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Chromosome aberrations induced by 5-aminouracil

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The effect of 5-aminouracil (5 AU) on the ovular tissue cells of *Trillium kamtschaticum* Pall. was tested by means of absorption of 250, 500, 750 and 1000 ppm 5 AU solution from the cut stems of plants.

Any appreciable retardation of cell cycle was not observed except when 1000 ppm solution was applied. In all the concentrations tested, chromosome aberrations, mainly chromosome breakage, were induced. The frequency of aberrations was higher when cells were given further 21 hours duration after chemical treatment. Some cells were subjected to severe pulverization of chromosomes.

Five-aminouracil, a pyrimidine analogue, has been utilized since it brings about partial synchronization of cell cycle. WAGENAAR (1966) reported that in *Allium cepa* root tip cells treatment with 100 ppm 5AU for 24 hours gives rise to 60% mitotic index 12 hours after removal of chemical. SMITH *et al.* (1963) obtained a peak of division stage, 62.5%, 14 hours after treatment with 700 ppm 5AU for 24 hours in *Vicia faba*. CHU (1965) reported in Chinese hamster cells *in vitro* treatment with 3×10^{-3}M 5AU brings about a peak of mitotic activity 8 hours after removal of treatment solution.

Besides these synchronizing effects, it has been reported that 5AU itself induces chromosome aberrations. BREWEN (1964) reported that *Vicia faba* treated with 750 ppm 5AU for 24 hours produced 3.3~6.0 aberrations per 100 cells. SWIETLINSKA (1972) observed frequent chromosome gaps in *Vicia faba* metaphase chromosomes after treatments with 750 ppm 5AU. CHU (1965) recorded in Chinese hamster cells 0.1 breaks per cell after treatment with 3×10^{-3} M 5AU.

The purpose of the present experiment is to study the ability of 5AU to produce chromosome aberrations at a concentration which is not able to bring about any appreciable retardation of cell cycle.

Materials and Methods

The stems of plants (*Trillium kamtschaticum* Pall., $2n=10$) at flowering were cut about 20 cm below the flower just prior to the chemical treatment, and the freshly cut stems were immersed in 5AU solutions for 24 hours. The concentrations of 5AU applied were 250, 500, 750, and 1000

ppm (7.86×10^{-3} M). Ovules from half of the material plants were fixed and stained immediately after chemical treatments. Remaining half of the material plants were allowed to recover from the effect of 5AU by means of immersing them in tap water for further 21 hours (recovery period). Ovular tissue cells were fixed in La Cour 2BE fluid and slides were made by usual Feulgen squash method.

To examine the effect of 5AU on cell cycle, mitotic index was counted by observing 1000 cells from 3 to 4 plants in each treatment. Chromosome aberrations were observed in 100 metaphase and 100 anaphase cells from 3 to 4 plants.

Results

The effect of 5AU treatments on the mitotic index is listed in Table 1. The mitotic index of the ovular tissue cells at flowering without 5AU treatment fell within the range from 7.5% to 12.5%. The concentrations lower than 750 ppm seems not to bring about any retardation of cell cycle after 21 hours 5AU treatments. The treatments with 1000 ppm 5AU clearly suppressed mitotic activity.

Absorption of even 250 ppm solution, which could not bring about suppression of mitotic activity, caused chromosome aberrations. The frequency of aberrations was increased by higher concentrations of 5AU (Table 2). At the concentration of 1000 ppm mitotic cells decreased, and only a few metaphase cell was observable. Therefore, another experiment was performed for 1000 ppm 5AU (Table 7).

Table 3 shows the distribution of aberrations among chromosomes.

TABLE 1. Mitotic index after 5 AU treatments. (/1000 cells)

Concentration of 5 AU (ppm)	250	500	750	1000
Without recovery period	116	119	103	46
With 21 hours recovery period	81	84	74	15

TABLE 2. Frequency of metaphase cells with chromosome aberrations (/100 cells)

Concentration of 5 AU (ppm)	250	500	750
Without recovery period	3	7	16
With 21 hours recovery period	5	11	20

TABLE 3. Frequency of metaphase chromosomes with aberration

Treatment	Chromosome	Concentration of 5 AU (ppm)			Total
		250	500	750	
Without recovery period	A	1	8	12	21
	B	0	1	2	3
	C	2	1	4	7
	D	0	0	3	3
	E	1	2	1	4
	Total	4	12	22	38
With 21 hours recovery period	A	4	8	16	28
	B	0	1	6	7
	C	0	5	3	8
	D	0	1	3	4
	E	1	1	5	7
	Total	5	16	33	54

TABLE 4. Frequency of each type of aberration at metaphase

	Chromosome		Chromatid		Total
	Breakage	Gap	Breakage	Gap	
Without recovery period	26	13	5	1	45
With 21 hours recovery period	71	14	11	4	100
Total	97	27	16	5	145

TABLE 5. Frequency of anaphase cells with chromosome aberrations (/100 Cells)

Concentration of 5 AU (ppm)	250	500	750	1000
Without recovery period	2	7	12	16
With 21 hours recovery period	13	23	55	60

TABLE 6. Frequency of each type of aberration at anaphase

Bridge	Fragment	Gap	Lagging chromosome
11	617	14	3

TABLE 7. Frequency of cells with chromosome aberrations at metaphase and anaphase after 24 hours treatment with 1000 ppm 5 AU and 24 hours recovery time

