breakage and certain other nuclear disturbances. *Exptl. Cell Research*, (Suppl. 2): 245-267.
- SPARROW, A. H., M. J. MOSES, and R. STEELE, 1952 A cytological and cytochemical approach to an understanding of radiation damage in dividing cells. *Brit. J. Radiol.* **25**: 182-189.
- WHITING, A. R., 1940 Sensitivity to X-rays of different meiotic stages in unlaidd eggs of *Habrobracon*. *J. Exptl. Zool.* **83**: 249-269.
- 1945a Effects of X-rays on hatchability and on chromosomes of *Habrobracon* eggs treated in first meiotic prophase and metaphase. *Am. Naturalist* **79**: 193-227.
- 1945b Dominant lethality and correlated chromosome effects in *Habrobracon* eggs X-rayed in diplotene and in late metaphase I. *Biol. Bull.* **89**: 61-71.
- 1950 A modification of the Schmuck-Metz whole-mount technic for chromosome study. *Stain Technol.* **25**: 21-22.
- 1955 Androgenesis as evidence for the nature of X-ray-induced injury. *Radiation Research* **2**: 71-78.