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Chromosome Studies on *Trillium kamtschaticum* PALL. and Its Allies. XXIX.

Effects of ATP and DNA on the rejoining of chromosome breaks induced by X-raying.* **

By

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1. Introduction

Based on a series of studies of X-ray-induced aberrations with *Vicia* seeds soaked in various chemical solutions, WOLFF and his collaborators (WOLFF '54; WOLFF & ATWOOD '54; WOLFF & LUIPPOLD '55, '56) concluded that oxidative metabolism with concurrent formation of ATP is necessary for chromosome breaks to rejoin, as shown in cases of the application of ATP just prior to irradiation. BEATTY et al. ('56) studying the yield of two-hit aberrations in *Tradescantia* microspores after 400 *r* X-ray irradiation under either anoxic (in helium) or oxygenated condition, suggested that in the normally oxygenated condition the respiratory system maintains the energy supply, which promotes

* The outline of this study was already reported at the 26th Meeting of the Botanical Society of Japan held in October, 1961, at Tokyo.

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the breaks to restitute, and that in the anoxic condition, a store of such metabolites as ATP which usually exists in cells, is largely used up unless there is extraneous supplementation, and then the breaks accumulate so that, when the oxygen supply is restored, they form exchanges. BEATTY & BEATTY ('60 a, b) tested the effect of ATP in the same material and ascertained that at 30°C and 40°C ATP brought about a remarkable decrease in the yield of two-hit aberrations. Further, they showed that the supplementation of potassium gluconate and 6-phosphogluconate is also capable of promoting restitution, a finding indicating that both the glycolytic and hexose monophosphate pathways are effective in releasing energy for rejoining of the broken ends.

Recently, WOLFF ('59, '60) proposed that the protein synthesis is necessary for broken ends to rejoin, on the basis of a finding that chloramphenicol, a protein synthesis inhibitor, administered between two doses of X-rays, prevented the breaks from the rejoining as indicated by interaction between the breaks caused by the first dose and those by the second one. WOLFF further supposed that nucleic acid synthesis does not involve in the repair of breaks. However, many informations are now being accumulated which indicate the importance of nucleic acids in the maintenance of chromosome integrity. It has been shown that in maize (STADLER & UBER '42) and in *Tradescantia* (KIRBY-SMITH & CRAIG '57) the action spectra for chromosome breakage by ultra-violet rays are almost identical with the nucleic acid absorption curve. And KAUFMANN ('60) reported that the DNase, when injected into *Drosophila* male, produced considerable number of lethal mutations as well as chromosomal rearrangements.

In our preceding experiment we showed that mitomycin C, an inhibitor of nucleic acid polymerization, administered at the time of X-ray irradiation, has profound effects in increasing two-hit aberration yield (MATSUURA et al. '62) and this seems to indicate the involvement of nucleic acid synthesis in the process of recovery of breaks. It is expectable, therefore, that the supplementation of available DNA source to cells might have some effects on the recovery of breaks. In order to make a further step on this problem, we attempted in the present work the treatments with ATP and DNA, both separate and simultaneous, in combination with the X-radiation.

2. Material and Method

Material plants used in the present experiments were obtained from natural population at Nakagoya, about 30 km north-east from Sapporo, and were transplanted to the experimental garden of our Laboratory.

Cut stems of the material plants bearing the flower just after flowering were placed in each experimental solution for 24 hours prior to the X-ray irradiation and rendered to absorb the

chemicals. Treatments were continued further six hours after irradiation and then, the plants were replaced in the beakers containing water. Experimental solutions were renewed once during the total 30 hours treatments. The plants of control group were placed in water instead of experimental solutions.

The concentrations of ATP (disodium salt, Nutr. Biochem. Co.) were 2×10^{-3} mol (ATP-H) and 2×10^{-4} mol (ATP-L) and those of DNA (sperm, Nutr. Biochem. Co.) were 0.1% (DNA-H) and 0.01% (DNA-L), H and L denoting high and low concentrations respectively. For the experiments of simultaneous application of ATP and DNA, four combinations of high and low concentrations of them were prepared, that is, DNA-H + ATP-H, DNA-H + ATP-L, DNA-L + ATP-H, DNA-L + ATP-L. The initial pH of each solution was adjusted nearly to 7.0 by HCl or NaOH. A slight change of pH was observed after 15 hours in ATP solutions but possible effects due to the pH change were minimized by the renewal of solutions.

Total dose of 60 *r*, which seems to be, in the light of the preceding works, an appropriate dose for *Trillium* yielding one to two aberrations per cell in average, was delivered at the intensity of 8.1 *r* per minute.

Forty-eight hours after irradiation, the ovular tissues were fixed with La Cour 2BE and stained by Feulgen's method.

Observation of aberrations was totally confined to metaphase cells whose chromosomes were distinguishable completely and the loci of breaks could be determined. The aberrations scored were simple chromatid breaks (sB′), isochromatid breaks (sB″) and two-hit aberrations (T). The frequency of exchanges were translated into the number of original breaks and expressed in abbreviated form as rB′ and rB″. The sum of aberrations obtained from three to five plants in each group was recalculated into the values per 100 cells in order to facilitate the comparison.

In order to investigate the reaction of heterochromatin region to radiation and also to the modifying effect of ATP and DNA, each chromosome arm was divided conveniently into segments with nearly equal length, making the length of a segment of each chromosome arm approximately equal among chromosome arms (Fig. 1). For example, in the submedian-

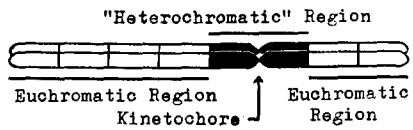

Fig. 1. Diagrammatic representation of "heterochromatic" and "euchromatic" regions (**B**-chromosome).

kinetochored chromosome **B**, the long arm was divided into five segments and the short arm into four segments. The aberrations were recorded in each of these segments thus divided. In *Trillium* species occurring in Japan the heterochromatin region is confined to the region proximal to the kinetochore in each chromosome arm. Since, however, the heterochromatin region is discernible only when the plants were exposed to cold temperature, the precise determination of locus of a break whether it falls into heterochromatic or euchromatic region is nearly impossible, in the present experiments which were carried out at room temperature, and hence the segment most adjacent to the kinetothore in each chromosome arm was designated as 'heterochromatic region'. It must then be noted that the 'heterochromatic region' thus de-

signated might include the whole part of true heterochromatin and the kinetochore, and furthermore, some of euchromatin part in addition.

