MODIFICATION OF THE FREQUENCY OF CHROMOSOMAL REARRANGEMENTS INDUCED BY X-RAYS IN DROSOPHILA. III. EFFECT OF SUPPLEMENTARY TREATMENT AT THE TIME OF CHROMOSOME RECOMBINATION

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THE two preceding papers of this series have described the effects of near infrared radiation (λ ca. 10,000 Å) and of ultraviolet radiation (λ 2,537 Å) in modifying the frequency of chromosomal rearrangements induced by X-ray treatment of the spermatozoa of *Drosophila melanogaster* (KAUFMANN, HOLLAENDER, and GAY 1946; KAUFMANN and HOLLAENDER 1946). In the course of the first series of experiments, females that had been inseminated by X-rayed males deposited eggs during the period of exposure to the near infrared radiation. Larvae developing from these eggs provided material for determining the effects of such exposure on chromosome recombination. In order to compare the effect of temperatures within the range customarily used in raising laboratory cultures of Drosophila, other groups of females inseminated by males receiving identical X-ray treatment were kept at either 18°C or 28°C during the period of fertilization and early cleavage.

Drosophila provides suitable material for measuring the influence of temperature and other conditions on chromosome recombination apart from any effect on the initial production of the breaks by X-rays. According to the interpretation of MULLER (1940) and KAUFMANN (1940, 1941), based on studies of fractionation of the X-ray dose, as well as collateral lines of evidence, the potential breaks induced by irradiation of the mature spermatozoa of the adult male are not utilized in forming new combinations of chromosomes until after the sperm nucleus has penetrated the egg in fertilization. Thus, in the experiments here reported, to measure the effect of temperature on chromosome recombination, several males were treated with a 4,000-roentgen dose of X-rays, mated with virgin females of the same stock (Oregon-R), and kept at 18°C for about 36 hours. The males were then discarded, and the inseminated females divided into three groups, one of which was put into an 18-degree incubator, another into a 28-degree incubator, and the third exposed to near infrared radiation during the period of oviposition. Subsequently the eggs were transferred to the 18-degree cold room for completion of the embryonic and larval development. Eggs were collected at approximately 12-hour intervals; no effort was made to handle them individually so as to determine accurately the termination of the period of syngamy and early cleavage during which chromosome recombination is presumably effected. Thus, varying proportions of the earlier embryonic development in individuals of two of the three groups were completed either at 28 degrees or while exposed to the near infrared radiation. Determination of the fre-

quency of chromosomal rearrangements and the number of breaks involved was made by a cytological analysis of the salivary glands of the third-instar female larvae.

MULLER and PONTECORVO (MULLER 1940) found that temperatures as different as 13°C, 24°C, and 29°C at the time of fertilization produced no significant differences in the frequencies of X-ray-induced translocations between the second and third chromosomes of *D. melanogaster*. Analysis by genetic methods readily permitted detection of translocations between these two chromosomes, but furnished no indication of the complexity of the rearrangements. The cytological method used in the present study offers an opportunity for the detection of breaks in all the chromosomes, except those breaks restricted to heterochromatin, so that intrachromosomal rearrangements as well as interchromosomal exchanges may be considered.

RESULTS

Analysis of 906 pairs of glands provided the data presented in table 1. The group of eggs exposed to the near infrared radiation provided 37.50±2.98

TABLE 1

Frequency of chromosomal rearrangements induced by 4,000 r of X-rays.

TEMPERATURE DURING FERTILIZATION	TOTAL SPERMS TESTED	REARRANGEMENTS IN SPERMS TESTED		% SPERMS SHOWING CHANGES	NO. OF BREAKS OBSERVED	BREAKS PER 100 SPERMS	MEAN BREAK NUMBER
		ABSENT	PRESENT				
18°C	329	232	97	29.48±2.51	238	72.34	2.45
28°C	313	216	97	30.99±2.61	253	80.83	2.61
Near infrared	264	165	99	37.50±2.98	266	100.76	2.69

percent of rearrangements, a value that differs significantly from the 29.48 ±2.51 obtained in the 18-degree sample ($\chi^2=4.253$; N=1; P=ca. .036). Differences were not significant between the 18- and 28-degree samples, or between the latter and the near infrared group, with respect to the frequency of rearrangements. A further test, using the χ^2 method, was made by comparing the frequency of rearrangements in the near infrared material with that obtained in a group of about 1,800 pairs of glands from a series of experiments in which temperatures of 18°C or 28°C were maintained during syngamy and early cleavage stages. The results, presented in table 2, indicate that the probability is low (P=ca. .02) that the differences are due entirely to sampling errors. It appears, therefore, that near infrared radiation, applied at the time that chromosome recombination presumably is effected, increases the frequency of rearrangements that are detectable by analysis of salivary-gland cells.

