THE EFFECT OF LOW TEMPERATURE UPON THE FREQUENCY OF X-RAY INDUCED MUTATIONS

EDWARD D. KING
Harvard University

Received November 15, 1946

INVESTIGATIONS of the combined effect of temperature and X-rays on mutation rate and chromosomal aberrations have given conflicting results. The early work of STADLER (1928, 1930) on Hordeum and that of MULLER (1930) on Drosophila admitted the possibility of a temperature effect. Negative results were obtained by TIMOFÉEF-RESSOVSKY (1934) on mutation rate in Drosophila and by MAKHIJCINI (1944) on chromosomal aberrations. However, MEDVEDEV (1935) did find a higher mutation rate in flies irradiated at low temperatures, and higher frequencies of chromosomal aberrations were found by PAPALASHIVILI (1935), MICKEY (1939) and HERSKOWITZ (1946). In order to clear up any doubt on this fundamental question the author has reinvestigated the problem by using a different technique and a broad range of dosages.

The stocks selected for this investigation were (1) Canton-Special (*C.S.*) for the wild genotype because of its low spontaneous mutation rate and high X-ray sensitivity (DEMEREC 1937) and, (2) sc^8 *B In-S* w^a, known as *Muller-5* (*M-5*) for the crossover-suppressor. This inversion stock possesses certain advantages over the *ClB* used in all earlier lethal-induction investigations. Unlike *ClB*, it is homozygous-viable; sc^8 is a well-known inversion involving nearly the entire genetic map of chromosome 1; *In-S* is an inversion within sc^8. The combination of an inversion within an inversion reduces crossing over to practically nil, which is not true for the *ClB* stock. The major sc^8 inversion contains the dominant duplication mutant Bar eye (*B*) and the recessive mutant apricot eyecolor (w^a). Also, unlike *ClB*, all socalled nondisjunctional females can be eliminated with absolute certainty on the basis of the recessive apricot eyecolor.

The *C.S.* males for raying, both experimental and control, were removed from the stock cultures and isolated in culture bottles for three days prior to treatment, in order to build up a large mature sperm reservoir. For treatment these males were placed in small gelatin capsules. The experimentals were placed in a refrigeration unit and the controls into an identical unit but with no refrigeration.

These units consisted of paraffined cylindrical paper "ice cream" containers. A pint-size container with a paraffin floor was fixed firmly into the deep paraffin floor of an outer gallon-size container. Each container top was made into a cellophane window. The unit for the cold series was kept in a 0.5°C constant temperature vault and on the day for raying, the space between the containers was filled with crushed ice.

The capsules containing the *C.S.* males for the cold series were placed in the refrigerator unit 30 minutes prior to raying, in order to lower their temperature to 0.5°C. For raying, the units were placed, one at a time, under a standard COOLIDGE X-ray tube with the flies 20 cm distant from the target. The five dosages used were 600 r units and multiples up to 3600 r units, with the exception of 3000 r units. No filter was used.

Virgin *M-5* females were mated with X-rayed *C.S.* males. The F_1 *M-5/C.S.* females were "caged" with *M-5* males for several days, after which they were transferred to shell vials, one female to two males per vial. In F_2, if no lethal were induced, the males should be of two very distinct phenotypes, namely, *C.S.*, round red eyed, and *M-5*, Bar apricot. On the other hand, were a lethal induced, the males should be only Bar apricot.

The X-ray controls were irradiated at room temperature. Although the parents of both experimentals and controls were not discarded during the following ten-day period, only the heterozygous F_1 females of the first six days emergence were chosen for study.

The results are shown in table 1. The average frequency of lethal mutations for all dosages was found, on the average, to be 2.30 times greater for the low temperature series than for the room-temperature series. For each dosage alone this excess was as follows:

600r units—3.09×
1200r units—2.07×
1800r units—2.17×
2400r units—1.84×
3600r units—2.33×

Plotting the frequencies of lethal mutations against dosage for each of the two series two divergent straight-line curves result, lines on which the frequency of any dosage whatsoever under the same experimental temperature should with reasonable expectancy fall. All the mutation values recorded are in terms of total lethality and do not include borderline semi-lethals.

No lethal appeared in either the nonirradiated control series so low temperature alone under the conditions of the experiment did not induce lethal mutations.

Sterility in the cold temperature series gives added proof of the effect of low temperature. Cold treatment without irradiation seemed to improve viability, compared with the flies irradiated at room temperature. Referring to the table it will readily be seen that for cold treatment with irradiation a sharp drop in viability results with increase in dosage, whereas the viability fluctuations in the warm series give no such curve. Although a different type of curve, the variation for viability at the low temperature is equally as smooth as that for mutation frequency.

