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THE EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME ABERRATIONS

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Most of the experimental work on temperature effects during or following irradiation shows that the higher temperatures cause more injury to living cells than low temperatures. Greater injury at higher temperatures was found in irradiated Drosophila eggs by Packard, in Ascaris eggs x-rayed at different temperatures by Dognon, and in chick embryos which were incubated at various temperatures after irradiation by Strangeways and Fell.[1] In a review of the temperature coefficient of radiation effects on living cells Clark also refers to the greater effectiveness of irradiation with high temperatures in producing erythema or in killing tumor cells.

On the other hand Mottram[2] found that the growth of *Vicia faba* roots was inhibited more when treated with gamma rays at low temperatures than when irradiated at high temperatures. Spear[3] also found that tissue cultures exposed to gamma rays at 1°C. and at 37°C., respectively, showed slower recovery at the low temperature. According to Muller,[4] Paplashvilli found more chromosome rearrangements in Drosophila when x-rayed at low temperatures. Mickey[5] also found more translocations in Drosophila rayed at low temperature. The frequency of translocations was almost twice as great when the flies were irradiated at 4°C. than when rayed at 28°C. to 33°C.

The frequency of chromosome aberrations is increased by high temperatures or by x-rays. The apparent reduction of chromosome aberrations in cells irradiated at high temperatures calls for further analysis. Such an analysis may also have some bearing on the nature of gene changes since there is some evidence that the temperature effect on x-ray induced mutations is similar to the temperature effect on x-ray induced chromosome aberrations.

Experimental Results.—The experimental work is based on an analysis of x-ray induced chromosome aberrations in Tradescantia microspores. The flowering stalks were placed in thermos bottles or pasteboard cartons which were filled with water at the desired temperatures. The flower stalks were placed in the containers five minutes before raying and, in most cases, were not removed for an hour after raying. They were then placed in vials of spring water and held at room temperature.

The microspores were fixed at 24 hours, 48 hours and 5 days after raying. The division figures obtained at 24 hours were from irradiated prophase nuclei when each chromosome consists of two sister chromatids. The chromosome aberrations at this time include only chromatid breaks, either simple deletions caused by single hits, or chromatid exchange or fusion between different chromosomes which are caused by two independent hits. The divisions obtained 48 hours after raying were from irradiated nuclei at very early prophase when splitting of chromosomes was beginning. The aberrations induced at this stage include both 1-hit and 2-hit chromatid aberrations, and chromosome aberrations induced before chromosome splitting. The latter are almost exclusively 2-hit breaks. The chromosomes fixed 5 days after raying were in the resting stage when rayed and the aberrations include dicentric and ring chromosomes which are 2-hit aberrations. Very few 1-hit aberrations appear at metaphase from irradiated resting nuclei, where the chromosomes are in the form of single threads. This timing of the nuclear cycle applies to the spring months. It is shorter in the summer. All exposures were made at 134 K. V. 10 ma. The radiation intensity was varied by changing the target distance. Each experiment included flowers from a single plant or a clonal line.

TABLE 1

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME ABERRATIONS

300 r 6 min. Fixed 5 days after raying.

TEMPERATURE	TOTAL CHROMOSOMES	BREAKS	% BREAKS
30°C.	1938	152	7.8
3°C.	1746	238	13.6

In the first experiment the flower buds were irradiated at 3° and at 30°C., respectively, for 6 minutes at 50 r/m. The flower stalks were removed from the cartons a few minutes after raying and kept at room temperature. The frequency of chromosome breaks at the two temperatures is shown in table 1. The chromosome breaks were nearly twice as frequent at the lower temperature.

TABLE 2

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMATID BREAKS

180 r $2^1/_2$ min. Fixed at 25–26 hrs.

TEMPERATURE	TOTAL CHROMOSOMES	BREAKS SINGLE		EXCHANGE		TOTAL
3°C.	1326	90	6.8%	58	4.4%	11.2%
30°C.	1746	52	3.0%	38	2.2%	5.2%

CHROMOSOME BREAKS

540 r $7^1/_2$ min. Fixed at 5 days.

TEMPERATURE	TOTAL CHROMOSOMES	BREAKS DICENTRIC	RINGS	TOTAL	
3°C.	6600	872	256	1128	17.1%
30°C.	6432	420	136	556	8.6%

The experiment was repeated, with both high and low dosage, so that both chromatid and chromosome breaks could be analyzed in adequate numbers. The buds were placed in the cartons 10 minutes before raying and removed to room temperature 10 minutes after raying. The results are shown in table 2. In the material fixed at 25–26 hours after raying both the single and exchange chromatid breaks were twice as frequent at the lower temperature. For the analysis of chromosome breaks the dosage was increased to 540 r, since the resting stage of the nucleus is less sensitive than the prophase. Again the chromosome aberrations were twice as frequent in the buds rayed at the lower temperature.

