MODIFICATION OF X-RAY MUTAGENESIS IN DROSOPHILA.
I. REUNION OF CHROMOSOMES IRRADIATED DURING
SPERMIOGENESIS¹

I. I. OSTER

Department of Zoology,² Indiana University, Bloomington, Indiana

Received February 7, 1955

MULLER (1938, 1939 a, b, 1940) studied the effect of variations in the timing of X-irradiation applied to mature *Drosophila* spermatozoa stored in the seminal receptacles and spermathecae of females and found no difference in the frequency of induced translocations whether the total dose of 2500r was delivered within a period of 25 minutes, divided into four fractions separated by intervals of a week, or extended throughout a month at an intensity of .01r per minute. His finding that such radical differences in timing did not alter the frequency of rearrangements led him to conclude that the broken ends of the spermatozoon's condensed chromosomes do not undergo union before the time of fertilization. Later, using similar techniques, DEMPSTER (1941) and KAUFMANN (1941) confirmed these findings. On the other hand, CATSCH and RADU (1943) presented evidence that union of broken ends does occur in not fully "mature" sperm. In their work irradiation of males with a dose of 4000r delivered within an interval of either 1 hour, 5 hours, or 10 hours and the subsequent testing for translocations in sperm released 4 to 6 days after being X-rayed resulted in the production of fewer translocations in the series in which the X-ray dose was applied over a protracted period of time. They interpreted this to indicate that some of the broken ends of chromosomes produced during the first few hours of the experiment had undergone union before having had a chance to unite with the ends from breaks which were produced during the last few hours of treatment.

Recent experiments by LÜNING (1952 a, b, c, d) and the similar but more definitive ones by AUERBACH (1954) have shown that the less mature stages of spermiogenesis are less resistant to radiation-induced damage than the more mature sperm and that even younger sperm are more resistant or at least exhibit fewer detectable changes. AUERBACH also found that the method and frequency of breeding which was used to obtain samples of sperm for analysis largely determined whether this period of increased sensitivity was detected at all. On the basis of these findings it is possible that the differences observed by CATSCH and RADU may have been due solely to variations in sperm utilization causing the sample analyzed for translocations to come from the more resistant sperm in the fractionated series and from more sensitive sperm in the other series. Because of this and because CATSCH and RADU failed to detect any increase in the frequency of translocations in sperm released a few days

¹ Adapted from a thesis presented for the partial fulfillment of the requirements for the degree of Doctor of Philosophy at Indiana University. This work was supported by a grant, for work of PROFESSOR H. J. MULLER and associates, from the Atomic Energy Commission (At (11-1)-195), and by a grant from the British Empire Cancer Campaign administered by C. AUERBACH.

² Contribution No. 581.

after irradiation in any of their work it was felt desirable to redetermine the time of union of breaks produced in immature spermatozoa by using a technique less liable to be complicated by the variations mentioned above.

It was thought that irradiation of spermatozoa stored in females should provide us with a more nearly homogeneous sample of mature sperm and that irradiation of pupae accurately classified according to age in which there is a preponderance of one or another stage of germ-cell development should provide us with a sample of the earlier stages of spermiogenesis. AUERBACH had shown the latter technique to be ideally suited for treating a given stage of gametogenesis and to be independent of the complications arising from the irradiation of mature males containing all the stages of spermat- and spermiogenesis and the resulting mixing of sperm which was at different stages of development at the time of treatment and less subject to variations in its utilization.

METHODS

Flies of a stock containing a ring-X chromosome (X^a) in the male and yellow (y), forked (f) attached-X chromosomes in the female ($yf:=$) were allowed to oviposit for 1 hour on standard *Drosophila* medium. Five days later males which were in the so-called prepupal stage characterized by a fully formed but colorless puparium were collected and separated into two groups. As this stage of pupal development lasts less than 1 hour it serves as an accurate time mark from which to date the age of the pupae. One group of pupae was irradiated with an X-ray dose of 800r (135 kV and 1 mm thick aluminum filter) at 48 ± 2 hours after the onset of pupation (series I) together with a similarly collected group of pupae which was 72 ± 2 hours after puparium formation old (series II). Upon eclosion at 96 ± 2 hours after pupation, the treated (series I & series II) and untreated (series 0) imagoes were mated by placing 1 male with 3 y *In49 v; bw; e* virgin females in separate vials for 2 days (brood 1). After this period the males and females were separated and the inseminated females from series 0, I, and II were irradiated simultaneously with a dose of 1800r. These females were again allowed to lay eggs until their supply of sperm was exhausted (brood 2). The F_1 males were tested for translocations between autosomes II and III by being backcrossed to y *In49 v; bw; e* virgin females and examining the F_2 offspring for the absence of independent assortment between brown (*bw*) and ebony (*e*) which are not linked in normal flies and the F_1 females were tested for lethals by being mated to y *In49 v; bw; e* males and examining the F_2 offspring for the absence of non-yellow males which would indicate that a lethal had been induced in the paternal X chromosome. All the F_1 cultures which did not yield an adequate number of flies to enable us to determine whether or not a lethal or translocation was present were retested.

