TEMPERATURE EFFECTS ON X-RAY INDUCED CHROMOSOME ABERRATIONS

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SEVERAL investigations have shown that the frequency of X-ray induced chromosome aberrations may be changed by varying the temperature at which the nuclei are irradiated. Irradiation of Tradescantia microspores at high temperatures have produced less aberrations than did the same X-ray dose at low temperatures (SAX and ENZMANN 1939; FABERGÉ 1940; CATCHESIDE, LEA, and THODAY 1946). Such results are not attributed to the temperature effect on the incidence of breakage, but to changes in the frequency of new associations of the broken chromosomes. DARLINGTON and LACOUR (1945), on the other hand, find no temperature effect on the frequency of X-ray induced chromsome aberrations and attribute the earlier described results to the effect of temperature on the time scale of nuclear development or to changes in the nucleic acid metabolism.

The utilization of X-rays in relation to dosage, the time-intensity factor, stage of nuclear development and temperature effects should provide data of value in interpreting the nature of chromosome breakage and reunion which is a normal event in meiosis. Temperature experiments have been continued in an attempt to determine more exactly the relation between temperature and X-ray effects.

Microspores of *Tradescantia paludosa* were maintained at different temperatures shortly before—for about ten minutes—and during irradiation by immersing inflorescences in a carton of water at a given temperature. Longer temperature exposures were maintained by immersing the cartons in large bowls of water which was maintained at a relatively constant temperature. After the temperature treatment and irradiation the inflorescences were kept in cartons of water by inserting the cut stems through holes punched in the covers. These were kept in the greenhouse until the time of fixation. The greenhouse temperatures varied considerably, but each series of experiments was subjected to comparable environmental conditions. The microspores were fixed at four or five days after irradiation.

Microspores were smeared on a dry slide, fixed in alcohol-acetic and stained with acetocarmine. Analysis of chromosome aberrations was based on an examination of five or six slides with the total number of division figures ranging from 1800–3000 for the lower X-ray doses to 450–600 for microspores which had received heavy dosage. Such an analysis gives aberration frequencies which, under comparable experimental conditions, rarely deviate more than ten percent.

The experiments were designed to test the frequency of X-ray induced chromosome aberrations when the microspores were subjected to different temperatures before, during and after irradiation respectively. Microspores were subjected to the different temperatures for about ten minutes before, during, and for several minutes after irradiation. Aberration frequency of the ring and dicentric chromosomes was three to four times as large for the cold series as for the microspores irradiated at 36°. The frequency at 22° was more nearly that of the 3° series although at 400 r the number of aberrations were intermediate between the 3° and 36° series. These results, shown in table 1, are in accord with previous experiments. At 3° the aberration frequency in-

TABLE 1

The effect of different temperatures during irradiation on X-ray induced aberrations. Combined data of four experiments. 14,748 chromosomes.

DOSE R. UNITS	% RINGS AND DICENTRICS ×2			% ROD DELETIONS	
	3°	±22°	36°	3°	36°
100	1.8	1.8	0.6	0.9	0.8
120	2.2	—	0.7	—	—
200	7.1	5.5	2.3	1.8	1.4
240	11.6	—	3.3	—	—
400	29.0	18.2	6.7	4.2	3.8
Ave.	10.3	8.5	2.7	2.3	2.0

creases approximately as the square of the dosage while at 36° the exponent of the dosage curve is approximately 1.5. The high temperature seems to have an effect on X-ray dosage response similar to low X-ray intensity or fractional dosage (SAX 1941).

The simple rod deletions, which presumably arise from single breaks, differ from the 2-hit aberrations in two respects. They increase in frequency in direct proportion to X-ray dosage and they show little or no response to different temperatures during irradiation. These results are in accord with those of CATCHESIDE *et al.* and support the earlier conclusions that the incidence of breaks induced by X-rays is independent of temperature.

