REDUCTION OF RADIATION DAMAGE TO TRADESCANTIA CHROMOSOMES BY ADENOSINE TRIPHOSPHATE, PROLINE, AND HISTIDINE¹

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IN 1963, we reported that *Tradescantia* microspores treated with histidine or proline prior to irradiation in helium showed fewer chromosome aberrations than the controls. In 1960 and 1962, similar results were obtained when material was pretreated in ATP, agreeing in general with the results that WOLFF and LUIPPOLD (1956) found in their work with *Vicia faba*. In 1960, we demonstrated by postirradiative treatments that the ATP effect was on the recovery process. In this report, similar experiments have demonstrated that histidine and proline also are operative during the recovery process.

Numerous experiments have been performed (BEATTY and BEATTY 1960, 1962) to demonstrate the extent of the biological effects of ATP in irradiated cells. Pre- or posttreatment in an ATP solution for 1½ hours at 30°C in helium reduces the aberration yield by 50%. Treatment in ATP in helium reduces to 41% the aberration yield due to treatment in helium alone for 400 min. Aberration yield due to irradiation at 40°C in helium is reduced to 29% by treatment in an ATP solution.

Recently we have carried out biochemical and radiobiological experiments involving ATP (BEATTY and BEATTY 1965). Using the luciferin-luciferinase assay for ATP under several experimental conditions, the ATP content in the protoplasm of *Tradescantia* microspores was found to be relatively constant. However, 1½ hours treatment in an ATP solution will double the amount of ATP in the liquid surrounding the microspores, the periplasmodium. Conditions such as anoxia which deplete the ATP in the periplasmodium, increase the aberration yield. Since the amount of ATP within the microspores seems to be constant, the firefly technique cannot be used to demonstrate the passage of materials from the periplasmodium into the microspores. Radiobiological studies, using ATP³² alpha, beta, gamma-labeled, showed radioactivity in the protoplasm of the microspores in all materials treated with radioactive ATP solutions, but more than twice the activity in the material which was also irradiated.

The present report deals with a more detailed study of the ATP, histidine, and proline effects on radiation recovery using both aberration yield and the luciferin-luciferinase assay for ATP as experimental procedures.

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MATERIALS AND METHODS

Inflorescences of *Tradescantia paludosa* having one or more nodes within a 2-inch region below the cyme were selected for study. The lower half-inch of the peduncle was slit longitudinally before being placed in a chemical solution. X-irradiation was given in atmospheres of helium, carbon monoxide, air, 5% oxygen in helium, and 5% oxygen in carbon monoxide at 30°C for 8 minutes at 50r/min and posttreated 15 min in the same gas. Acetocarmine preparations were made of the microspores 96 hr after irradiation and chromosome aberrations of the ring and dicentric types were scored. At least 500 cells were examined in each experiment.

Details of the luciferin-luciferinase method of ATP assaying are given by BEATTY and BEATTY (1965). Briefly, 30 anthers were removed, squashed in ice water between two watch glasses, anther walls strained out and the fluid analyzed. Equal volumes of ATP solutions and of soluble extract from dried firefly lanterns were used. A Farrand Photoelectric Fluorometer used for readings was calibrated and the data are given in mg/liter ATP equivalents.

Adenosine triphosphate, crystalline disodium (No. 9861), L-histidine (No. 9130), and L-proline (No. 4556) were obtained from Nutritional Biochemicals and the firefly materials from Sigma Chemical Company.

RESULTS AND DISCUSSION

To study the effect of ATP, histidine, or proline on the cell cycle, two types of experiments were done. First, progress through the stages (BEATTY and BEATTY 1953) in the developing microspores was studied, and the chemicals used were found to have no effect. Second, the populations of treated cells going through the microspore division were checked for aberration yields on the 3rd, 4th, and 5th days following irradiation. The yields were the same on the 3rd and 4th days, with a slight lessening effect of the chemicals on the 5th day, resulting in a slightly higher aberration yield. The populations used in the experiments in this paper were derived from materials fixed 96 hours after irradiation.