The increased frequency of rearrangement is not attributable to selective recombination of specific chromosome regions. The breaks are distributed among the chromosomes approximately in proportion to their lengths, and the χ^2 test indicates that the probability is high (P = ca. .72) that differences in distribution among the 18-degree, 28-degree, and near infrared groups may be attributed to errors of sampling.

DISCUSSION

The effect of near infrared radiation applied during the period of oviposition in increasing the frequency of X-ray-induced chromosomal rearrangements contrasts with that produced by temperatures within the range of 18 to 28°C. The efforts of MULLER and PONTECORVO to modify the frequency of chromosome recombination at the time of fertilization were carried out within the

TABLE 2

Frequency of chromosomal rearrangements induced by 4,000 r of X-rays. Comparison of effect of near infrared radiation during syngamy and early cleavages (264 pairs of glands) with effect of temperatures in the range from 18 to 28°C (1,842 pairs of glands).

	DISTRIBUTION AMONG SPERMS TESTED		
	WITHOUT REARRANGEMENT	WITH REARRANGEMENT	TOTAL
Observed	165	99	264
Expected	(181.33)	(82.76)	
Observed	1,281	561	1,842
Expected	(1,264.67)	(577.24)	
	$\chi^2 = 5.326$ N = 1 P = ca. .02		

temperature range customarily used for raising cultures of *D. melanogaster.* Most of the previous experiments designed to measure the effect of temperature on X-ray-induced breaks in Drosophila have been concerned with the period during which the X-ray treatment was administered, in order to determine whether the ionization initiated chemical reactions that were modifiable by temperature, or whether there was a more direct effect on the chromonemata. The earlier studies of PAPALASHWILI (1935) and MICKEY (1939), which indicated that low temperatures increase the frequency of chromosomal rearrangements, were not confirmed by the extensive work of MULLER and MAKHIJANI (MULLER 1940) on translocations between the second and third chromosomes and on sex-linked lethal mutations, which are known to include some chromosomal rearrangements. The latter finding, also reported earlier by MULLER (1930) has been confirmed by TIMOFÉEFF-RESSOVSKY and ZIMMER (1939). In addition, PLOUGH and EHRENFELD (PLOUGH 1941) found that the production of translocations is not appreciably influenced by temperature, or by temperature shocks (when larvae are treated).

The more extensive data provided by studies on plant chromosomes are complicated by the fact that in such a genus as Tradescantia breakage and

recombination occur concurrently. Lower temperatures (ca. 3°C) at the time of irradiation generally result in increased frequency of aberrations as compared with higher temperature (ca. 33°C), probably by delaying restitution so that the broken ends are available for establishing rearrangements (SAX and ENZMANN 1939; FABERGÉ 1940; RICK 1940), although SAX and ENZMANN report that a reversed effect of temperature occurs when the chromosomes of Tradescantia are irradiated during the earliest prophase stages.

Near infrared radiation also effects an increase in the frequency of chromosomal rearrangement when used prior to X-rays. Various possible modes of action have been considered in the first publication of this series (KAUFMANN, HOLLAENDER, and GAY 1946). The amount of energy available (ca. 1.2 electron volts for λ 10,000 Å) is perhaps sufficient to induce molecular rotation and to initiate low-grade chemical reactions. Because of these properties, the effective action of radiation of this type is probably not limited to an extension of the range of temperature within which fertility and viability may be maintained in Drosophila, but may depend on selective action on certain cellular components (KAUFMANN, HOLLAENDER, and GAY 1946). For a more specific definition of these properties, an extension of our knowledge of infrared absorption spectra in biological materials will be required, as well as studies of the effects of various other wave length regions of the infrared. In more general terms, any cellular changes induced by absorption of near infrared radiation that facilitate chromosome movement at the time that the potential breaks become available to participate in recombination will provide the physical basis for increased frequency of recombination as compared with that of untreated material.

SUMMARY

Eggs deposited by females of *Drosophila melanogaster* that had been inseminated by males previously exposed to 4,000 roentgens of X-rays were kept either at 18°C or at 28°C, or exposed to near infrared radiation (λ ca. 10,000 Å) during the period of syngamy and early cleavage in order to measure the effect of these agents on chromosome recombination.

The frequency of chromosomal rearrangements detected by analysis of salivary gland chromosomes was higher in the group exposed to the near infrared radiation than in the groups kept at 18 or 28°C (table 1). The χ^2 test indicates that the values obtained following the use of near infrared are significantly higher than in the large group of about 1,800 pairs of glands secured in a series of experiments in which temperatures of 18°C or 28°C were maintained during syngamy and early cleavage stages.

Although it increases the temperature within the organism during the period of exposure, the effective action of near infrared radiation in facilitating recombination among the breaks induced by the ionizing radiation is probably not limited to an extension of the range of temperature within which fertility and viability may be maintained in Drosophila, but may depend on selective action on certain cellular components.

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