The greater frequency of chromatid and chromosome aberrations when the nuclei are rayed at the lower temperature may be due either to greater frequency of induced breaks, or to differences in frequency of fusions of broken ends of chromatids and chromosomes. Since the initial break presumably is caused by a photochemical reaction, it is probable that the temperature effect is due to differences in the rate of fusion of broken ends of chromosomes. This interpretation can be tested by irradiating at high and at low temperatures and then reversing the temperatures of half the flower buds in each series immediately after raying. The results of this experiment are shown in table 3.

TABLE 3

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME BREAKS

160 r 2 min. exp. 5 day.

TEMPERATURE	TOTAL CHROMOSOMES	CHROMOSOME BREAKS	
3°C.	972	48	4.9%
38°C.	822	12	1.5%
3°→38°	942	26	2.8%
38°→3°	978	22	2.2%

The buds were irradiated for 2 minutes at 80 r/m in the water at 3° and 38°C. Two minutes after irradiation half the flower stalks in each carton were transferred, those irradiated at 3° were placed in a carton of water at 38°, and those rayed at 38° were transferred to a carton of ice water. The original series and the transfers were left in the containers for an hour before they were put in vials at room temperature. The nuclei irradiated at 3° showed about three times as many chromosome aberrations as those irradiated at 38°. The shift from cold to hot water reduced the frequency of chromosome aberrations to a point about intermediate between the frequencies of the original hot and cold series, but the shift from hot to cold after irradiation produced little increase in frequency of aberrations. These results confirm the assumption that the primary effect of x-rays in inducing chromosome aberrations is practically independent of temperature, and that the temperature effect is concerned with a secondary reaction.

TABLE 4

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMATID AND CHROMOSOME BREAKS

320 r 4 min. 3°C. and 38°C. for 1.5 hrs. Fixed 48 hrs. after raying.

TEMPERATURE	TOTAL CHROMOSOMES	CHROMATID BREAKS 1 HIT		2 HIT		CHROMOSOME BREAKS	
3°	3030	46	1.5%	6	0.2%	252	8.3%
3°→38°	3648	169	4.6%	36	1.0%	164	4.5%

The effect of changing temperature after irradiation was repeated and microspores were fixed at 48 hours after raying in order to get both chromatid and chromosome breaks at the same time. The results are shown in table 4. The frequency of chromosome breaks shows the same trend as the previous experiments, but the frequency of chromatid breaks is much greater in the cells subjected to high temperature after irradiation.

TABLE 5

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMATID AND CHROMOSOME BREAKS

160 r. 2 min. exposure. At 24 and 48 hours after raying.

TEMPERATURE	TIME	TOTAL CHROMOSOMES	CHROMATID BREAKS 1 HIT		2 HIT		CHROMOSOME BREAKS	
3°	24 hr.	3486	126	3.6%	90	2.6%	..	...
38°	24 hr.	3084	56	1.8%	50	1.6%	..	...
3°	48 hr.	3276	26	0.8%	4	0.1%	90	2.7%
38°	48 hr.	3324	36	1.1%	14	0.4%	18	0.5%

This unexpected increase of chromatid breaks at the higher temperature led to another experiment to compare the effect of temperature on chromatid breaks at 24 and 48 hours after irradiation. The results are shown in table 5. The frequency of chromatid breaks induced at mid-prophase

was about twice as high at 3° as at 38°, but the nuclei rayed at early prophase when the chromosomes were beginning to split show slightly more aberrations at the higher temperature. The frequency of chromosome aberrations, induced before chromatid formation, was very much more frequent at the lower temperature. Apparently there is a marked change in the temperature effect of x-ray induced breaks at earliest prophase, which is opposite that found in both earlier and later stages of nuclear development.