RESULTS

The genetic analysis of the three differently treated series of flies yielded the results shown in tables 1 and 2.

Our data are in agreement with the findings of LÜNING and AUERBACH in showing that irradiation with 800r of the stage of gametogenesis present in 48-hour old pupae

TABLE 1
Frequency of sex-linked lethals

Brood 1	Series 0	Series I	Series II
	(Unirradiated)	(Irradiated 48 ± 2 hour old pupae—800r)	(Irradiated 72 ± 2 hour old pupae—800r)
Lethal X chromosomes	0	46	54
Total tested X chromosomes	500	798	2149
% lethals	0	5.76	2.51
Brood 2	(Irradiated mature spermatozoa—1800r)	(Irradiated mature spermatozoa—1800r)	(Irradiated mature spermatozoa—1800r)
Lethal X chromosomes	49	43	25
Total tested X chromosomes	703	284	325
% lethals	6.97	15.14	7.69

TABLE 2
Frequency of translocations involving chromosomes II and III

Brood 1	Series 0	Series I	Series II
	(Unirradiated)	(Irradiated 48 ± 2 hour old pupae—800r)	(Irradiated 72 ± 2 hour old pupae—800r)
Translocations of chromosomes II and III	0	44	17
Total tested sets of paternal chromosomes	0	763	1565
% Translocations	0	5.77	1.09
Brood 2	(Irradiated mature spermatozoa—1800r)	(Irradiated mature spermatozoa—1800r)	(Irradiated mature spermatozoa—1800r)
Translocations of chromosomes II and III	43	25	38
Total tested sets of paternal chromosomes	505	225	257
% Translocations	8.73	11.11	15.95

leads to the production of a higher frequency of lethals and translocations than the application of an equivalent dose of X-rays to older pupae does; these increased frequencies are nearly equal to those produced by treating more mature spermatozoa with a dose of 1800r. Histological examinations by KHISHIN (AUERBACH 1954 and unpublished) have demonstrated that the anterior portions of the testis of 48-hour old pupae contain mainly spermatid and spermatid transforming stages and that irradiation of such individuals results in the production of a high frequency of changes in their cells. Upon eclosion, these treated cells, being at the end of the testis closest to the genital opening are released first with little or no admixture of cells which were at an earlier stage of development (and hence less sensitive) at the time of irradiation.

When the immature spermatozoa of 48-hour pupae were irradiated and then these cells were given an additional dose of X-rays when they had become transformed into

mature spermatozoa, the frequencies of lethals and translocations were increased in only an additive manner. A non-synergistic effect for sex-linked lethal mutations after a fraction of the dose was applied to pupae and the other portion to the sperm in the male was reported previously by TIMOFÉEFF-RESSOVSKY (1934), TIMOFÉEFF-RESSOVSKY and ZIMMER (1935), and KERKIS (1938). However, judging from the relatively low mutation rates obtained after the pupal treatments, their experiments correspond to our series II in which the initial radiation was applied to nearly mature spermatozoa in 72-hour old pupae with similar results. While at the doses used an appreciable number of the lethal mutations are probably not due to 2-break deficiencies and thus an additive result for them is not too decisive, the fact that in our experiments even the translocations were additive indicates that the breaks produced by the first dose had already undergone union by the time that those produced by the second dose were able to reunite.

On the other hand, series II illustrates the inability of breaks induced by irradiation of the almost mature spermatozoa of the 72-hour pupae to undergo recombination. For in this case the translocation frequency, arising when this treatment was followed by irradiation of the same spermatozoa later in the female, was significantly greater than a merely additive result would have given. This shows that breaks produced by the 72-hour pupal irradiation were still "open" at the time when those produced by the irradiation of the sperm in the female were able to undergo union, for cross-union with the latter. Since the earlier evidence, previously cited, shows the latter breaks undergo union only after fertilization, it might be concluded that those induced in the 72-hour pupae likewise remain open until that stage.

