in only chromatid aberrations including a considerable number of simple chromatid deletions and occasional chromatid exchanges

Ultra-violet radiation of the generative nucleus in the pollen tube induces only simple chromatid deletions. The loss of only one of the two chromatids is in accord with the genetic observations that ultra-violet radiation produces primarily fractional endosperm deficiencies in maize. No configurations representing an interchange of chromatin between non-homologous chromosomes were found.

The qualitative difference between the types of breaks induced by x-ray and ultra-violet radiation is tentatively explained by assuming that the sphere of influence of a single x-ray quantum is much greater in area than that of a single ultra-violet quantum. The vast difference in energy values, and the difference in the physical behavior attendant to absorption of the respective quanta supply a possible physical and chemical basis for this variation in degree of effectiveness.

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# *CYTOLOGY AND DEVELOPMENT OF THE EMBRYOS OF X-RAYED ADULT DROSOPHILA MELANOGASTER*

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In 1927 Muller[1] conclusively demonstrated that mutations could be induced in animals by means of x-rays. Exposure of the germ cells of *Drosophila melanogaster* to relatively heavy doses of x-rays resulted in the production of large numbers of mutations of different types, including recessive lethals, semi-lethals and visibles of various kinds. "In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X and in the other chromosomes. Since the zygotes receiving these never developed to maturity, such lethals could not be detected individually, but their number was so great that through egg counts and effects on the sex ratio evidence could be obtained of them en masse." Dominant lethals are, thus, a class of lethals which can effect the death of zygotes even though present in single dose, i.e., introduced by but one of the gametes.

Hanson,[2] shortly thereafter, subjected Drosophila males to x-rays and made counts to determine the extent of the effects of the radiations. Mortality of the $F_1$ eggs, larvae and pupae when compared to the death rate among the controls was found to be significantly increased. He inferred that dominant lethal mutations had been induced in the treated sperm, resulting in death of the zygotes at various stages in their ontogeny. Timofeef-Ressovsky,[3] Gowen and Gay,[4] Sonnenblick,[5] Demerec, Kaufmann and Hoover,[6] Kaufmann[7] and others have also noted the marked increase in mortality among the progeny of irradiated adult Drosophila. Furthermore, the induction of non-transmissible dominant lethals in Habrobracon has been reported.[8, 9]

Although the progeny of treated Drosophila die at various levels of development, the high death rate among the embryos is especially striking. In those zygotes which initially commenced development with one haploid complement of untreated chromosomes and with one irradiated chromosomal complement, the majority of the inviable alterations and rearrangements introduced by the treated gametes are thus eliminated prior to hatching of the young larvae. This report, based on egg counts and the study of preparations of approximately one thousand eggs and embryos, will indicate the nature of the inviable chromosomal alterations and their effects on embryonic development.

A wild type Oregon-R strain of *Drosophila melanogaster* was used in the experiments. Tests with this vigorous stock indicate that more than 96 per cent of the deposited normal eggs hatch as larvae. But 55 of 1420 zygotes deposited by untreated parents failed to emerge from the embryonic envelopes. Five-day-old virgins were employed throughout all of the work. The flies were put into small gelatin capsules which were placed on a sheet of cardboard at a distance of 34 cm. from the tungsten target. The tube was operated at 200 kv. and at 30 ma. current. Under these conditions, the output of the machine, as measured in air, was 390 r per minute. Dosages ranging from 195 r to 4680 r were used. The various exposures were given by stopping the machine at measured intervals and removing the samples in sequence. Males and females, in separate capsules, were simultaneously subjected to the radiations and, following the exposure, mated to untreated virgins. Eggs were collected on the moist inside surface of ripe banana skins and at timed periods of development were fixed and sectioned, securing organisms which were representative of all levels of embryonic development for comparison with controls.