MULLER (1930) observed what seemed to be a temperature effect as expressed in lethal frequency of flies irradiated at about 2300 r units at a low temperature (8°C) and at a high temperature (34°C), 8.2 percent and 6.2 percent respectively. At that time he was inclined to interpret it as a secondary radiation effect but was not satisfied with that explanation. Had MULLER ir-

radiated his flies at or close to 0°C his results would have been much more convincing in reference to the effect of low temperature. However, his values are in accord with what might be expected for an intermediate temperature. Following the suggestion of MULLER, MEDVEDEV (1935) repeated the experiment and found that a definite difference in lethal mutation frequencies did exist between his cold series at 0°C and at room temperature. Using three

TABLE 1

Data on X-ray induced mutations at high and low temperatures.

DOSAGE	TEMPERATURE	AVERAGE $M5/+$ PER CULTURE BOTTLE	% VIABILITY	CHROMOSOMES TESTED	LETHALS	% LETHALS
Control	25°	265	88.33	300	0	0
Control	0°	280	94.33	322	0	0
600	23°	201	67.00	118	1	0.85
600	0°	239	98.74	304	8	2.63
1200	27°	271	90.33	254	10	3.94
1200	0°	125	41.67	245	20	8.16
1800	23°	332	110.67	384	22	5.73
1800	0°	68	22.67	185	23	12.43
2400	26.8°	334	111.33	280	26	9.29
2400	0°	59	19.67	111	19	17.12
3600	28°	213	71.00	741	85	11.52
3600	0°	16	5.33	153	41	26.80

dosages 2500 r, 6000 r and 7000 r he obtained approximately twice as many lethal mutations at low as compared with room temperature; for low temperature 5.9 percent, 10.00 percent and 13.24 percent respectively as compared with room temperature 3.48 percent, 5.75 percent and 8.60 percent respectively. Independently, PAPALASCHWILI (1935) found that the combined action of X-rays and low temperature, 0° and lower, increased the frequency of translocations as compared with the action of X-rays alone. MICKEY (1939) likewise, following the implications contained in the investigations of PAPALASHWILI, found that, as with lethal mutations, low temperature combined with X-ray produced approximately twice as many translocations as X-ray at room temperature.

Since irradiation at low temperature produces an increase of both lethal mutations and chromosomal aberrations there is reason to believe that the two events may be related. Not only is low temperature more effective in producing X-ray induced mutations and chromosomal aberrations, but the dosage relations are linear in both cases. The recent work of LEA and CATCHESIDE (1945)

and of HERSKOWITZ (1946) shows that the two responses—lethal mutation and chromosome breakage—are related and that the great majority of X-ray induced lethals are probably produced at points of breakage. It is therefore to be expected that the frequency of both lethal mutations and chromosomal aberrations should respond in a similar manner to irradiation at low temperatures and show similar relations to X-ray dosage.

In response to criticisms relative to dosage effect of ice as opposed to air, tests indicate only 5.6 percent increase due to the use of ice in the apparatus.

SUMMARY

Special stocks of Drosophila were irradiated at various dosages from 600 to 3600 r at room temperature and at 0.5°C. For all dosages the frequency of induced lethal mutations were greater at the low temperature, with an average increment of $\times 2.3$. The relation between dosage and frequency of lethal mutations was linear at both temperatures.

ACKNOWLEDGMENT

The author wishes to acknowledge his gratitude to DR. H. J. MULLER for the use in these investigations of his inversion stock.

LITERATURE CITED

DEMEREC, M., 1937 Frequency of spontaneous mutations in certain stocks of Drosophila melanogaster. Genetics **22**: 469–478.

DEMEREC, M., 1937 The Gene. Carnegie Institution of Washington, Y.B., p. 45.

HERSKOWITZ, I. H., 1946 The relationship of X-ray induced recessive lethals to chromosomal breakage. Amer. Nat. **80**: 588–592.

LEA, D. E., and D. G. CATCHESIDE, 1945 The relation between recessive lethals, dominant lethals, and chromosome aberrations in Drosophila. J. Genet. **47**: 10–24.

MAKHIJANI, J. H., 1944 The ineffectiveness of temperature in influencing the production of mutations by X-rays. Bombay University Journal **13(16)**: 1–13.

MEDVEDEV, N. N., 1935 The contributary effect of cold with irradiation in the production of mutants. Acad. des. Sci. URSS, Comptes Rendus **4(9)**: 283–285.

MICKEY, G. H., 1939 The influence of low temperature on the frequency of translocations produced by X-rays in Drosophila melanogaster. Genetica **21**: 386–407.

MULLER, H. J., 1930 Radiation and genetics. Amer. Nat. **64**: 220–251.

PAPALASHWILI, G., 1935 The effect of a combined action of X-rays and low temperature on the frequency of translocations in Drosophila melanogaster (in Russian). Biologicheskii Zhurnal **4**: 587–591.

STADLER, L. J., 1928 The rate of induced mutation to dormancy, temperature, and dosage. (Abstract) Anat. Record **41**: 97.

STADLER, L. J., 1930 Some genetic effects of X-rays in plants. J. Hered. **21**: 3–19.

TIMOFÉEF-RESSOVSKY, N., 1934 The experimental production of mutations. Biological Reviews **9**: 411–457.