3. Results

As expected, a small amount of breaks favorable for analysis was yielded in the control material by radiation of the total dose of 60 *r*, e.g. 135 simple breaks (sB) and 36 breaks involved in exchanges (rB) per 100 cells (Table 1). The proportion of rB in the total number of breaks detected (TB, sB+rB) was 21%. The ratio of B′:B″ among the simple breaks is approximately 1 : 1.6, and 1 : 1.1 when rB was included in calculation. The above ratios of B′ : B″ seem to indicate that the observed cells were irradiated nearly at the critical period of effective split of chromosomes in the prophase stage.

The modification effect of ATP on the aberration yield was very striking. The frequency of sB reduced to the extent nearly half that in the control group, and that of rB decreased too, though slightly. The B′ : B″ ratio in the simple breaks was 1 : 2.0. The weight of B″ increased as compared with that in the control group. Superficially, this seems to indicate that ATP stimulated the process of cell division. Certainly it may be so. However, there is another possibility that under the conditions of extensive acceleration of restitution the breaks of chromatid type may be more favored than those of chromosome type. In fact, if rB was taken into consideration the ratio is converted to 1 : 1.1, which is similar to that in the control. The high and low concentrations of ATP appear not to bring about any appreciable difference in the aberration yield.

The DNA treatments also brought about a conspicuous effect. The yield of sB considerably reduced but the extent of reduction was a little smaller than that attained by the ATP treatments. In spite of such a remarkable reduction in the frequency of simple breaks, that of interchanges remained at the level comparable to the control group. This suggests that the effect of DNA on the rejoining mechanism may differ qualitatively from that of ATP. The high and low concentrations of DNA did not produce any appreciable difference in the yield of aberrations.

The combination of high concentrations of both ATP and DNA (DNA-H + ATP-H) gave the most remarkable results of all the treatments attempted in the present study. Especially the reduction of sB yield was conspicuous. However, the decrease of rB frequency could not attain to the level obtained in the single ATP treatments. The combination of low concentrations of both ATP and DNA (DNA-L + ATP-L) showed lower value of total aberration (TB) than that obtained by the single treatment with low concentration of DNA. It is notable, however, that the rB frequency was smaller than in the case of

TABLE 1. Aberrations per 100 cells in each experimental group.

Treatment	Control (81)*	ATP-H (101)	ATP-L (59)	DNA-H (79)	DNA-L (60)	DNA-H + ATP-H (60)	DNA-H + ATP-L (80)	DNA-L + ATP-H (55)	DNA-L + ATP-L (60)
sB′	51.9	25.7	25.4	27.1	35.0	23.3	21.3	21.8	25.0
sB″	82.7	52.5	54.2	61.0	56.7	30.0	38.8	38.2	51.7
sB′+sB″ (sB)	134.6	78.2	79.7	88.1	91.7	53.3	60.0	60.0	76.7
rB′	28.4	23.8	27.1	33.9	35.0	30.0	41.3	43.6	26.7
rB″	7.4	3.0	3.4	1.7	1.7	1.7	3.8	3.6	8.3
rB′+rB″ (rB)	35.8	26.7	30.5	35.6	36.7	31.7	45.0	47.3	35.0
sB+rB (TB)	170.4	105.0	110.2	123.7	128.3	85.0	105.0	107.3	111.7

* Total number of cells observed.

sB′ : Simple chromatid breaks.

sB″ : Simple iso-chromatid breaks.

rB′ : Chromatid breaks involved in interchanges.

rB″ : Iso-chromatid breaks involved in interchanges.

the single low concentration of ATP.

The results obtained from the combination groups of low and high concentrations of ATP and DNA (DNA-H + ATP-L, and DNA-L + ATP-H), were somewhat complicated. Though the decrease in the frequency of sB was greater than those in respective single treatments, rB frequency somewhat increased as compared with those in the single ATP and DNA groups and even in the control group.

A similar extent of decrease of sB′ frequencies were attained in all the ATP, DNA, and combination groups, while the decrease of sB″ frequency was conspicuous in the three combination groups (DNA-H + ATP-H, DNA-H + ATP-L, and DNA-L + ATP-H). In consequence, the decrease of sB (sB′ + sB″) frequency in each combination group was more than those in respective single treatments. This suggests that some additional effect was produced by the simultaneous use of DNA and ATP.

In the groups in which DNA was applied singly or in combination with ATP, the rB frequencies either remained at the level of the control or rather increased than that in spite of the extensive reduction of sB frequencies. It seems likely that DNA affects rejoining mechanism in a manner so as to leave a room for breaks to form interchanges. Such a manner of action was not recognized in the single ATP treatments.

These results suggest the following: (1) ATP is effective in promoting restitution, but it is not conceivable that ATP has a particular effect on the occurrence of interchanges; (2) DNA, too, promotes restitution but in a different manner from ATP, so as to leave a room for the formation of more two-hit aberrations; (3) the combination treatments of DNA with ATP seem to bring about an cumultive effect on restitution, though the extent of difference between these combination groups and single DNA and ATP groups was not statistically significant.

4. Analysis and Discussion

i) Effects on cell division

As reviewed by READ ('59), many workers (READ & WOLFF '48, MOTTRAM '36, FRITZ-NIGGLI '56, DEUFEL '51) investigated the effect of radiation on cell growth in terms of the percentage of cells which are in division at the time of investigation, e. g., at various periods of time elapsed after irradiation. Their results indicate that a high dosage of radiation suppresses cell division completely at several hours after irradiation followed by considerable recovery of mitotic rate after further several hours. A weaker dose, 170 *r* X-rays (READ & WOLFF

'48), however, could not bring about complete cease of cell division.

Though examination of possible changes in mitotic rate was not carried out, it may safely be said that the X-ray dose in the present experiments, 60 *r*, is low enough not to bring about any obstacle for the present purpose of investigation.

It has been demonstrated in *Tradescantia* (SAX '41, SWANSON '43) and in grasshopper neuroblasts (CARLSON '41) that the type of breaks shifts from chromosomal to chromatid according to the time elapsing after irradiation to metaphase stage. The cells irradiated sufficiently earlier in resting stage possess only chromosome type aberrations at metaphase, while those irradiated just prior to metaphase stage have mostly chromatid type aberrations. It is believed that the aberrations of chromatid type can be produced after the chromosomes undergo effective split. However, as the results of above-mentioned works have shown, the shift of aberration type is gradual rather than discontinuous. This may be interpreted by assuming that after completion of split, two daughter chromatids are held together for a while, allowing that an ionizing particle passes through both of them so as to produce iso-chromatid type breaks. Nevertheless, it is certain that there exists considerable duration of time in which the aberrations of both chromatid and chromosome types occur together.