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*Results:* (*a*) *Egg Mortality Counts.*—The testes of adult males carry an abundant supply of sperm[10] which are passed to the mates during copulation. Thus, radiation of the males involves exposure of gametes which had already undergone maturation. In the females, however, the situation is different. The physiological condition of the chromosomes at the time of exposure is not like that obtaining in the males. With the ex-

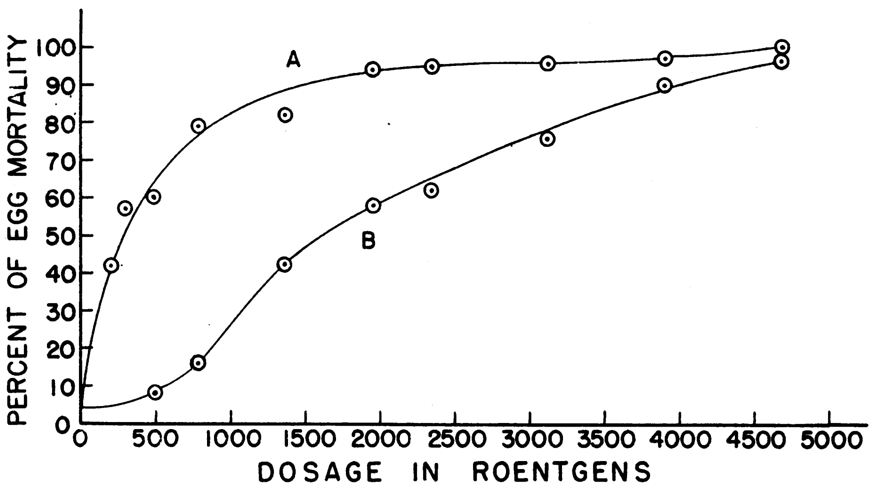
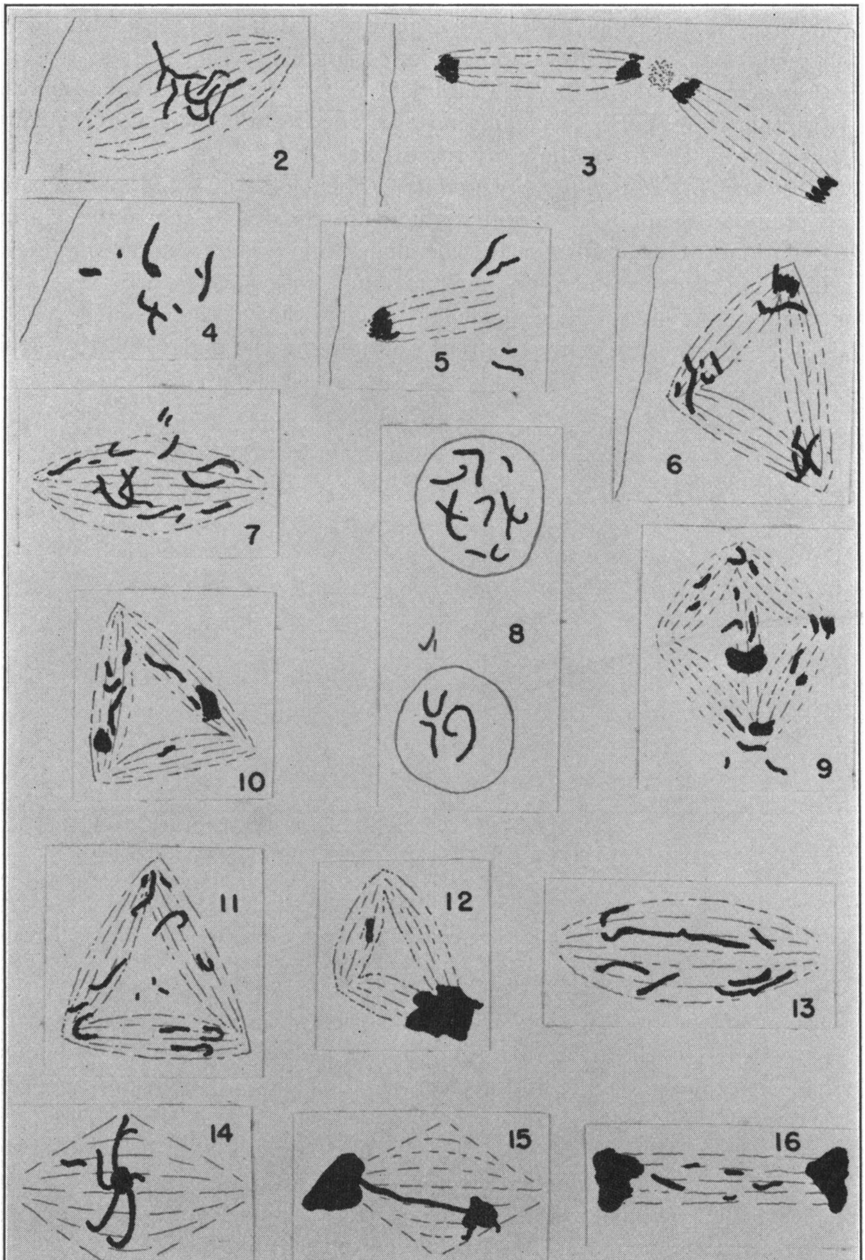