If the observed decrease in the frequency of chromosome aberrations at higher temperatures depends on a simple chemical reaction there should be a gradual decrease in the induced aberrations with increased temperature. Several experiments were set up to determine the effect of a series of different temperatures. The effect of various temperatures on the frequency of x-ray induced aberrations is shown in tables 6, 7 and 8. In the first experiment, which was done in February, there is a slight gradual decrease in the frequency of aberrations with temperature increases from 3 to 28°, but at 36° the percentage of aberrations drops suddenly to about a third of the frequency found at the lower temperatures. This led us to suspect that the critical temperature was at about 30°, so a second experiment was completed to test the upper temperature range. This experiment was done early in May. The results are shown in table 7. A very high frequency of aberrations was found in nuclei irradiated at 3°, but a greatly reduced frequency was found at 24° and a continued low but somewhat variable frequency at higher temperatures. In a third experiment we again attempted to determine more precisely the critical temperature. The results of this analysis, done in late May, are shown in table 8. The critical temperature is between 10° and 20°, with little significant variation in frequency of aberrations between 20° and 35°. These results, although not complete, show that there is a critical temperature at which x-ray effects are greatly modified. The fact that this critical temperature may vary from more than 28° to less than 20° probably can be attributed to seasonal effects on the nuclear condition. It is known that the microspore nuclear cycle requires nearly two weeks during the winter months and only one week during the summer.

TABLE 6

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME BREAKS

300 r 10 min. Fixed at 5 days.

TEMPERATURE	TOTAL CHROMOSOMES	BREAKS DICENTRIC	RING	TOTAL	%
3°C.	4752	252	138	390	8.2
12°C.	3714	194	104	298	8.0
20°C.	4704	234	76	310	6.6
28°C.	4944	218	88	306	6.2
36°C.	4014	82	10	92	2.3

TABLE 7

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME BREAKS

320 r 4 min. exp. 5 days.

TEMPERATURE	TOTAL CHROMOSOMES	CHROMOSOME BREAKS	% BREAKS
3°	2064	342	16.6
24°	1902	44	2.3
29°	3126	118	3.8
33°	2346	62	2.6
36°	3690	68	1.8

TABLE 8

EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOME ABERRATIONS

360 r Fixed at 4 days.

TEMPERATURE	TOTAL CHROMOSOMES	BREAKS	% BREAKS
3°	1404	70	10.0
10°	1896	103	10.9
20°	1242	40	3.2
25°	1500	48	3.2
30°	1464	62	4.2
35°	816	22	2.7

Interpretation of Results.—The experimental results show that, 1—x-ray induced chromosome aberrations produced at mid-prophase or the resting stage are much more frequent if the cells are subjected to low temperatures during or following irradiation, 2—the temperature effect is reversed when the chromosomes are irradiated at earliest prophase when the chromosomes split to form sister chromatids, 3—the frequency of x-ray induced breaks is independent of temperature and 4—the temperature effect is maximal at a critical temperature which appears to vary with seasonal effects on the nuclear cycle.

Since the production of breaks in the chromosomes by x-rays is independent of temperature, the temperature effect must be related to the union of broken ends of chromosomes. It has been shown that these broken ends can remain in an unstable condition and capable of fusion for as long as one hour at room temperature and that many of the x-ray induced breaks reunite in the original position so that no aberration is produced.[6] The effect of high temperature during irradiation apparently is an acceleration of fusion of broken ends so that many of the unions restore the chromosome to its normal condition. At low temperatures the union of broken ends is delayed so that there is greater chance for the broken ends to get out of alignment and fuse in illegitimate associations which produce the chromosome aberrations. This interpretation is supported by the experiments on temperature effects following irradiation (table 3).

The reversed effect of temperature on x-ray induced aberrations at earliest prophase is difficult to explain. This stage in the chromosome cycle is a critical one, since crossing-over occurs at this stage of chromosome development. Crossing-over is decreased with increased temperatures in the range from 13° to 25°, but at higher temperatures crossing-over is increased as compared with the standard condition of 25°.[7] Stern[8] finds that the frequency of somatic crossing-over in Drosophila is low at 30° but high at both 25° and 17°C. Just how these varied results can be reconciled is not clear at present, but possibly both induced and natural breaks at this stage of chromosome development are affected by the stresses imposed by relational coiling of the newly formed sister chromatids. If the torsion is relatively great the broken ends may be thrown out of alignment before refusion is possible, leading to new associations of chromatids. The degree of torsional strain imposed by relational coiling may be dependent upon temperature.

In many respects the breaks induced by x-rays are similar to the natural breaks which occur at every chiasma during meiosis. The breaks do not occur at random, they presumably do not produce genic alterations, and a broken end almost invariably fuses with another broken end. There must be natural breaking points in the chromosome which form the loci of both spontaneous and induced breaks. The chromosome has been pictured as a micelle of long polypeptide molecules in parallel alignment, differentiated longitudinally by sequences of amino-acid molecules.[9] The linkage bonds between the genes must not be very stable since they can be broken and re-established with no injury to the chromosome.