Previous results (BEATTY and BEATTY 1960) demonstrated that postirradiative treatments with ATP in a helium atmosphere were effective, and thus that ATP was operating during the recovery process. Similar experiments in the present series were done using histidine and proline, and results indicate that posttreatments are as effective as pretreatments in reducing the aberration yield from the control value of 23 ± 1 per 100 cells. For example, preirradiative treatment in histidine gave an aberration yield per cell of 0.12 ± 0.02 while postirradiative treatments gave 0.13 ± 0.02 . Preirradiative treatments in proline gave 0.16 ± 0.01 while postirradiative treatments gave 0.17 ± 0.02 . Since pre- and posttreatments gave the same results, pretreatments were used because of the greatly simplified experimental procedure they afforded.

To determine whether ATP, histidine, or proline have any radiomimetic properties, inflorescences were placed in the most concentrated solution used for each chemical for $11\frac{1}{2}$ hours. In each experiment, 500 metaphase configurations were examined and no aberrations were found.

In order to explore the activity of each chemical compound in modifying the chromosome aberration yield, a wide range of concentrations was used for each one. As ATP concentrations were increased (Table 1), aberrations proportionally decreased, until a concentration of maximum effectiveness was reached at 16 mg/100 ml water. Concentrations of proline reached maximum effectiveness at 36 mg, and histidine at a concentration of 16 mg/100 ml.

TABLE 1

Interchanges obtained when inflorescences were treated for 1½ hours in air in various concentrations of either ATP, proline, or histidine, then X-rayed for a total dose of 400r at 50r per minute in a helium atmosphere

Chemical	mg/100 ml of water	Number of cells	Average interchanges per cell $\pm$ SE
He control	...	1800	0.23 ± 0.01
ATP	250	500	0.11 ± 0.02
	124	500	0.12 ± 0.02
	62	500	0.12 ± 0.02
	32	500	0.12 ± 0.02
	16	500	0.13 ± 0.02
	6	350	0.14 ± 0.02
	3	600	0.15 ± 0.02
	0.6	500	0.20 ± 0.02
Proline	36	1050	0.16 ± 0.01
	18	500	0.17 ± 0.02
	9	500	0.19 ± 0.02
	4.6	450	0.23 ± 0.02
	2.3	500	0.23 ± 0.02
Histidine	47	400	0.13 ± 0.02
	32	500	0.12 ± 0.02
	16	450	0.13 ± 0.02
	8	500	0.18 ± 0.02
	3	450	0.18 ± 0.02
	1.6	450	0.18 ± 0.02

In Figure 1 some of the experimental data from Table 1 involving ATP, proline, and histidine are plotted. The graph based on the ATP experiments indicates that an increase in the amount of ATP gave a decrease in aberration yield until the maximum effectiveness was reached at approximately 0.12 aberrations per cell. Neither proline nor histidine showed this proportional response to differ-

FIGURE 1.—Average aberrations per hundred cells plotted against concentration of chemical. (Data from Table 1)

TABLE 2

Interchanges obtained when inflorescences were treated in air in one concentration of either ATP, proline, or histidine for different periods of time, then X-rayed in a helium atmosphere for a total dose of 400r at 50r per minute

Chemical	mg/100 ml of water	Treatment (hours)	Number of cells	Average interchanges per cell $\pm$ se
He control	...	...	1100	0.23 ± 0.01
ATP	124	$\frac{1}{2}$	500	0.20 ± 0.02
		$\frac{1}{4}$	500	0.11 ± 0.02
		1	500	0.16 ± 0.02
		$1\frac{1}{2}$	500	0.12 ± 0.02
		3	1000	0.12 ± 0.01
		5	500	0.13 ± 0.02
		9	500	0.13 ± 0.02
Proline	36	$\frac{1}{2}$	500	0.22 ± 0.02
		1	450	0.23 ± 0.02
		$1\frac{1}{2}$	1050	0.16 ± 0.02
		3	450	0.16 ± 0.02
Histidine	32	$\frac{1}{2}$	450	0.17 ± 0.02
		1	500	0.17 ± 0.02
		$1\frac{1}{2}$	500	0.12 ± 0.02
		3	400	0.13 ± 0.02

ences in concentration, but proline showed an initial plateau in which the next higher concentration did not initiate a greater response. This may indicate that a certain concentration must be built up within the cells before any effect can be realized. Once this concentration was reached, additional proline caused a decrease in aberration yield, until a leveling off occurred around 0.16 per cell. After an initial reduction in aberrations, the plateau produced by histidine covers a wider range of concentrations than that found with proline, and occurs at the points where 0.6 to 8 mg/100 ml result in 0.18 aberrations per cell.