TABLE 2. The ratio of chromatid breaks (B′) and iso-chromatid breaks (B″) in each experimental group.

Treatment	sB′ : sB″	sB′+rB′ : sB″+rB″
Control	1 : 1.6	1 : 1.1
ATP-H	1 : 2.0	1 : 1.1
ATP-L	1 : 2.1	1 : 1.1
DNA-H	1 : 2.3	1 : 1.0
DNA-L	1 : 1.6	1 : 0.8
DNA-H + ATP-H	1 : 1.3	1 : 0.6
DNA-H + ATP-L	1 : 1.8	1 : 0.7
DNA-L + ATP-H	1 : 1.8	1 : 0.6
DNA-L + ATP-L	1 : 2.1	1 : 1.2

In the control group of the present experiments, the ratio of B′ : B″ was 1 : 1.6 in the simple breaks but 1 : 1.1 in the total breaks detected. The ratios in each experimental group are listed in Table 2. The middle column shows the ratios only among simple breaks. The decrease of B″ relative to B′ in the DNA-H + ATP-H group may be understood as the result of conspicuously

advanced restitution. In the ATP groups and DNA-H group and also in the DNA-L + ATP-L group, the weight of B″ is somewhat enhanced. However, as mentioned briefly in the foregoing section, it may not be regarded that the increase of B″ relative to B′ in these groups immediately implies the acceleration of division cycle, since in the condition promotive of restitution, B′ may be more favored in restitution than B″, and the final yield of simple breaks would be affected also by the extent of interchange formation. The B′ : B″ ratios taking rB into consideration are listed in the right column of Table 2. In three combination groups a drop of the weight of B″ is recognizable. Since, however, it is hard to accept that ATP or DNA affects so as to bring about a retardation of cell growth, it is reasonable to suppose that the acceleration of restitution favoring particularly for B″ due to some unknown additional effect produced by the simultaneous use of ATP and DNA is responsible for the down of B″ weight in these groups. Thus, in such modification experiments as in the present paper, the changes in the ratio of B′ to B″ cannot be regarded as implying simply the stimulation or retardation of division cycle. Even if it was accepted that these changes in B′ : B″ ratios truly indicate the stimulation or retardation of cell growth, these changes may not introduce so severe confusion that makes the present examinations impossible.

Biochemical studies have revealed that the DNA synthesis occurs during interkinesis in somatic cells (WALKER & YATES '52; HOWARD & PELC '52; TAYLOR & MCMASTER '54; MOSES & TAYLOR '55) and before zygotene stage in meiotic cells (TAYLOR '53; SWIFT '50). A correlation was recognized to exist between the kind of X-ray-induced breaks and the DNA doubling (THODAY '54; MITRA '56, TAYLOR '57). However, SWANSON ('57) hold that radiation is not so sufficiently delicate as to permit the determination of the multiplicity of chromosome structure. Nevertheless, it is conceivable enough that both breaks of chromatid and iso-chromatid types can be involved in interchanges. If one assumes that the phase of chromosome duplication directly corresponds to that of DNA synthesis, the chromatid type aberrations must be due to breakage and reunion occurred after DNA synthesis. In this connection very interesting is the fact that irradiation to meiotic chromosomes after diplotene stage, even at early metaphase I, induces aberrations of half-chromatid type (MITRA '58; WILSON et al. '59; MATSUURA et al. '62). The time of irradiation in these experiments which induce two-side-arm bridges is obviously out of the DNA synthetic phase. In spite of this, the reunion of broken ends does actually take place. These findings indicate that the chromosome itself or the nucleus is constitutionally provided with an ability to recover the continuity of chromosomes at any time when they are broken, and usual cytological events such

as crossing over, translocation, inversion, etc., as well as the formation of aberrant chromosomes and restitution from breaks after radiation treatments are all dependent on this ability.

ii) Response of heterochromatin

A number of works have dealt with the response of heterochromatin to radiation damage as well as the action of various chemicals. KAUFMANN ('39) studied the distribution of radiation-induced breaks in the X-chromosome of *Drosophila* and found that one third to one fourth of total number of breaks occurred in the proximal heterochromatic region which was estimated to occupy approximately one third of total length in both meiotic and mitotic chromosomes. He found also that there are intercalary sections which show specifically high frequency of aberrations, and supposed that these regions include heterochromatin. In general, however, his *Drosophila* data indicated no or little difference in breakability between heterochromatin and euchromatin (KAUFMANN '46). This finding makes a marked contrast with *D. virilis* where the totally heterochromatic Y-chromosome had a break frequency only half as much as the X, in spite of nearly equal length of the two (BAKER '49). Recently, EVANS and BIGGER ('61) found non-random distribution of chromatid aberrations among chromosomes in *Vicia* roots which were irradiated by gamma-rays, and showed that the aberrations are frequent in heterochromatic regions and in regions near by heterochromatin. GRAF ('57) demonstrated that in corn root tip cells the number of anaphase bridges induced by maleic hydrazide is highly correlated with the number of heterochromatic knobs in the varieties used and interpreted this as due to a selective breaking effect of some chemicals on heterochromatic regions, while the aberrations induced by X-rays did not show any correlation with the number of heterochromatic knobs. FORD ('48) studied the effect of N-N-di-(2-chloroethyl)-methylamine on *Vicia* chromosomes and found that the induced breaks are not distributed at random indicating certain specificity for heterochromatin. Further confirmation was obtained by LOVELESS & REVELL ('49). DARLINGTON & MCLEISH ('51) and MCLEISH ('54) demonstrated that the chromosome breaks induced by maleic hydrazide appear to be associated with heterochromatic regions producing only a few breaks in other regions. REVELL ('52) reported that some chemicals with radiomimetic effect cause breaks in heterochromatic regions more than at random. OEHLKERS ('52) has shown that in *Vicia* root tip cells and *Oenothera* meiotic cells the chemically induced breaks did not show random distribution. SMITH & LOTFY ('55) observed in *Vicia* that beta-propiolactone produced mainly large akinetic fragments, and suggested that these large fragments had resulted from breakages selectively

occurred in the proximal heterochromatic region. Aethylmethansulfonate and myleran, which are known as mutagens, were also found to break chromosomes in some favor of heterochromatin (RIEGER & MICHAELIS '60, MICHAELIS & RIEGER '60). It is obvious from these results that heterochromatin reacts differentially to various chemicals, possibly owing to its biochemical specificity.