Stage	No. of cells observed	Frequency of cells with						% of abnormal cells
		1 B′	1 B″	2 B″	3 B″	4B″	Many breaks	
Mataphase	100	6	6	3	2	4	2	23.0%

Stage	No. of cells observed	Frequency of cells with								% of abnormal cells
		1 f	2 f	3 f	4–5 f	5–6 f	1 g	1 g+1 f	Many fragments	
Anaphase	104	8	6	4	6	2	4	1	2	31.7%

B′ : Chromatid breakage or gap
B″: Chromosome breakage or gap
g : Gap
f : Fragment

The largest chromosome, A, appeared to be most frequent in aberrations. As shown in Table 4, chromosome type breakage predominates over chromatid type aberrations indicating that 5AU already affected chromosomes before DNA replication. In Tables 5 and 6, the frequency of aberrations observed at anaphase is listed. It is clear that aberrations at anaphase are more frequent that those observed at metaphase. It is also noteworthy that some cells suffer severe pulverization of chromosomes (Table 7, Plate 1).

Discussion

It has been reported that 5AU blocks DNA synthesis. However its effect is not complete in contrast to FUdR (fluorouracil deoxyriboside) which completely suppresses DNA synthesis (SCHEUERMANN and KLAFFKE-LOBIEN 1973). Further it is noted that effect of 5AU prolongs after removal and therefore results in a partial synthronization of mitotic phase after several hours of recovery period. In *Allium cepa* root cells, treatment of 100 ppm 5AU for 24 hours brought about 60% synchronization of mitotic cells after 12 hours (WAGENAAR 1966). With *Vicia faba* concentrations used to obtain synchronization, were 500 ppm for 15 hours (BREWEN 1964) and 750 ppm for 24 hours (SWIETLINSKA 1972). In the present experiment, in order to test the effect of 5AU on the ovular tissue cells of *Trillium*, 5AU solution was absorbed from cut stems of flowering plants. As a result, concentrations under 750 ppm could not induce any appreciable depression of mitotic activity and only when 1000 ppm solution was applied conspicuous decrease of mitotic

index was obtained. It is considered that, in contrast to the root tip cells in which 500 or 750 ppm 5AU, and even 100 ppm in the case of *Allium cepa* are effective to suppress mitotic activity, in the present experiment with *Trillium* even 750 ppm 5AU cannot affect the cell cycle, is due to indirect absorption of chemical solution from cut stems. In other words, real concentrations in ovular tissue cells were far lower than those of chemical solutions. The fact that 21 hours duration after 5AU absorption tend slightly to suppress mitotic activity may imply that action of 5AU was prolonged.

In the present experiment, it is ascertained that low concentrations of 5AU which cannot affect the cell cycle, are able to induce chromosome aberrations. Increase of aberration frequency at anaphase compared with those observed at metaphase indicates that many invisible lesions are concealed in chromosomes. The frequency of aberrations at metaphases and anaphase induced by application of 1000 ppm 5AU was obtained from another experiment (Table 7). After 24 hours recovery period, 23% of metaphase cells possessed chromosome aberrations and total 50 or more breakages were counted in 100 cells. At anaphase 32% of cells exhibited aberrant figure and total 80 or more breakages (mainly fragmentation) were observed in 104 cells.

Sometimes cells were subjected to moderate or severe pulverization of chromosomes. It is suspected that real concentration of 5AU in cells attained abnormally high level by an unknown reason, or effect of 5AU on a critical stage of transformation of chromosome structure, results in pulverization of chromosomes.

The fact that main type of aberration at metaphase chromosome is chromosome breakage or gap suggests 5AU affect chromosomes before DNA replication. It is also noted that exchange type aberration is very few suggesting that repair system which is responsible for the production of exchange aberration is also affected by 5AU.

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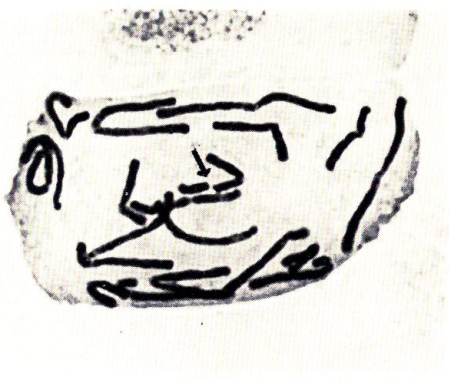
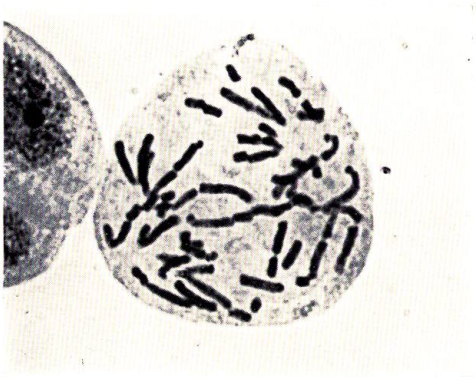
Plate I.

Chromosome aberrations at metaphase and anaphase in the ovular tissue cells of *Trillium kamtschaticum* PALL. induced by 5-aminouracil treatments. Arrows indicate chromosome or chromatid breakages. The lower right and lower left photographs show moderate pulverization of chromosomes.