

optics) and one fiber appears to pull away from the other, the former becoming convoluted. Weaker solutions of acetic acid produce only a slight separation of the fibers. Twenty-five percent solutions of formic or lactic acid as well as a 1:1 solution of acetic acid and ethyl alcohol act similarly to that of aqueous acetic acid. On the other hand, a 1:3 solution of acetic acid in ethyl alcohol, hydrochloric acid, butyric acid, ammonium hydroxide, and formaldehyde have no effect on the tail. We have also found that the enzyme pronase produces a separation of the fibers of the sperm tail but with this treatment there is only a partial separation (i.e., sections of the tail seem to be affected) and both fibers appear to be non-spiralized. However, application of a 25% solution of acetic acid to such separated fibers causes one element of each pair to become convoluted. This uniform differential reaction to acetic acid indicates that the spiralization of one of the fibers of the tail following immediate acid fixation is not somehow related to the actual separation which takes place, but reveals a structural difference between the two elements.

Among the few previously reported accounts of a similar nature on insect spermatozoa were those made by Ballowitz (1890, *Zeit. für Wissen. Zool. Leipzig*) and Retzius (1909, *Biologische Untersuchungen, Neue Folge XIV, Stockholm*). Ballowitz had found that subjecting macerated samples of beetle spermatozoa to hypertonic salt solutions or osmic acid for several days revealed a number of fibers in the tail. We have also tried such drastic treatments with *Drosophila*, but at best only obtained a partial splitting accompanied by much cross-wise fragmentation of the tails of the spermatozoa.

After numerous observations of the preparations, it was noticed that the tails of *Drosophila* spermatozoa which had only been in Ringer's solution often exhibited separation along small areas of their length. In these cases neither fiber appeared spiralized. The slight degree with which this occurs makes it evident why it had not attracted the attention of other investigators who had studied non-fixed material.

At the present time it seems probable to suppose that one of the fibers represents an elongated mitochondrion. On the other hand, it may yet be too early to rule out the possibilities that either the two fibers correspond to the axial filament and its sheath or to any (or all) of the groups of bundles which are discernible with the electron microscope. The functional aspects of these structural relations have yet to be elucidated. However, the fact that a portion of the spermatozoon which had seemed impregnable to further observation by visible light has been dissociated enhances the possibility that the sperm head and the orientation and state of the chromosomal material located therein might also be amenable to such observation provided the proper techniques can be developed. In addition, it should be of interest to determine the nature of these tail elements in spermatozoa of other genotypes (particularly in those bearing two Y chromosomes or disarrangements of the Y chromosome), in spermatozoa which had been treated with various mutagens, and in spermatozoa of other species of *Drosophila*.

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Oster, I. I. The Institute for Cancer Research. Improved strains for detecting somatically-induced damage.

Experiments involving treatment of immature stages of *Drosophila melanogaster* with physical as well as chemical mutagens led us to suggest that the resulting increase in mortality of both the pre-imaginal and post-

imaginal stages was largely due to chromosome breakage followed by loss of essential parts of the genome (Muller, H. J., 1958, Conf. on the Genetic Aspects of Life Shortening by Radiation, Dec. 13 and 14, 1958, Ames, Iowa; Oster, I. I., 1958, Proc. Sec. Austral. Conf. on Rad. Biol.; Oster, I. I., 1959, Science; Oster, I. I., 1960, Science; Oster, I. I., 1960, in Proc. of Conf. on Research on the Radiotherapy of Cancer; Oster, I. I., 1961, The Sec. Int. Conf. of Human Genetics; Oster, I. I. and A. Cicak, 1958, DIS). Several genetical schemes, involving comparisons between males and females, rod-X- and ring-X-bearing males, and normal rod-X-carrying males and attached-X females, were utilized to obtain evidence for this view. Soon thereafter, additional confirmatory data was obtained by Ostertag and Muller (1959, Science), who also extended the work to include comparisons of normal individuals with those heterozygous for small deficiencies (Ostertag, 1963, *Zeit. für Vererbungsl.*).

Based on our previous results we had developed a standard technique for determining whether any particular agent is capable of causing life-shortening via a genetic mechanism. This had consisted of comparing the mortality rates between two strains of males, one carrying a normal rod-X chromosome and the other a ring-X chromosome, which had been similarly treated during one of the pre-imaginal stages. Although this method proved to be very reliable, we thought that it would be useful to devise an even more sensitive one.

This can now be done by combining a ring-X chromosome with two autosomal deficiencies in the following manner (phenotypes are shown in parenthesis):

$$P_1 \ Y/X^{c2}, y \ B; e^{11} \ \sigma\sigma \ (y \ B \ e) \times$$

Cy,InsO

T(2;3)CyD#2

D InsCXF e

$$sc^8 \cdot Y/y \ f; \text{-----} \text{ooooo}$$

$$\text{vg}^{-D}$$

$$\text{ooooo} \text{oooooooooooo} \text{-----} \text{♀♀} \ (y^+ \ f \ Cy \ D \ \text{vg}^{-D} \ Ly)$$

$$\text{oooooooooooooooooooo}$$

$$Ly$$

F₁: Select non-yellow, non-ebony ($y^+ e^+$) larvae for treatment. Third instar larvae should be used since ebony (e) cannot be recognized in the younger stages. These will be males of the composition: $sc^8 \cdot Y/X^{c2}, y \ B; \text{-----} \text{ooooo}$ which can be recognized because

$$\text{vg}^{-D} \quad Ly$$

their mouth parts are black (y^+) and their spiracle sheaths are normal colored (e^+). On the other hand, their sisters will have brown mouth parts and their brothers which do not carry the deficiencies will have darkly-colored spiracle sheaths. Re-combinants with only one of the deficiencies will die during early embryogenesis because of aneuploidy.

Males for the P₁ cross can be maintained with attached-X females carrying a normal Y chromosome while P₁ females can be kept as a balanced stock with males having the same autosomal constitution. Larvae for comparison can be obtained by crossing males carrying a normal rod-X with females which do not contain the two deficiencies. Otherwise these stocks should carry the same markers in order to avoid the introduction of undesirable viability differences. The translocation, T(2;3)CyD#2, between a second chromosome containing $dp^{tx1} \ Cy, InsO \ pr \ cn^2$ and a third chromosome containing $ru \ h \ D \ InsCXF \ e$ was produced especially for this scheme.

The F₁ resulting from this cross offers the advantage of being more sensitive to the induction of somatic damage than individuals containing either only a heterozygous deficiency or a ring-X chromosome. As in the previous methods which we had developed for utilizing the larval stages the present one allows for a more accurate measure of the numbers being actually treated than those involving flies which are not marked suitably. By introducing those few characteristics which can be detected in the immature stages, it is possible to select for treatment and observation only those individuals which possess the desired genotype. This also permits us to study the pattern of damage which may ensue following a particular treatment. In addition, the use of heterozygotes reduces the inviability which may arise due to homozygosis, thereby increasing the background mortality to undesirable (and often unpredictable) levels.

Preliminary tests, using low doses of X rays and gamma rays, have confirmed our expectations for the proposed scheme.

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