LA COUR & PELC ('58) suggested that heterochromatic segments may be replicated at a different time from the euchromatic parts, on the basis of the autoradiographic studies on the incorporation of tritiated thymidine into chromosomes of *Vicia* root meristematic cells. LIMA-DE-FARIA ('59) carried out a similar studies with the chromosomes of *Melanoplus* and *Secale*, and found that heterochromatic regions of the sex chromosome of *Melanoplus* undergoes the synthesis of DNA later than the euchromatin does, and also that in *Secale* the heterochromatin synthesizes DNA at a different period of time from the euchromatin. However, PELC & LA COUR ('60) utilizing a similar method in *Fritillaria* and *Scilla* demonstrated that the DNA synthesis initiates at the end of chromosomes and progresses towards the kinetochores. Since each of eight of the twelve *Scilla* chromosomes has a distal heterochromatin region, they concluded that DNA synthesis progresses from the distal to the proximal with no disparity between eu- and hetero-chromatin.

The distribution pattern of heterochromatin in chromosomes has been well-established in *Trillium*, *Fritillaria*, *Vicia* and so forth. In *Trillium* species growing in Japan, the heterochromatic region is entirely confined to the proximal region in each chromosome arm. When *Trillium* plants are exposed to low temperature, the heterochromatic region reveals itself as a thin, Feulgen-negative threads in metaphase cells (HAGA & KURABAYASHI '53), probably owing to the nucleic acid starvation in cells due to low temperature, as suggested by DARLINGTON & LA COUR ('40). In the present study, the first proximal segment in each chromosome arm was conveniently designated as 'heterochromatic region' (cf. Fig. 1) and the aberrations occurring in 'heterochromatic' and euchromatic segments were separately scored and listed in Table 3. The expectation was calculated by dividing the sum of aberrations in the proportion of 22.3 : 77.7, the former being the proportion of sum of heterochromatic segments in the total chromosome length in a cell.

If there exists any difference in the yield of aberrations between heterochromatic region and euchromatic one, the following surmises will be possible as to its causality. (1) The frequency of initial damage may not be even among them. It has been known that such chemicals as maleic hydrazide induce breakage specifically in heterochromatin. Since several workers obtained inconsistent results as to the radiation-induced breakage, it cannot be concluded

TABLE 3. Distribution of aberrations with respect to 'heterochromatic' ('H') and euchromatic (E) regions. (per 100 cells)

Treatment	Control		ATP-H		ATP-L		DNA-H		DNA-L	
Region	'H'	E	'H'	E	'H'	E	'H'	E	'H'	E
Obs.	19.8	114.3	15.0	63.3	15.3	64.4	22.0	66.1	20.0	71.6
sB Exp.	30.0	104.6	17.4	60.8	17.8	61.9	19.6	68.5	20.4	71.3
χ^2-test	non-sig.		non-sig.		non-sig.		non-sig.		non-sig.	
Obs.	14.8	21.0	14.9	12.0	18.6	11.9	13.6	22.0	10.0	26.7
rB Exp.	8.0	27.8	6.0	20.7	6.8	23.7	7.9	27.7	8.2	28.5
χ^2-test	Sig.		Highly Sig.		Highly Sig.		non-sig.		non-sig.	
Obs.	34.6	135.8	29.9	75.2	33.9	76.3	35.6	88.1	30.0	98.3
TB Exp.	38.0	132.4	23.4	81.6	24.6	85.6	27.6	96.1	28.6	99,7
χ^2-test	non-sig.		non-sig.		non-sig.		non-sig.		non-sig.	

Treatment	DNA-H + ATP-H		DNA-H + ATP-L		DNA-L + ATP-H		DNA-L + ATP-L		Mitomycin C	
Region	'H'	E	'H'	E	'H'	E	'H'	E	'H'	E
Obs.	10.0	43.3	7.5	52.5	5.5	54.5	10.0	66.7	17.0	94.0
sB Exp.	11.9	41.4	13.4	46.6	13.4	46.6	17.1	59.6		
χ^2-test	non-sig.		non-sig.		non-sig.		non-sig.			
Obs.	8.3	23.3	15.0	30.0	9.1	38.2	15.0	20.0	50.0	43.0
rB Exp.	7.1	24.6	10.0	35.0	10.5	36.8	7.8	27.2		
χ^2-test	non-sig.		non-sig.		non-sig.		non-sig.			
Obs.	18.3	66.7	22.5	82.5	14.5	92.7	25.0	86.7	67.0	137.0
TB Exp.	19.0	66.0	23.4	81.6	23.9	83.4	24.9	86.8		
χ^2-test	non-sig.		non-sig.		non-sig.		non-sig.			

at present they are distributed randomly in these two regions. (2) The mechanism of restitution and the reaction to modifying factors are different between them. The extent of initial breaks caused by radiation as well as of restitution from them can merely be estimated by means of analysis of changes in the harvested type and frequency of aberrations from various modification designs. It has been concluded by SAX ('38, '40), FABERGÉ ('40) and others, and generally accepted that only a few of damages initially caused by radiation remain open as breaks until the time of fixation and observation.

It is simply considered that a condition stimulating restitution from breaks reduces, as in the present experiments, the frequencies of both of simple breaks and two-hit aberrations, the decrease of the latter being a consequence of rapid diminution of broken ends which are available for interchange formation. On the other hand, a factor preventing the breaks from rejoining will bring about an increase of simple breaks and also of two-hit aberrations. For example, SAX ('43) applying centrifugation which seems to prevent restitution from X-ray-induced breaks recorded an increase in both of exchanges and iso-chromatid aberrations. CONGER ('48) also demonstrated certain increase in the frequency of chromosome exchanges as well as deletions by means of applying sonic vibration during the X-irradiation. If a modifying factor is operative so as to prevent breaks moderately from rejoining for a prolonged period of time, the frequency of interchanges will increase greatly after removal of preventive effects, and when the increase of interchanges is quite large, the yield of simple

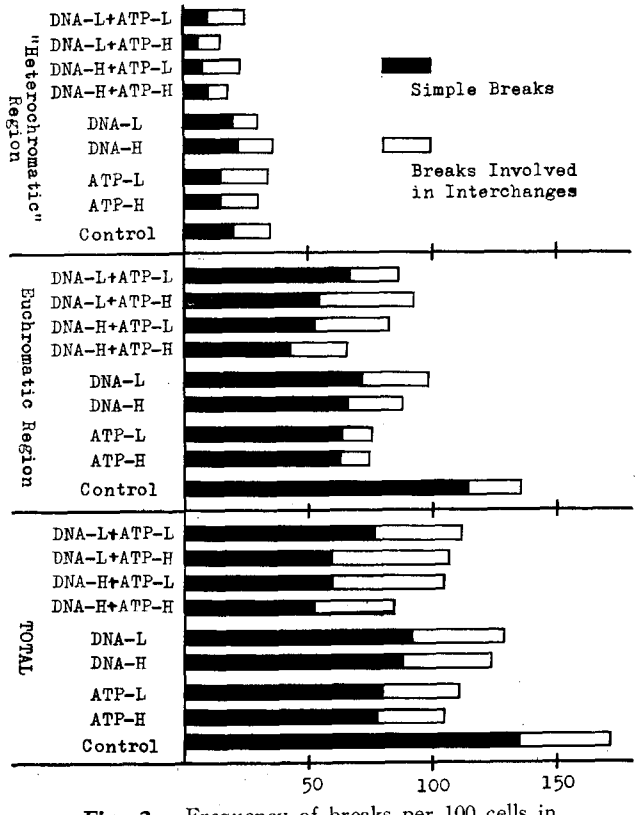

Fig. 2. Frequency of breaks per 100 cells in each experimental group.

