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Chromatid Aberrations Induced by π^- Mesons in *Vicia faba* Root Meristem Cells*

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SUMMARY

Chromatid aberrations were induced in *Vicia faba* root meristem cells by 31, 92, and 117 rads of π^- mesons at the peak of the depth-dose distribution. The irradiation was carried out under both aerobic and anoxic conditions. The yields of various types of aberrations were obtained in cells at 4-hour intervals up to 24 hours after irradiation. An oxygen enhancement ratio of 1.8 was found. In cells irradiated at the peak of the depth-dose distribution the aberrations were not distributed among cells according to random expectations. Too many cells had no aberrations and too many had multiple aberrations. This is a reflection of the way in which energy is dissipated when a π^- meson stops and produces stars in the medium.

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I. Introduction

π^- mesons deposit their energy in an unusual way. The fraction of the dose deposited at the entrance of a π^- meson beam is relatively small and has a low LET (0.3 keV/μ), similar to that of conventional radiations. At the peak of the depth-dose distribution, however, because of star formation, the LET spectrum is very broad, covering a range of 0.2 keV/μ to 1000 keV/μ. Here, nearly 25 per cent of the dose is from events with an LET value greater than about 20 keV/μ (Curtis and Raju 1965). Because of the unusual characteristics of π^- mesons, there is great interest in their potential as a radiotherapeutic tool. (Fowler and Perkins 1961, Fowler 1965, Richman et al. 1966, Raju and Richman 1969). Consequently, in order to define the radiobiological effects of π^- mesons, a series of experiments has been conducted at the 184 inch synchrocyclotron at Berkeley. The reduction of root growth in Vicia faba exposed to π^- mesons at the peak of their depth-dose distribution was reported earlier (Raju et al. 1970). This paper summarizes the results of a cytological analysis of the chromosome aberrations induced in root tips exposed to π^- mesons.

2. Material and Methods

Vicia faba seeds (Sutton's Longpod) were germinated in running water, grown in vermiculite and then transferred to an aerated water tank maintained at 19°C. The details of the physical characteristics of the π^- meson beam, the irradiation configuration, and the π^- meson dosimetry have been described earlier (Raju et al. 1970). Ten-day-old primary roots of Vicia faba were arranged in special Lucite boxes

through which air or N_2 was bubbled to maintain the roots in either aerobic or anoxic conditions. The boxes were positioned in the π^- meson beam at the peak of the depth-dose distribution and doses of 31, 92, and 117 rads were given at a dose rate of 30 rads/hr. Also, some root tips were given an exposure of 95 rads at the beam entrance.

After exposure, the bean roots were returned to the growth tank and maintained at 19°C. To collect cells in metaphase where aberrations could be scored, the root tips were given a 4 hour treatment in an aerated 0.05 per cent solution of colchicine and then fixed in Ford's fixative at 4 hour intervals from 4 to 24 hours after irradiation. Five root tips were fixed for each time. Permanent slides were made of each root tip by the Feulgen squash method (see Wolff 1964 for details). Only chromatid aberrations were observed. They were classified as chromatid deletions, isochromatid deletions, exchanges, and tri-radials.

3. Results and Discussion

The frequencies of various aberrations obtained at different fixation times after exposure under aerobic conditions are given in table I and plotted in figure 1. The frequencies obtained under anoxic conditions are given in table II and plotted in figure 2. As may be seen in the figures, the yield of chromatid aberrations varies with fixation time. This is a manifestation of the differential stage sensitivity of cells distributed in S and G_2 of the cell cycle coupled with radiation-induced mitotic delays and reversions (Wolff 1968). Such patterns are quite common and have been observed for Vicia faba root tip cells exposed to 14 MeV neutrons (Savage 1968).

The variability with fixation time made it difficult to obtain a meaningful comparison of effects at any one fixation. Consequently it was necessary to obtain the mean aberration yield over the entire period of fixation. The values are given in table III and plotted in figure 3. The curves were fit to the equation $Y = kD^n$, and the values of the exponents are also presented (figure 3).

The data shown in figure 3 show that at the peak of the depth-dose distribution for π^- mesons, simple chromatid breaks and tri-radials increase linearly with dose, whereas exchanges are formed largely with two-hit kinetics and increase as the 1.7 power of the dose. Isochromatid deletions increase as an intermediate power of the dose. With densely ionizing radiations, exchanges would be expected to be one-hit and increase linearly with dose. The shape of the curve for exchanges shows that even at the peak of the depth-dose distribution of π^- mesons a significant fraction of the dose deposited is of low LET. This is in conformity with the physical characteristics of pions. Since tri-radials are a form of exchange, they too would be expected to increase with the same dose kinetics as did simple exchanges. We believe the reason they did not is that the yields of tri-radials were very low, under which conditions one-track events usually predominate in the formation of two-break events (Abrahamson et al. 1971).