The temperature effect on the 2-hit aberrations seems to be due to the effect on the union of broken ends of chromosomes in new associations. Since the incidence of X-ray breakage seems to be independent of temperature the effect of temperature differences should occur after the breaks are induced. Fractional dosage and dosage intensity experiments indicate that induced breaks either undergo restitution or form new associations within a relatively short time although some breaks may remain open for perhaps hours. However most exchanges occur within a few minutes of the occurrence of the breaks. If microspores were subjected to different temperatures soon after irradiation a temperature effect might be expected. Experiments were de-

signed to test the temperature effect both during and after irradiation. The results are shown in table 2. In these experiments any change of temperature after irradiation was made as rapidly as possible but it required several minutes to transfer the inflorescences to the cartons at different temperatures and for the effective temperature change of the microspores. The results of temperature differences during irradiation were consistent and in accord with previous experiments. The effects of altered temperatures after irradiation were not consistent. The experiments were repeated many times and the average frequencies of aberrations indicate little or no effect of temperature

TABLE 2

The effect of different temperatures during and after irradiation on the frequency of X-ray induced chromosomal aberrations. Combined data of 27 experiments—97,200 chromosomes. Frequency in percent of dicentric and ring chromosomes ×2. X-ray dose 320 r at 70 r/m.

TEMPERATURE AFTER IRRADIATION	TEMPERATURE DURING IRRADIATION			AVE.
	3°	±22°	36°	
3°	21.2	15.3	9.2	15.2
22°	20.3	17.0	7.7	15.0
36°	22.5	18.1	9.8	16.8
Ave.	22.0	16.8	8.9	

after irradiation. The most variable results were obtained when the microspores were irradiated at 36° and then transferred to 3°. If there is a temperature effect after irradiation it must be limited primarily to a period of several minutes after X-ray treatment. Perhaps the variability in the results of different experiments was due in part to the variation in time of transfer from one temperature to another, but the cause of some of this variation is obscure.

If the temperature effect on aberration frequency is due to changes induced in the chromosomes the temperature changes before irradiation might be responsible for changes in aberration frequency. In our previous experiments the differential temperature treatment was started about ten minutes before irradiation and terminated several minutes after exposure. DARLINGTON and LACOUR (1945) have suggested that our earlier temperature effects were due to the effect of temperature on the nuclear cycle and nucleic acid metabolism. This conclusion seems rather naive in view of the duration of the temperature treatment, the differential response of 2-hit and 1-hit aberrations, and the apparent absence of any significant change in rate of nuclear development resulting from temperature changes after irradiation. Nevertheless we subjected Tradescantia microspores to different temperatures for one hour. They were then transferred to cartons of water at room temperature for ten minutes before raying. The results are shown in table 3, There is little or no effect of the pretreatment in relation to aberration frequency, especially when com-

pared with the effects of the same temperatures during irradiation. It is possible of course that several days or weeks of pretreatment would have an effect on X-ray sensitivity, but in our experiments the relatively short duration of temperature shock before raying seems to play no part in altering the chromosomes in relation to aberration frequency produced by X-rays.

TABLE 3

Effect of temperature differences before irradiation. Rayed at 21°, 240 r at 70 r/m—5184 chromosomes.

PRE-TREATMENT (1 HR.)	RAYED AT	% RINGS & DICENTRICS
3°	22°	13.2
36°	22°	11.1

SUMMARY

An analysis of X-ray induced chromosomal aberrations of Tradescantia microspores shows that—(1) The frequency of ring and dicentric chromosomes is increased several times by subjecting microspores to low temperatures during irradiation although temperature differences have little effect on the frequency of rod deletions. (2) Temperature changes induced several minutes after irradiation have no consistent effect on aberration frequency. (3) Low temperatures for as long as an hour before irradiation have comparatively little or no effect on aberration frequency. The effect of temperature on X-ray induced chromosomal aberration frequency appears to be limited to the period during, or very shortly after, irradiation.

LITERATURE CITED

CATCHESIDE, D. G., D. E. LEA, and J. M. THODAY, 1946 The production of chromosome structural changes in Tradescantia microspores in relation to dosage, intensity and temperature. J. Genet. **47**: 137–149.

DARLINGTON, C. D., and L. F. LACOUR, 1945 Chromosome breakage and the nucleic acid cycle J.Genet. **46**: 180–251.

FABERGÉ, A. C., 1940 An experiment on chromosome fragmentation in Tradescantia by X-rays J. Genet. **39**: 229–248.

SAX, KARL, 1941 Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spring Harbor Symp. on Quant. Biol. **9**: 93–101.

SAX, KARL, and E. V. ENZMANN, 1939 The effect of temperature on X-ray induced chromosome aberrations. Proc. Nat. Acad. Sci. **25**: 397–405.