FIGURE 2.—Average aberrations per hundred cells plotted against duration of treatment periods in chemicals before irradiation. (Data from Table 2)

The data recorded in Table 2 and plotted in Figure 2 are from experiments in which the concentration of ATP, proline, and histidine was held constant, but the length of treatment was varied. As ATP-treatment time was lengthened, the aberration yield decreased linearly up to $1\frac{1}{2}$ hours, and was maintained through 9 hours of treatment. The data from experiments with proline and histidine do not show this immediate linear relationship with length of treatment and decrease in aberration yield. Treatment with proline results in a plateau effect during the first hour similar to that which appeared when different concentrations were used, and reaches its maximum effect in $1\frac{1}{2}$ hours. Histidine treatments, as in the concentration experiments, resulted in an initial decrease in aberrations, followed by a plateau during the first hour, and reached a period of maximum effectiveness at $1\frac{1}{2}$ hours.

For the data recorded in Table 3 and plotted in Figure 3, the same concentrations of ATP, proline, and histidine were used as in Table 2, and the minimum time for maximum effectiveness, $1\frac{1}{2}$ hours, was selected. After the $1\frac{1}{2}$ hr treatment in the chemical, the inflorescences were washed and placed in water for varying periods of time before they were irradiated. This experiment was designed to investigate whether the effects of treatment given immediately before irradiation will be of sufficient duration to carry into the recovery period following irradiation. Since the irradiation is given in 8 min, it is evident that the treatment effects of ATP and proline, lasting 2 hr for ATP and almost 2 hr for proline, operate well into the recovery period. Even for histidine, although its effect disappears after 15 min, there is a short period when it could be affecting recovery. Also, since both pre- and posttreatment with ATP, proline, and histidine give the same aberration yield, it is reasonable to attribute the effects to recovery. The

TABLE 3

Effects of ATP, proline, or histidine in reducing radiation damage (400r in helium) which are residual in inflorescences in the interval following removal from treatment chemicals to the time of irradiation

Chemical	mg/100 ml of water	Interval (hours)	Number of cells	Average interchanges per cell $\pm$ se
He control	...	..	1600	0.23 ± 0.01
ATP	124	0	500	0.12 ± 0.02
		$\frac{1}{4}$	500	0.11 ± 0.02
		$\frac{1}{2}$	500	0.13 ± 0.02
		1	650	0.13 ± 0.01
		2	500	0.16 ± 0.02
Proline	36	0	1050	0.16 ± 0.02
		$\frac{1}{4}$	550	0.18 ± 0.02
		$\frac{1}{2}$	500	0.17 ± 0.02
		1	500	0.19 ± 0.02
		2	500	0.23 ± 0.02
Histidine	32	0	500	0.12 ± 0.02
		$\frac{1}{4}$	450	0.22 ± 0.02
		$\frac{1}{2}$	500	0.22 ± 0.02
		1	1000	0.22 ± 0.02
		2	500	0.22 ± 0.02

FIGURE 3.—Average aberrations per hundred cells plotted against residual effects of chemicals. (Data from Table 3)

results from these experiments, involving different periods after removal from the solutions, perhaps indicate to some extent the rate at which these chemicals are metabolized. The immediate reduction in aberration yield associated with the low concentrations and duration of treatment in histidine, as well as the rapidly diminishing effect after removal from histidine solution, may be associated with a histidine-feedback mechanism, as described by AMES and HARTMAN (1962) for *Salmonella*. MOYED and FREEDMAN (1959) found in *Escherichia coli* that a 10^{-4} M concentration will produce histidine inhibition. This concentration of histidine is one of those used in obtaining data resulting in the plateau in Figure 1 at 18 aberrations per 100 cells. One would have to assume that this biosynthetic pathway exists in *Tradescantia* and that an energy balance in favor of rejoining would result from histidine inhibition and histidine repression.