It was found that root tips exposed under anoxic conditions and transferred to colchicine immediately had very few cells in mitosis if the time in nitrogen was 3 hours or more. For the longest exposure times in nitrogen there appeared to be a mitotic delay of about 12 hours since no cells were found in division at the 4 hour and 8 hour fixations (table II).

Although the dose delivered by π^- mesons at the region where they stop and produce stars is expressed as 31, 92, and 117 rads, it should be noted that these are doses measured on a macroscopic scale by means of ionization chambers. Actually the dose will not be distributed randomly, and on a microscopic level the local dose will be much higher. Since this is the case, it seemed probable that the chromatid aberrations would not be distributed randomly among the cells scored. Table IV contains the fits of the data to the Poisson formula of $e^{-m} \cdot m^r/r!$, where m is the mean number of aberrations per cell and r indicates whether a cell has 0, 1, 2, 3, etc. aberrations. Although chromatid aberrations induced by sparsely ionizing radiations do fit a Poisson distribution (Lea 1946), the aberrations produced by π^- mesons at the peak of the depth-dose distribution do not. It was observed that too many cells had no aberrations or multiple aberrations, as might be expected from the fact that the energy deposited by π^- stars is highly localized and is of high LET. For the doses used, not as many cells would be hit by these star events as when the dose is distributed randomly. Furthermore when the star is produced, enough energy is deposited in the small volume of a cell to cause multiple chromosome breaks. In contrast, the abberrations produced by the pion beam at its entrance, where the energy dissipation is random, do fit a Poisson distribution.

3.1 Oxygen Enhancement Ratio (OER)

The low dose rate of 30 rads/hr obtained from the synchrocyclotron necessitated exposure times up to 4 hours to achieve the doses used in the experiments. Root tips remaining in a nitrogen atmosphere for

such times showed a mitotic delay. For an accurate comparison (over comparable parts of the cell cycle) of individual types of aberrations in aerobic and anoxic conditions, fixation times beyond 24 hours are necessary after exposures in nitrogen. Nevertheless, the ratio of the aberration frequencies produced by a given dose in air and nitrogen at a single fixation time has often been used as an index of the influence of oxygen (Larsson and Kihlman 1960). If a similar procedure is followed with these data and a comparison made of the initial peak values for chromatid deletions for 92 and 117 rads, a value of 1.7 to 1.9 is obtained for the OER. A comparison of the initial peak values of isochromatid deletions for the same two doses leads to the same value of 1.8. Although the value of 1.8 obtained is not as rigorous as could be obtained if whole curves could be compared, it is relatively independent of dose rate. The OER obtained from growth measurements is dependent on dose rate and had a value of 1.35 (Raju et al. 1970). This value increased to 1.5 (Raju et al. 1970) when the root tips were exposed at a very low temperature (4°C), under which conditions the OER for root growth becomes relatively independent of dose rate.

3.2 Relative Biological Effectiveness (RBE)

The present experiments were not designed to obtain the relative biological effectiveness of π^- mesons. Nevertheless, a rough comparison of the yields of π^- meson induced chromatid deletions observed in our experiments could be made with X-ray induced chromatid deletions observed by others and with the data obtained for the π^- mesons at the entrance of the beam. Chromatid deletions were chosen for the comparison because they increase linearly with dose.

At 6 hours after irradiation the chromatid deletions produced by a dose of 31 rads of π^- mesons is 0.07/cell. The equivalent yield is found after a dose of 95 rads of 250 kV X-rays (Revell 1966). Therefore the RBE is approximately 3.

The mean chromatid deletion frequency for the entire period of fixation is 0.047/cell for root tips exposed at the entrance of the pion beam. The corresponding dose of π^- mesons at the peak of the depth-dose distribution is 25 rads. Thus a value of 3.8 is obtained for the biological effectiveness of π^- mesons at the peak when compared with those at the entrance.