FIGURE 1

Relation between dosage and the percentage of egg mortality. (*A*) Adult females irradiated. (*B*) Adult males irradiated.

ception of the one egg which may be present in the uterus, the other components of the ovarian strings are in a stage not further advanced than metaphase of the initial meiotic division.[11] Following exposure, therefore, the oöcytes and oögonia have yet to pass through the two maturation divisions. Since almost all of the eggs used in this study were collected during the twenty-four-hour period after treatment (a few were collected on the second day), such eggs were the most mature, i.e., closest to first meiotic metaphase, when exposed to the radiations.

Eggs were collected as described above and counts were made in order to determine the number which eventually hatched as larvae. The data are given in table 1, while in figure 1 the percentage of egg mortality is shown plotted against dosage in roentgens. The curves suggested by the




FIGURES 2–16

2, 3. Normal metaphase and telophase of first and second meiotic divisions.

4, 5, 6. Aberrant meiotic figures in eggs deposited by treated females.

points are non-linear and indicate the increase in egg mortality with increasing dosage. The *Y* intercept is at 3.9, representing the average egg mortality in the controls.

There is a decided difference in the sensitivity of the sperm and mature oöcytes to the radiations. The effects of small dosages on the female gametes are very striking. This is evident in the graph, for with dosages up to 780 r the curve *A* rises steeply; from this point the slope diminishes and the curve gradually approaches the maximum. These data follow closely those of A. R. Whiting[12] who irradiated Habrobracon females and determined from daily egg counts that mortality was highest for eggs laid on the first day following the treatment. Curve *B*, representing the variations in egg mortality following exposure of the sperm, has a gradual slope and only at the highest dosage tends to approximate the other curve. The number of affected male gametes, while not directly proportional to dosage, increases gradually.

TABLE 1

| | ADULT FEMALES IRRADIATED (X UNTREATED MALES) | | | ADULT MALES IRRADIATED (X UNTREATED FEMALES) | | |
|---|---|---|---|---|---|---|
| DOSAGE IN ROENTGENS | EGGS DEPOSITED | EGGS NOT HATCHING | % EGGS NOT HATCHING | EGGS DEPOSITED | EGGS NOT HATCHING | % EGGS NOT HATCHING |
| 195 | 458 | 192 | 42.1 | .... | ... | .... |
| 293 | 752 | 432 | 57.4 | .... | ... | .... |
| 488 | 1080 | 656 | 60.7 | 444 | 36 | 8.1 |
| 780 | 564 | 448 | 79.4 | 296 | 48 | 16.2 |
| 1365 | 538 | 454 | 82.2 | 498 | 212 | 42.5 |
| 1950 | 568 | 536 | 94.6 | 620 | 360 | 58.1 |
| 2340 | 772 | 734 | 95.1 | 740 | 460 | 62.1 |
| 3120 | 552 | 528 | 95.7 | 1224 | 928 | 75.8 |
| 3900 | 530 | 516 | 97.4 | 594 | 528 | 88.9 |
| 4680 | 144 | 144 | 100.0 | 251 | 242 | 96.5 |

(*b*) *The Maturation Divisions.*—Entrance of the sperm normally induces the completion of the first meiotic spindle. The chromosomes on the first polar spindle advance as far as metaphase and then pause until the sperm enter the egg, whereupon the divisions proceed. The anastral maturation divisions occur close to the dorsal surface of the egg in an island of protoplasm situated approximately one-fourth of the distance back from the anterior end. The chromatic complexes which separate during the first meiotic division form no vesicular nuclei, but, after a few minutes pass directly upon the second polar spindles (Figs. 2 and 3). The second division proceeds, resulting in four haploid nuclei of which the innermost becomes the female pronucleus. The other three are the polar body

FIGURES 2-16 (*Continued*)

7–16. Types of cleavage figures observed in eggs with treated chromosomes. Fragmentation, chromosome clumping, multipolar configurations are commonly seen. Similar aberrations are noted whether sperm or egg contributes the irradiated chromosomes.

nuclei; these are not extruded from the egg, but remain in the antero-dorsal island and there gradually disintegrate.