breaks may decrease. In fact, the mitomycin treatment combined with X-irradiation produced a conspicuously increased number of interchanges and a slightly decreased frequency of simple breaks (MATSUURA et al. '62). This indi- cates that the block of DNA synthesis prevents the restitution from breaks for

TABLE 4. Statistical analysis of differences in aberration frequency among experimental groups.

Groups in Comparison	Control: ATP-H			Control: ATP-L			Control: DNA-H			Control: DNA-L		
Region	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)
sB (sB′+sB″)	—	H-S	H-S	—	H-S	H-S	—	H-S	S	—	S	S
rB (rB′+rB″)	—	—	—	—	—	—	—	—	—	—	—	—
TB (sB+rB)	—	H-S	H-S	—	H-S	H-S	—	S	S	—	—	—

Control: DNA-H + ATP-H			Control: DNA-H + ATP-L			Control: DNA-L + ATP-H			Control: DNA-L + ATP-L			DNA-H: DNA-H + ATP-H		
'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)
—	H-S	H-S	—	H-S	H-S		S-H	S-H	—	H-S	H-S	—	—	S
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	H-S	H-S	—	H-S	H-S	S	S	H-S	—	H-S	H-S	—	—	—

DNA-H: DNA-H + ATP-L			ATP-H: DNA-L + ATP-H			ATP-H: DNA-L			ATP-L: DNA-H + ATP-L			Control: ATP Groups		
'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)
S	—	—	—	—	—	—	—	—	—	—	—	—	H-S	H-S
—	—	—	—	H-S*	—	—	S*	—	—	S*	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	H-S	H-S

Control: DNA Groups			Control: Comb. Groups			ATP Groups: Comb. Groups			DNA Groups: Comb. Groups			ATP Groups: DNA Groups		
'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)	'H'	E	(T)
—	H-S	H-S	S	H-S	H-S	—	—	—	H-S	—	H-S	—	—	—
—	—	—	—	—	—	—	H-S*	—	—	—	—	—	S*	—
—	H-S	S	S	H-S	H-S	S	—	—	S	—	S	—	—	—

Comparisons which were found to have no significant difference in all respects are not presented in this table.

H-S: Difference is highly significant.
S: Difference is significant.
—: Difference is non-significant.
'H': 'Heterochromatic region'.
E: Euchromatic region.
(T): Total.
* indicates that the latter one of the two groups in comparison shows higher frequency than that of the former.

a considerably prolonged period of time, resulting in enhancing the chance of interchange formation after the inhibitor was removed. The data of Table 3 must be read keeping in mind the above-mentioned changes in such relationships between the types of aberrations.

Further, in order to analyse the data in each treatment group, statistical analysis of differences between groups was carried out and the results are presented in Table 4. From the comparison of experimental groups with the control (cf. Fig. 2), it is obvious that the reduction of aberrations in each group is mainly due to the significant decrease in the frequency of simple breaks in euchromatic region, and also that both ATP and DNA, and their combinations, promote the repair of breaks especially in the euchromatic region. On the contrary, the frequencies of simple breaks in the heterochromatic region as well as those of ones involved in interchanges (rB) in both regions could not be regarded to differ significantly in the ATP and the DNA groups from those in the control. However, it must be noted here, as mentioned in the foregoing section, that the region designated as 'heterochromatic' in the present paper may include some portion of euchromatin, and therefore that the frequencies of aberrations attributed to 'heterochromatic' or euchromatic region wear certain obscurity.

In the control group the frequency of rB in the heterochromatic region was significantly higher than that in the euchromatin region, and this situation was kept also in the ATP groups and the DNA-H group. This is an indication that breaks in heterochromatin caused by X-rays cannot be repaired so readily as those in euchromatin. It is noteworthy that, though the frequency of aberrations in the euchromatin region in all the experimental groups except that DNA-L group is significantly low when compared with that of control, the aberration frequency in the heterochromatin region remained at the level comparable to the control in the simple DNA and ATP groups. Perhaps the single application of either DNA or ATP could not bring about any modifying effect on the aberrations occurring in heterochromatin in contrast to the pronounced effects in euchromatin. In the combination groups, however, a significant decrease of aberration frequency was demonstrated in the heterochromatic region.

Further precise examination of the data in Table 3 also reveals that in the combination groups which involve the high concentration of ATP the frequency of rB as well as of total breaks (TB) was a little decreased in the heterochromatic region. This seems to indicate that the supplementation of DNA source in combination with high dose of ATP introduces some accelerating effect of restitution on heterochromatin. It is also to be pointed out that in

the DNA-H + ATP-L and DNA-L + ATP-H groups, the rB frequency in the euchromatic region is significantly higher than that in their respective ATP groups.

The result obtained from the preceding work applying mitomycin C (MATSUURA et al. '62) was inserted in the last two columns of Table 3. In this experiment the mitomycin treatment was given for 14 hours, and at the time 8 hours after the initiation of the treatment 60 *r* of X-rays was given. The chemical treatment was followed by 42 hours immersion of cut stems in water in order to remove the effect of mitomycin and then the materials were fixed. The results indicate that mitomycin affected so as to increase the two-hit aberrations especially in heterochromatic regions. This was interpreted as due to certain inhibitory effect of mytomaycin on the rejoining system acting more effectively in heterochromatin than in euchromatin. This result is quite suggestive of specificity of heterochromatin in relation to the metabolism of nucleic acids.

The conclusions suggested in this section may be summarized as follows. (1) The breaks induced in heterochromatin seem not to be repaired so readily as those in euchromatin allowing the formation of more two-hit aberrations, probably due to a differential metabolic rate involved in the synthesis of nucleic acids. (2) ATP or DNA, when applied singly, is capable of promoting restitution from damages in the euchromatin region but not in the heterochromatic region, indicating differential response of heterochromatin to the supplementation of energy or DNA source. (3) Simultaneous supply of DNA and high dose of ATP seems to promote restitution of breaks in heterochromatic regions, too.