In order to characterize further the activities of these chemical compounds in

TABLE 4

The effects of ATP, histidine, or proline on aberration yield when X-irradiation (400r) is given in different gas atmospheres

Chemical	mg/100 ml water	He	CO	Air	5% O ₂ -95% He	5% O ₂ -95% CO
Control		0.23 ± 0.01 (1600)	0.23 ± 0.02 (500)	0.77 ± 0.03 (1400)	0.46 ± 0.03 (500)	0.72 ± 0.04 (500)
ATP	124	0.12 ± 0.02 (500)	0.14 ± 0.02 (600)	0.66 ± 0.04 (500)	0.36 ± 0.03 (500)	0.48 ± 0.04 (500)
Histidine	32	0.12 ± 0.02 (500)	0.13 ± 0.02 (500)	0.76 ± 0.04 (500)	0.43 ± 0.03 (500)	0.74 ± 0.04 (500)
Proline	36	0.16 ± 0.01 (500)	0.15 ± 0.02 (500)	0.75 ± 0.04 (500)	0.48 ± 0.03 (500)	0.73 ± 0.05 (350)

Average interchanges per cell ± SE are given in the body of the table. Number of cells on which each point was based is given in parenthesis.

modifying the aberration yields, a comparison was made between irradiations given in atmospheres of He, CO, air, 5% O₂-95% He, and 5% O₂-95% CO (Table 4). Inflorescences were treated for 1½ hours before irradiation in the same concentration of chemicals given in Table 2. In all gas atmospheres, treatment in ATP resulted in a considerable reduction in chromosome aberrations—to 52% in helium and to 86% in air of the control damage (Table 4). This was not the case, however, in material treated with histidine or proline. Both reduced the aberration yield only when materials were irradiated under anoxic conditions in either He or CO. In the remaining three gas mixtures, each of which contained some oxygen, the aberration yields were the same as the untreated control.

Since a histidine or proline effect was found only when the materials were subjected to anoxia, several experiments (Table 5) were carried out in helium to determine whether these chemicals would have any effect on the amount of ATP in the periplasmodium surrounding the microspores. (BEATTY and BEATTY [1965] found that the amount of ATP in the protoplasm of the microspores is relatively constant, but as the amount of ATP in the periplasmodium increases the aberration yield decreases. The converse is also true. If, after depletion of ATP under conditions of anoxia to a concentration at which the maximum aberration yield is obtained, the inflorescences are returned to an air atmosphere, and then tested after a 40-min period, the ATP content is nearly normal—indicating a rather rapid restoration.) Treatments in ATP solutions resulted in an increase in ATP content in the periplasmodium, the greatest increase being shown by those treated in ATP and irradiated. None of the experimental material treated with histidine or proline showed any increase in ATP.

SUMMARY

Adenosine triphosphate, proline, and histidine have no effect on the rate of progress of the developing *Tradescantia* microspore through its successive stages, nor do they have any radiomimetic properties. There are minimum and maximum concentrations of all three chemicals, between which they are effective in reducing damage from X rays. ATP shows a linear relationship between both duration of treatment and concentration and the reduction of damage, while proline, and also histidine after an initial reduction, exhibit thresholds during which limited or no effect is shown. Proline and ATP are still effective up to 2

TABLE 5

Effect of ATP, histidine, and proline on ATP content (in mg/liter) of the periplasmodium, as determined by luciferin-luciferinase assay

Treatment solutions	Untreated (control)	Treated but not irradiated	Treated and irradiated
ATP	1.25	2.10	4.65
Histidine	1.15	1.25	1.15
Proline	1.15	1.25	1.25

hours after removal from treatment solutions, while the effect of histidine is greatly reduced in 15 minutes; pretreatment with any of the three, however, remains effective in reducing aberrations well into the recovery period following irradiation. ATP reduces radiation damage in all gas atmospheres used—to 52% in HE, 61% in CO, 86% in air, 78% in 5% O²–95% He, and 67% in 5% O²–95% CO of the control damage—while histidine and proline are effective only in the absence of oxygen. Treatments in ATP solutions result in increased ATP in the periplasmodium within the anther, the greatest increase being in those also irradiated; treatment in histidine and proline does not affect the ATP content in the periplasmodium.

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