In the sections of eggs laid by treated females and fixed between five and sixteen minutes after deposition, various distortions of the maturation figures can be observed. No achromatic figure (Fig. 4) may be present, while the fractured chromosomes are scattered about in the clear protoplasmic island. That this egg was in the stage of polar body formation is indicated by the presence of an unresolved sperm head with its centriole and delicate sperm tail in another section. Occasionally a part of the spindle is missing. The remainder may not have formed at all or, having formed, disintegrated earlier. For example, in figure 5 only a half-spindle with a chromosomal mass at the remaining pole is present; several chromatic elements are lying free in the cytoplasm. In the treated material multipolar spindle configurations rather than the normal bipolar arrangements are sometimes present (Fig. 6). No sperm tails were seen in the island in which the division represented by figure 6 was occurring, thus precluding the possibility that a supernumerary spermatozoon may have established an independent spindle which combined with the first polar body spindle to upset the process of meiosis. The chromosomes and chromosome fragments are distributed over all three poles and it is difficult to establish whether the complex at any one pole is a balanced one. While the maturation divisions are occurring, one of the sperm heads normally becomes large and vesicular and eventually fuses with the resulting female pronucleus. However, if as a result of aberrant divisions no female pronucleus is formed, the sperm head may resolve itself into its haploid complement of chromosomes, but no instance was ever observed in which a haploid sperm complement formed a mitotic figure and began to cleave. In one hundred preparations of early normal eggs, selected at random, only one aberrant meiotic figure was noted. Within the sections of sixty-nine organisms whose mothers had been treated with 2340 r, there were, on the other hand, sixteen with various meiotic abnormalities.

(*c*) *The Early Cleavages.*—During the early cleavages, the egg is a syncytium with the nuclei uniformly scattered throughout the cytoplasm. The nuclei divide synchronously and, at the eighth cleavage, begin their migration to the periphery, arriving in the posterior polar plasm slightly prior to their appearance in the other portions of the egg. Globules of posterior polar plasm, each containing a nucleus and a number of the polar granules, are pinched off; these are the primordial germ cells.

In these normal eggs the mitotic figures are bipolar and equivalent chromosomal groups separate at anaphase. This orderly mode of mitosis is frequently found upset, however, in zygotes developing with treated chromosomes.[13] In such zygotes, chromosome fragmentation, chromatin bridges, asymmetrical divisions, clumping of the chromosomes, multipolar figures of the tripolar and quadripolar types and cytoplasmic vacuolization have been observed. Within the sections of a single egg, there may be found chromatic and achromatic aberrations of various kinds, as well as apparently normal figures. Furthermore, similar aberrations appear in the eggs, regardless of whether sperm or ovum contributed the irradiated chromosomes. It would be impossible to indicate all of the abnormalities noted in the zygotes and the varied modes of interference with the progress of normal development. Therefore, a representative group has been selected for discussion.

A first cleavage figure, with no evidence of gonomery, is indicated in figure 7. Fracture of one or more chromosomes had occurred, and elimination of some fragments appears to be taking place, since some are off the spindle and lying free in the cytoplasm. Other fragments are passing to the poles and may be incorporated in a daughter nucleus. In a slightly later developmental stage, late prophase of the second division, the nuclear membranes are beginning to disappear but the chromosomes are distinct (Fig. 8). An asymmetrical distribution had evidently occurred during the progress of the first division, for the volume of chromatin present in one of the nuclei is definitely greater than in the other nucleus. Lying close to the lower figure are some fragments which stained lightly and which had not been included in the reconstruction nucleus.