As already mentioned in this section there exist conflicting results about the behavior of heterochromatin in relation to DNA synthesis. The contradiction, however, seems to be partly due to the difficulties underlying in the methodology. Further, it is not certain that the rejoining of chromosome breaks directly correlates with the DNA synthesis at the time of chromosome replication. Nevertheless, the present results indicate that heterochromatin reacts differently from euchromatin to stimulative action of ATP and DNA in the recovery process of breaks.

iii) Effect of ATP

Application of ATP in the radiation study of chromosomes was attempted first by WOLFF & LUIPPOLD ('56). Preirradiative treatment with ATP for 1/2 hour was effective in reducing the period within which an intensity dependence of the two-hit aberration appears. This means that the promotion of rejoining of chromosome breaks resulted in the decrease of effective ends capable of

taking part in the formation of two-hit aberrations. The effect of ATP on the repair of damages has been demonstrated by SERMONTI & MORPURGO ('59) studying the somatic segregation of mutants induced in *Penicillium* with nitrogen mustard or X-rays. They found that the treatment with 1% $MnCl_2$, which is known to stimulate ATP synthesis in mitochondria, decreased the segregation of mutants. Later, BEATTY & BEATTY ('60 a, b) also obtained satisfactory results with ATP in *Tradescantia* microspores. The pre- and postirradiative application of ATP at 30°C gave a similar extent of promotive effect on restitution, suggesting that ATP affects the recovery mechanism but not the breakage event. BEATTY & BEATTY ('59, '60 a) have suggested that besides the oxidative metabolism the glycolytic and hexose monophosphate pathways may furnish the energy for chromosome rejoining.

In the present experiments, such effect of ATP as promotive for rejoining breaks is further confirmed in *Trillium*. It is obvious that the rejoining of chromosome breaks involves metabolic processes requiring energy supply.

The simple ATP treatments produced nothing or only slight effect in heterochromatic region in contrast to the pronounced effect in euchromatin regions. It is reasonable to suppose that heterochromatin is provided with a weak metabolic activity related to the nucleic acid synthesis and mere energy supply cannot proceed the biochemical processes in it.

iv) Effect of DNA

The results of the present experiments with the application of DNA may be summarized as follows. (1) The single DNA treatment promoted restitution, but the frequency of interchanges remained as comparable as in the control, in contrast to the single ATP treatment which brought about a slight down of interchange frequency. Further it was demonstrated that the single DNA treatment cannot affect the aberrations in heterochromatic regions as in the case of single ATP treatment. (2) In combination with ATP, a more conspicuous decrease of simple breaks (promotion of restitution) was obtained, while the frequency of interchanges remained as comparable to or even increased from the level of the control and of the single DNA groups. The frequency of simple breaks in 'heterochromatic regions' was lower in all the combination groups than those in the single DNA and single ATP groups, while the frequency of interchanges in 'heterochromatic regions' either remained as comparable to the control, ATP, and DNA groups, or was slightly decreased, making a contrast to that in euchromatic regions showing certain increase in some combination groups. Thus, the simultaneous application of DNA and ATP seems to produce a promotive effect on restitution in heterochromatin, which could

not be obtained by single treatments of DNA or ATP. (3) Since the experimental groups which contained DNA showed frequencies of interchanges comparable to or larger than in the control, it is assumed that DNA promotes restitution in some way leaving a room for the formation of interchanges.

As to the way how the extraneously supplied DNA affects the process of radiation induction of chromosomal aberrations, the following three possibilities will a priori be assumed: (1) high concentration of DNA in cells, or DNA rich state of chromosomes at the time of radiation may act protectively against radiation damages, (2) DNA acts indirectly on the rejoining of breaks through promoting the synthesis of ATP or proteins, and (3) the supply of DNA promotes restitution by providing materials for the reconstruction of chromosome structure.

LURIA & EXNER ('41) studied the inactivation of bacteriophage by radiation and found that phages suspended in water or NaCl solution were inactivated fastly than those suspended in meat-extracts or gelatin. This protective effect was attributed to the antagonistic action against the indirect effect of radiation due to activated H_2O. LEA et al. ('44) also have shown that the dose of radiation to produce the same extent of inactivation of tobacco-mosaic virus is larger to viruses in protein solution of high concentrations or in gelatin than to those in the solution of low concentration. FRIEDEWALD & ANDERSON ('40, '41) also have demonstrated similar effects in rabbit papilloma virus. Thus, the high concentration of substances of high molecular weight may act protectively against radiation damage antagonizing against the indirect action due to the activation of H_2O by radiation. In the present experiments, if DNA is absorbed sufficiently enough to increase the concentration in cells, such a protective effect is probable. The cut stem immersion method may be more favorable for the absorption of chemicals of relatively high molecular weight than the root immersion method, for the substances can directly be absorbed into vascular system and readily transferred to the tissues.

BURNETT et al. ('51, '52) studied the effect of sodium hydrosulfite and related substances on the radiation damages and found that reducing agents are effective in protection of bacteria against damages. RILEY ('55) also confirmed in *Allium cepa* root tip cells the protective effect of sodium hydrosulfite and 2-3-dimercaptopropanol against the gamma-ray-induced chromosomal damages. The decrease of damages was interpreted as resulted from the elimination of indirect effect caused by the formation of H_2O_2, HO_2, H and OH radicals which are produced when H_2O containing O_2 is irradiated (cf. DALE '43).

WAGNER et al. ('50) reported that biochemical mutations are produced in *Neurospora crassa* at a rate significantly higher than the control rate by treating

the conidia with nutrient broth which have been previously irradiated with ultra-violet light, and also by treating the conidia directly with H_2O_2 and with potassium cyanide. STONE et al. ('48) and WYSS et al. ('47) has also demonstrated a similar effect of ultra-violet light in bacteria.

Another line of evidence also indicates a possibility of protection against radiation damages. KAUFMANN & HOLLAENDER ('46) showed that, in contrast to infrared light (cf. KAUFMANN et al. '46), post-irradiative ultra-violet light decreases the X-ray-induced chromosomal aberrations in *Drosophila*. SWANSON ('44) also demonstrated in the pollen tube chromosomes of *Tradescantia* that ultra-violet irradiation just prior or just after X-ray irradiation conspicuously decreases the frequency of chromatid-interchanges, and the number of chromatid breaks, though ultra-violet light itself is capable of producing chromatid breaks. Thus ultra-violet light seems to facilitate the chromatid breaks to restitute and decrease the chance of two-hit aberrations. SWANSON suggested that ultra-violet strengthens the nucleic acid matrix of chromosomes so as to prevent the initial breaks from interchange formation and from becoming established breaks.