In general our results tend to confirm the previous findings of MULLER who found that the condensed chromosomes of mature spermatozoa do not undergo union before fertilization and CATSCH and RADU who reported that the ends of chromosomes broken in less "mature" spermatozoa recombine prior to fertilization. However, as mentioned previously, the latter's results may have been fortuitous and are subject to an alternative explanation, i.e., that in reality they were comparing sensitive with less sensitive cells. Also, it is not possible to rule out as an interpretation of their work the one which LANE has presented to explain a similar reduction in the frequency of translocations. In his plant material he found a seemingly antagonistic effect of fractionated treatments and from this he reasoned that an earlier irradiation may increase the resistance of chromosomes to damage by a later irradiation. On the other hand, our experiments on the induction of lethal mutations and translocations in the same individuals, which yielded additive frequencies for the former and more than an additive one for translocations in the series involving irradiation of spermatids, indicate that an enhancement of radio-resistance was not produced.

Although our present experiments were not designed to throw light on the factors responsible for the increased sensitivity of spermatids to X-rays, it is possible that the drastic physical changes which occur during spermiogenesis are one of the main underlying causes. That is, during the elongation of the spherical spermatid to form the conical spermatozoon and the consequent moving of the chromosomes and probable separation of their parts if broken, reunion rather than restitution would be favored.

SUMMARY

The ends of chromosomes broken during spermiogenesis undergo reunion before fertilization while the breaks produced in mature spermatozoa remain open and reunite during fertilization. In these stages of germ cell development prior irradiation does not lead to an alteration in their reactions to subsequent doses of X-rays.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to PROFESSOR H. J. MULLER for his constant encouragement and helpful suggestions during the course of these experiments. I would also like to thank DR. C. AUERBACH for many stimulating discussions along the lines of the present investigation.

LITERATURE CITED

- AUERBACH, C., 1954 Sensitivity of the *Drosophila* testis to the mutagenic action of X-rays. Zeit. Ind. Abst. Ver. **86**: 113-125.
- CATSCH, A., and GH. RADU, 1943 Die Abhängigkeit der röntgeninduzierter Translokationsrate bei *Drosophila melanogaster* von der Intensität der angewandten Bestrahlungsdosis. Naturwissenschaften **31**: 419-420.
- DEMPSTER, E. R., 1941 Absence of a time factor in the production of translocations in *Drosophila* sperm by X-irradiation. Am. Naturalist **75**: 184-187.
- KAUFMANN, B. P., 1941 The time interval between X-irradiation of sperm of *Drosophila* and chromosome recombination. Proc. Nat. Acad. Sci. U. S. **27**: 18-24.
- KERKIS, J. J., 1938 (On the possibility of increasing the gene mutability by X-ray treatment of the organism at the early stage of development.) Izv. Akad. Nauk SSSR. [Otd. mat.-est., Ser. biol.]: 67-74.
- LANE, G. R., 1951 X-ray fractionation and chromosome breakage. Heredity **5**: 1-35.
- LÜNING, K. G., 1952a X-ray induced dominant lethals in different stages of spermatogenesis in *Drosophila*. Hereditas **38**: 91-107.
- 1952b X-ray induced chromosome breaks in *Drosophila melanogaster*. Hereditas **38**: 321-338.
- 1952c Studies on the origin of apparent gene mutations in *Drosophila melanogaster*. Acta Zool. **33**: 193-207.
- 1952d Studies on X-ray induced mutations in various stages of spermatogenesis in *Drosophila melanogaster*. Doctoral Dissertation, University of Stockholm, A. Bonnier's Press, Stockholm.
- MULLER, H. J., 1938 The remaking of chromosomes. Collecting Net (Woods Hole) **13**: 181, 183-195, 198.
- 1939a Report of investigations with radium. Med. Res. Council Special Report Ser. **236**: 14-15.
- 1939b The mechanism of structural change in chromosomes of *Drosophila*. (Abstr.) Proc. 7th Internat. Genet. Congr., Edinburgh, (J. Genetics Suppl.), 221-222. (1941)
- 1940 An analysis of the process of structural change in chromosomes of *Drosophila*. J. Genet. **40**: 1-66.
- TIMOFÉEFF-RESSOVSKY, N. W., 1934 Einige Versuche an *Drosophila melanogaster* über die Beziehungen zwischen Dosis und Art der Röntgenbestrahlung und der dadurch ausgelösten Mutationsrate. Strahlentherapie **49**: 463-478.
- TIMOFÉEFF-RESSOVSKY, N. W., and K. G. ZIMMER, 1935 Strahlen genetische Zeitfactorversuche bei *Drosophila melanogaster*. Strahlentherapie **53**: 134-138.