Irregularity in the mechanics of mitosis is well manifested through the frequently observed multipolar figures which are found in the sections of eggs of treated parents. Several of these atypical spindle arrangements may be observed within one egg. The chromosomes and fragments are usually found distributed over the various spindles (Figs. 9, 10 and 11), but are also observed clumped at one of the poles (Fig. 12). With the exception of a rod-like fragment, all of the chromosomes in the latter figure are massed at one pole, forming an irregularly shaped clump which stained intensely. Clumping of the chromosomes is often noticed in the experimental material. The radiations alter the consistency of the chromosomes and the change is usually manifested at anaphase when the viscid strands are about to separate. The pycnotic masses take various shapes (Figs. 12, 13, 14, 15 and 16). At times the clumped masses may be connected by chromosomal strands, but often they appear as more or less solid complexes in which the identity of the individual components is lost. It has not been possible to determine whether the complexes at the poles in figures such as have just been referred to can continue to divide. If restitution nuclei do form and cleavage does continue, nuclei with varying chromosome combinations would result.

Indication has already been given above that irregularities in the mitotic mechanism are by no means restricted to one particular type in any one organism. A tabulated presentation of the occurrence of such abnormalities in five organisms which were fixed during the early cleavage divisions is given in table 2. It is interesting to note that among the degenerate aberrant figures lie some which are apparently normal and appear to be capable of continuing to divide.

TABLE 2

| SEX OF TREATED PARENT | DOSAGE | NUMBER AND TYPE OF MITOTIC ABNORMALITIES |
|---|---|---|
| Male | 3900 r | 11 figures containing clumped chromatic masses; 1 quadripolar figure |
| Male | 3900 r | 3 figures with fractured chromosomes; 1 normal figure |
| Male | 3120 r | 1 tripolar figure; 3 figures with clumped masses; 1 normal figure |
| Female | 2340 r | 3 figures with chromosomes and fragments |
| Female | 2340 r | 2 figures with chromosomes and fragments; 1 normal figure |

(*d*) *Later Embryonic Stages.*—Development and morphogenesis proceed at such a rate in Drosophila that after twelve hours of incubation at 25°C. the egg has passed from first cleavage to a relatively highly complex, differentiated organism. During this period, the syncytial cleavages occur, the primordial germ cells are segregated, the malpighian tubules and nervous tissue have made their appearance, metamerism has occurred, the definitive number of salivary gland cells is present, the major portion of the yolk has been enclosed in the mid-gut and a pair of gonads are in their permanent position in the postero-dorsal region of the embryo. Later, other structures appear and during the twentieth and twenty-first hour of development a majority of the young larvae emerge from the embryonic cases. Among the offspring of treated adults are found organisms which apparently differ in no morphological aspect from controls of similar age, together with embryos in which extensive cellular proliferation but no differentiation has occurred. Although the latter are from twelve to sixteen hours of age, no sign of structural organization is noticed. Apart from the yolk inclusions which are concentrated in one or another region of the organisms, these embryos consist of massed aggregates of cells. The cells are of various sizes, often vacuolated and their membranes are frequently not intact, resulting in syncytial-like patches. The cells have no orderly arrangement, but are haphazardly dispersed throughout the cytoplasm. No embryo comparable to these was observed in the controls.

Eggs which had not hatched by the third day following deposition, i.e., some forty-eight hours past the normal modal hatching time, were selected at random and dechorionated. In some of the dechorionated eggs no evidence of development could be seen, but in others embryonic differentiations were visible. These embryos were segmented and larval jaws and tracheae were noted. When removed from the membranes, such embryos would sometimes move their jaws and muscles feebly; others remained in the position in which they were placed and showed no activity at all. In several instances, the larvae had difficulty in emerging wholly from the egg cases, for the envelopes still enclosed the posterior portion of these organisms. Careful removal of the membranes did not induce such individuals to move or show any other activity.

*Summary.*—When Drosophila males and females are exposed to x-rays and then mated with untreated flies, a high percentage of the embryos do not hatch as larvae. Especially susceptible to the radiations are the most mature oöcytes. The treatments have affected the gametes to an extent that, in many cases, orderly development becomes impossible. Varied distortions of the meiotic and mitotic divisions have been observed. Within the sections of single eggs, distortions of various types as well as apparently normal figures have been found. At an age when normal embryos are highly differentiated, some of the embryonic offspring of irradiated parents consist of structureless masses of cells. It is likely that there is a direct relationship between the observed cytologic and developmental abnormalities and the greatly increased percentage of mortality among the progeny of treated adult Drosophila.

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