Though the possibility of protective effect by DNA against radiation damage cannot be eliminated at present, the extent of the protection, if any, seems not to be so large, since the DNA treatments or combination treatments produced considerable number of interchanges which are comparable to or rather higher than that in the control group. If the protection was so effective, it would result in the decrease in the number of breaks and inevitably of two-hit aberrations.

The second possibility may be suggested by some biochemical studies. ALLFREY & MIRSKY ('57) reported that DNA and other polyanions are capable of stimulating ATP synthesis of isolated cell nucleus pretreated with DNase, and amino acid incorporation into them. Further, FRENSTER et al. ('61) have found that only DNA is promotive of amino acid incorporation into the proteins of isolated nuclear ribosomes. These studies are highly suggestive of the complicated nature of cellular metabolism. However, direct parallelism cannot be supposed to exist between the results of these *in vitro* experiments and those of our *in vivo* experiments since in the latter the supply of DNA was made in addition to the native DNA functioning normally and, therefore, it is doubtful whether the extraneous supply of DNA can further increase the synthesis of ATP or proteins.

Based on the fact that such protein synthesis inhibitors as chloramphenicol and aureomycin prevent breaks from rejoining, WOLFF ('59, '60) proposed that protein synthesis is necessary for rejoining, and further assumed that the nucleic acid synthesis does not involve in the rejoining mechanism. This latter assum-

ption was derived from the evidence that incorporation of tritiated thymidine into the nuclei of germinating *Vicia* root cells was not recognized at the particular time when WOLFF and his collaborators ordinarily irradiated and found breaks rejoining. He assumed further that the aberrations one observes in chromosomes after irradiation represent only intergenic alterations. However, there is no reason to assume that radiation induces breaks only at the intergenic portion of chromosomes and not within the genes, and that the rejoining of breaks involves the synthesis of only proteins, since it is a well-established fact that the chromosome consists mainly of proteins and nucleic acids, and many authors (SCHWARTZ '58, FREESE '59, CALLAN & MACGREGOR '58 & KAUFMANN '60) hold that the continuity of chromosome depends at least partly on DNA strands.

In the foregoing work of our laboratory (MATSUURA & TANIFUJI '62), it was demonstrated that a protein synthesis inhibitor, chloramphenicol, effectively inhibits the breaks from rejoining, as WOLFF ('59, '60) suggested. Therefore, the involvement of proteins in the intergrity of chromosomes and the necessity of protein synthesis for the rejoining of breaks seem to be evident. However, there is another possibility that the protein synthesis may be involved merely indirectly in the rejoining mechanism in such a way to provide some related enzymes, though the final conclusion must be awaited in future.

The nucleic acid synthesis inhibitor, mitomycin C, was also found to be effective in preventing breaks from rejoining (MATSUURA et al. '62) and this indicates the importance of nucleic acids for the maintenance of chromosome integrity and the involvement of nucleic acid synthesis in the rejoining process of breaks. Furthermore, the present experiments demonstrated that DNA effectively promotes restitution. DNA supplied in a polymerized form may not be incorporated intact into chromosomes. It is likely that DNA is once degradated at least to the level of nucleotides, and then incorporated. Many incorporation experiments indicate that the supply of nucleoside as thymidine is successful in demonstrating incorporation of labells into chromosomes. KIM & WOLF ('61) demonstrated that DNA and RNA are highly effective in supporting the growth of *Penicillium* as sole nitrogen source. This is an indication that cells are ready to degradate these compounds and utilize.

From these results, it may be concluded with safety that DNA is a part of chromosome integrity and the repair of breaks needs the synthesis of nucleic acids, thus supporting the chromosome model with alternate links of DNA and protein and denying the model which assumes a protein backbone with attached DNA.

v) Distribution pattern of breaks

A survey of literature on the distribution pattern of breaks produced by X-rays indicates that some investigators point to their random distribution along the chromosomes and/or their proportionality to chromosome length (e.g. KAUFMANN '46, THODAY '48, MATSUURA & HAGA '50, REVELL '52, Cf. READ '59), while the others claim to non-random distribution (e.g. SAX '38, SAX & MATHER '39, SWANSON '42, SETO '60). Such conflicting results should be considered in connection with heterochromatin distribution along the chromosomes, that is, whether ionizing radiations do react specifically with heterochromatin or not, a problem, as mentioned in the foregoing item (ii), which has not definitely been solved as yet. Another point to be considered here was presented by SAX ('42), who studied the distribution of breaks produced by the second dose of X-rays in the first-dose-induced akinetic fragments and kinetic chromosomes, and found that the breaks in akinetic fragments more readily restitute than those in kinetic chromosomes. This was interpreted by assuming that the movement of the kinetic chromosomes during the processes of cell division gives a strain to the breaks and that the absence of such a kind of strain in the akinetic fragments facilitates the breaks in them to rejoin.

The data obtained in the present experiments were rearranged in order to investigate the distribution pattern of aberrations among the five chromosomes of the complement (Table 5). The expectation on the basis of random distribution was obtained by dividing the sum of sB or TB in each group proportionately to the length of the five chromosomes. The χ^2 test revealed that the difference could not be regarded as incompatible with the random distribution in all the experimental groups and in the control group. As seen in the Table, however, the aberrations in the control group are more frequent in the largest chromosome and rather infrequent in the smaller chromosomes. This tendency disappeared in almost all treatment groups and therefore, may not be considered as having any implication. However, in the light of SAX's ('42) result, it is reasonable to assume that in the control group the larger the chromosome the stronger strain due to movement of the chromosome was given to the breaks preventing them from rejoining, while in the chemical treatment groups the effect promotive for restitution made obscure such a tendency relating to the chromosome length.

The two-hit aberrations harvested in the present study were analyzed in regard to the loci of two breaks involved in an interchange. As shown in Table 6, they are grouped into three categories: the interchanges between breaks both in heterochromatic regions ('H'-'H'), those between breaks both in euchromatic regions (E-E), and those between breaks in 'heterochromatic' and

TABLE 5. Distribution of aberrations among the five chromosomes.