If the temperature effect on x-ray induced chromosome aberrations depended upon a simple chemical reaction there should be a gradual decrease in aberrations with increased temperature. The decrease, however, is not gradual, but drops quickly within certain temperature ranges. This behavior suggests a critical temperature effect on the physical condition of the nucleus—possibly viscosity changes or changes in the stress of chromosome coiling.

The discrepancies in the various experiments with the temperature effect on irradiated cells probably can be attributed to the different effects produced by irradiation. It is known that x-rays or gamma rays affect all parts of the cell; the permeability of the cell membrane is altered, the cytoplasmic viscosity is changed, the mitochondria and golgi bodies become granular and the chromosomes are fragmented or fused. The lethal action of x-rays has been attributed to the action on oxidases, permeability of the cell membrane and electrolyte equilibrium, all of which are essential in the respiratory processes of the cell.[10] On the other hand the effects of x-rays on chromosome aberrations and mutations is well known, and either of these may be lethal at some point in the cell lineage. The lethal action of

irradiation on cells is often attributed to the chromosome changes induced by the direct effect on the chromosomes.[11]

The relative importance of the physiological effect and the genetic effect of irradiation depends on the nature of the cells. Radiation of plant cells shows the two effects very clearly. The first effect is the clumping of the chromosomes, disturbance of spindle formation and inhibition of nuclear division. At moderate doses these physiological effects are temporary and the cell recovers. The only permanent changes are the genetic effects caused by chromosome aberrations and mutation. Doses sufficient to produce numerous chromosome aberrations do not prevent cell division. On the other hand Drosophila eggs show 50 per cent killing at a dose which has little effect on the sperm. The fact that there are enormous differences in the lethal dose for different organisms, for different tissues or different stages of development in the same organism, or even the same tissue under different cultural or environmental conditions, indicates that the physiological effect of radiation may play an essential part in the lethal action of radiation. Presumably the effect of high temperatures in increasing the lethal action of radiation can be attributed to increased physiological response. On the other hand the greater effectiveness of radiation at low temperatures can be shown to be associated with an increase in chromosome aberrations. The physiological factor appears to be of great importance in radiation therapy.

There is some evidence that the temperature effect on x-ray induced mutations is similar to the temperature effect on x-ray induced chromosome aberrations. Although either high temperature or x-rays greatly increase the mutation rate, irradiation at high temperatures is no more effective than irradiation at low temperatures. In fact there is a tendency for more mutations to be produced when Drosophila is rayed at low temperatures. In two experiments by Timoféeff-Ressovsky more mutations were produced when the flies were irradiated at 10°C. than at 35°C., although the differences were not satistically significant. But Medvedev found nearly twice as many mutations in flies irradiated at 0°C. than in those irradiated at 20°C.[12] The mutation rate in Tradescantia, as measured by pollen grain variability, also appears to be greater when the plants are irradiated at low temperatures (Rick, unpublished). If these results are confirmed by further work, it appears that most mutations are caused by chromosome aberrations or that the temperature effect on x-rayed genes is similar to the effect of temperature on x-ray induced chromosome aberrations.

Summary.—Tradescantia microspores x-rayed at low temperatures show more chromosome aberrations than those irradiated at high temperatures. At high temperatures fusion of broken ends is accelerated and many of the breaks are reunited in the original position, while at low temperatures fusion is delayed and broken ends may fuse in illegitimate unions to pro-

duce many chromosome aberrations. This interpretation applies to nuclei irradiated at the resting stage and at mid-prophase, but the reversed temperature effect on early prophase chromosomes is difficult to explain. The temperature effect is maximal at a critical temperature which appears to vary with the seasonal effect on the nuclear cycle. The varied response of different types of cells to temperature effects during irradiation can be attributed to the relative effects of physiological and genetic factors.

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THE BEHAVIOR IN SUCCESSIVE NUCLEAR DIVISIONS OF A CHROMOSOME BROKEN AT MEIOSIS

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If a chromatid in maize is broken at a meiotic anaphase and if it enters a telophase nucleus, fusion between the two sister halves of this chromatid will result at the position of breakage.[1] Because of this fusion, the separation at a succeeding anaphase of the sister halves of this chromatid necessarily produces a bridge configuration. The pull on the chromatin of this bridge in the anaphase or telophase results in breakage and again a broken chromosome enters each telophase nucleus. The following questions immediately arise: Will this chromatid produce a bridge in the following mitotic anaphase by a similar fusion of sister halves at the position of the second break? Will this process continue indefinitely in each successive division or will the broken end of a chromosome eventually "heal" and continue through mitotic cycles without further fusions, bridges and breakages? It is stated in the previous publication that the evidence on this