Treatment		Observed and expected values of aberrations per 100 cells in chromosomes						χ^2-test
		A	B	C	D	E	Total	
Control	sB Obs.	58.0	25.9	19.8	21.0	11.1	134.6	non-sig.
	Exp.	41.7	25.6	23.8	25.6	17.9		
	TB Obs.	70.4	30.9	27.2	27.2	14.8	170.4	non-sig.
	Exp.	52.8	32.4	30.2	32.4	22.7		
ATP-H	sB Obs.	21.8	21.8	15.8	10.9	7.9	78.2	non-sig.
	Exp.	24.2	14.9	13.8	14.9	10.4		
	TB Obs.	31.7	24.8	20.8	14.9	12.9	105.0	non-sig.
	Exp.	32.6	20.0	18.6	20.0	14.0		
ATP-L	sB Obs.	27.1	18.6	13.6	16.9	3.4	79.7	non-sig.
	Exp.	24.7	15.1	14.1	15.1	10.6		
	TB Obs.	32.2	27.1	18.6	22.0	10.2	110.2	non-sig.
	Exp.	34.2	20.9	19.5	20.9	14.7		
DNA-H	sB Obs.	30.5	10.2	22.0	11.9	13.6	88.1	non-sig.
	Exp.	27.3	16.7	15.6	16.7	11.7		
	TB Obs.	40.7	13.6	27.1	16.9	25.4	123.7	non-sig.
	Exp.	38.3	23.5	21.9	23.5	16.5		
DNA-H + ATP-H	sB Obs.	33.3	11.7	26.7	10.0	10.0	91.7	non-sig.
	Exp.	28.4	17.4	16.2	17.4	12.2		
	TB Obs.	40.0	11.7	16.7	8.3	8.3	85.0	non-sig.
	Exp.	27.4	16.2	15.0	16.2	11.3		
DNA-H + ATP-L	sB Obs.	22.5	6.3	16.3	10.0	5.0	60.0	non-sig.
	Exp.	18.6	11.4	10.6	11.4	8.0		
	TB Obs.	35.0	16.3	23.8	20.0	10.0	105.0	non-sig.
	Exp.	32.6	20.0	18.6	20.0	14.0		
DNA-L + ATP-H	sB Obs.	20.0	10.9	12.7	7.3	9.1	60.0	non-sig.
	Exp.	18.6	11.4	10.6	11.4	8.0		
	TB Obs.	41.8	18.2	18.2	16.4	12.7	107.3	non-sig.
	Exp.	33.3	20.4	19.0	20.4	14.3		
DNA-L + ATP-L	sB Obs.	26.7	11.7	16.7	16.7	5.0	76.7	non-sig.
	Exp.	23.8	14.6	13.6	14.6	10.2		
	TB Obs.	41.7	13.3	26.7	20.0	10.0	111.7	non-sig.
	Exp.	34.6	21.2	19.8	21.2	14.9		

TABLE 6. Analysis of 114 two-hit aberrations with respect to the loci of two breaks involved in an interchange. 'H' indicates the 'heterochromatic' region and E indicates euchromatic region.

Region	'H'-'H'	'H'-E	E-E	Total
Observed No. of Interchanges	29	24	61	114
Expected Value (1)	5.7	39.6	68.9	
Expected Value (2)	14.8	52.4	46.7	

(1) Values calculated on the assumption that the breaks capable of forming interchanges are distributed randomly between two regions and there is no restriction to the occurrence of interchanges.

(2) Values obtained based on the observed frequency of rB (breaks involved in interchanges) in 'heterochromatic' region (82, 36.0%) and in euchromatic region (146, 64.0%), assuming that these frequencies represent the proportion of available breaks in two regions capable of forming interchanges and that no limitation exists in the formation of interchanges.

euchromatic regions ('H'-E). Clearly the occurrence of interchanges is not random between breaks in heterochromatic and euchromatic regions. The majority of interchanges represents inter-heterochromatic or inter-euchromatic ones. It is then assumed that the heterochromatic regions in resting nucleus are held together in close proximity and hence more interchanges are produced among them. Similar results have been obtained by REVELL ('52) and by THODAY ('48). A doubt may arise that a rather higher frequency of two-hit aberrations in 'heterochromatic regions' than in euchromatin observed in the control group and ATP groups is merely attributable to spacial proximity of heterochromatin in the resting nucleus and not to the differential response of heterochromatin to X-rays and to chemicals. However, the extreme increase of interchanges in heterochromatic regions by mitomycin treatment and also considerable decrease of aberrations in heterochromatic regions in the combination groups may not be interpreted without supposing a differential response of heterochromatin.

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5. Summary

1. The effect of extraneously supplied ATP and DNA on the X-ray-induced chromosomal aberrations was studied in the ovular tissue cells of

Trillium kamtschaticum PALL.

2. ATP decreases the aberration yield to a considerable extent, indicating that the rejoining of breaks involves metabolic processes requiring energy supply.

3. DNA also considerably decreases the frequency of aberrations. It is supposed that DNA is once degradated at least to the level of nucleotides and then incorporated for the reconstruction of chromosome continuity.

4. Simultaneous application of DNA and ATP seems to bring about an additional effect in reducing the frequency of simple breaks. Further, it is noted that in the groups which contains DNA singly or in combination with ATP the interchange frequency remains at or even increases than the level of the control.

5. The response of heterochromatic regions was analysed by means of separate scoring of aberrations occurring in 'heterochromatic' and euchromatic segments. The single ATP or single DNA treatments could not induce any appreciable changes in the frequency of aberrations in 'heterochromatic regions' in contrast to a remarkable decrease of aberrations in euchromatic regions. Thus, heterochromatin seems to react differently from euchromatin to extraneously supplied ATP and DNA.

6. The two-hit aberrations yielded in the present study were analysed in regard to the loci of breaks involved in the interchanges. The results indicate that in the resting nucleus the heterochromatic regions are held together in close proximity with each other so that more two-hit aberrations are produced between the breaks in them than between the breaks in heterochromatin and those in euchromatin.

7. The distribution of aberrations among the five chromosomes of the complement was analysed statistically and was not found to be incompatible with random distribution.

Postscript. During the press of the present paper, we were accessible to a paper by TAYLOR et al. (Proc. Natl. Acad. Sci. 48, ('62): 190–198) who observed that fluorodeoxyuridine (FUDR), a specific inhibitor of thymidylate synthetase, induces chromosome lesions which can lead to fragmentation, and thymidine or bromodeoxyuridine (BUDR) can reverse the effect of FUDR. In the experiments in combination with X-irradiation they ascertained that thymidine or BUDR, when supplied to cells deficient in thymidylate, is also effective in increasing interchange of chromosomes, a fact which implies that the reunion of broken ends requires the supply of thymidylate. Their conclusion is similar to ours in that at least DNA constitutes part of the linear axis of the chromosome, and the rejoining of broken ends involves DNA synthesis.

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