4. Conclusions

The LET spectrum of π^- mesons at the peak of the depth-dose distribution is very broad, covering a range of 0.2 keV/μ to 1000 keV/μ. Nearly 25 per cent of the dose is due to events greater than about 20 keV/μ in LET. Hence, from physical considerations one would expect aberrations characteristic of both low- and high-LET radiations. Thus the existence of cells with multiple aberrations and the nonrandom distribution of aberrations among cells from root tips exposed at the peak of the depth-dose distribution are indicative of the high-LET components, whereas the nonlinearity in the shape of the dose-response curves and a relatively high OER of 1.8 could be partially attributed to the effect of the low-LET components.

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TABLE I

Frequencies of various types of aberrations produced under aerobic conditions

π^- meson dose (rads)	Fixation time	Number of cells scored	Chromatid breaks	Isochromatid breaks	Chromatid exchanges	Tri-radials
31	4	99	8	5	0	0
(Peak)	8	99	7	14	2	5
	16	38	1	1	1	0
	20	104	5	8	1	5
	24	105	4	5	1	0
92	4	98	37	52	6	7
(Peak)	8	105	22	42	6	10
	12	99	8	24	7	9
	16	98	11	39	5	7
	24	91	4	12	1	0
117	4h45'	104	45	60	11	11
(Peak)	8h45'	102	32	71	20	14
	12h45'	113	21	50	10	9
	16h45'	90	16	56	14	10
	20h30'	101	11	27	8	6
	24h30'	81	9	15	2	2
95.5	8	65	3	14	3	1
(Entrance)	12	118	8	28	4	2
	20	102	6	19	5	0
	24	98	1	12	0	0

TABLE II

Frequencies of various types of aberrations produced under anoxic conditions

π^- meson dose (rads)	Fixation time	Number of cells scored	Chromatid breaks	Isochromatid breaks	Chromatid exchanges	Tri-radials
31	4	91	0	2	1	0
(Peak)	12	99	2	13	1	1
	16	97	2	6	4	2
	20	92	3	10	1	2
	24	45	1	1	0	0
92	4	No cells in mitosis				
(Peak)	8	110	24	25	9	2
	12	91	8	27	4	7
	16	103	11	26	7	4
	20	100	17	38	6	3
117	4h45'	No cells in mitosis				
(Peak)	8h45'	No cells in mitosis				
	12h40'	100	23	19	3	2
	16h40'	102	17	41	3	7
	20h40'	104	13	37	1	4

TABLE III

Mean aberration yields per cell produced under aerobic conditions of exposure

π^- meson dose (rads)	Number of cells scored	Chromatid breaks	Isochromatid breaks	Chromatid exchanges	Tri-radials
(Peak) 31	445	0.056	0.074	0.011	0.022
92	491	0.167	0.34	0.051	0.067
117	591	0.23	0.47	0.11	0.088
95.5 (Entrance)	381	0.047	0.19	0.031	0.008

TABLE IV

Number of cells containing 0, 1, 2, 3, and 4 or more aberrations:

π^- meson dose (rads)		Aberrations per cell 0	1	2	3	≥ 4	X^2 Test
31	Observed	389	44	8	3	1	$X^2 = 13.8$
	Expected	378	61.9	5.1	0.3	0	$n = 2$, $p = 0.001$
92	Observed	291	123	55	16	6	$X^2 = 20.7$
	Expected	263.2	164.1	51.2	10.6	1.86	$n = 3$, $p < 0.001$
117	Observed	298	161	71	36	25	$X^2 = 73.4$
	Expected	240.5	216.2	97.2	29.1	7.9	$n = 4$, $p < 0.001$
95.5 (Entrance)	Observed	294	73	11	1	2	$X^2 = 1.004$
	Expected	288.5	80.2	11.2	1	0.1	$n = 2$, $p = 0.6$

Peak

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FIGURE CAPTIONS

Fig. 1. Variation of the frequencies of different classes of chromatid aberrations with fixation time after exposure to π^- mesons at the peak of the depth-dose distribution under aerobic conditions. a) chromatid gaps, b) chromatid deletions, c) isochromatid deletions, d) exchanges and e) tri-radials. 31 rads Δ, 92 rads O, 117 rads ●.

Fig. 2. Variation of the frequencies of different classes of chromatid aberrations with fixation time after exposure to π^- mesons at the peak of the depth-dose distribution under anoxic conditions. a) chromatid deletions, b) isochromatid deletions, c) exchanges and d) tri-radials. 31 rads Δ, 92 rads o, 117 rads ●.

Fig. 3. Mean aberration yield per cell as a function of π^- meson dose. a) chromatid deletions, b) isochromatid deletions, c) exchanges d) tri-radials and e) all aberrations.

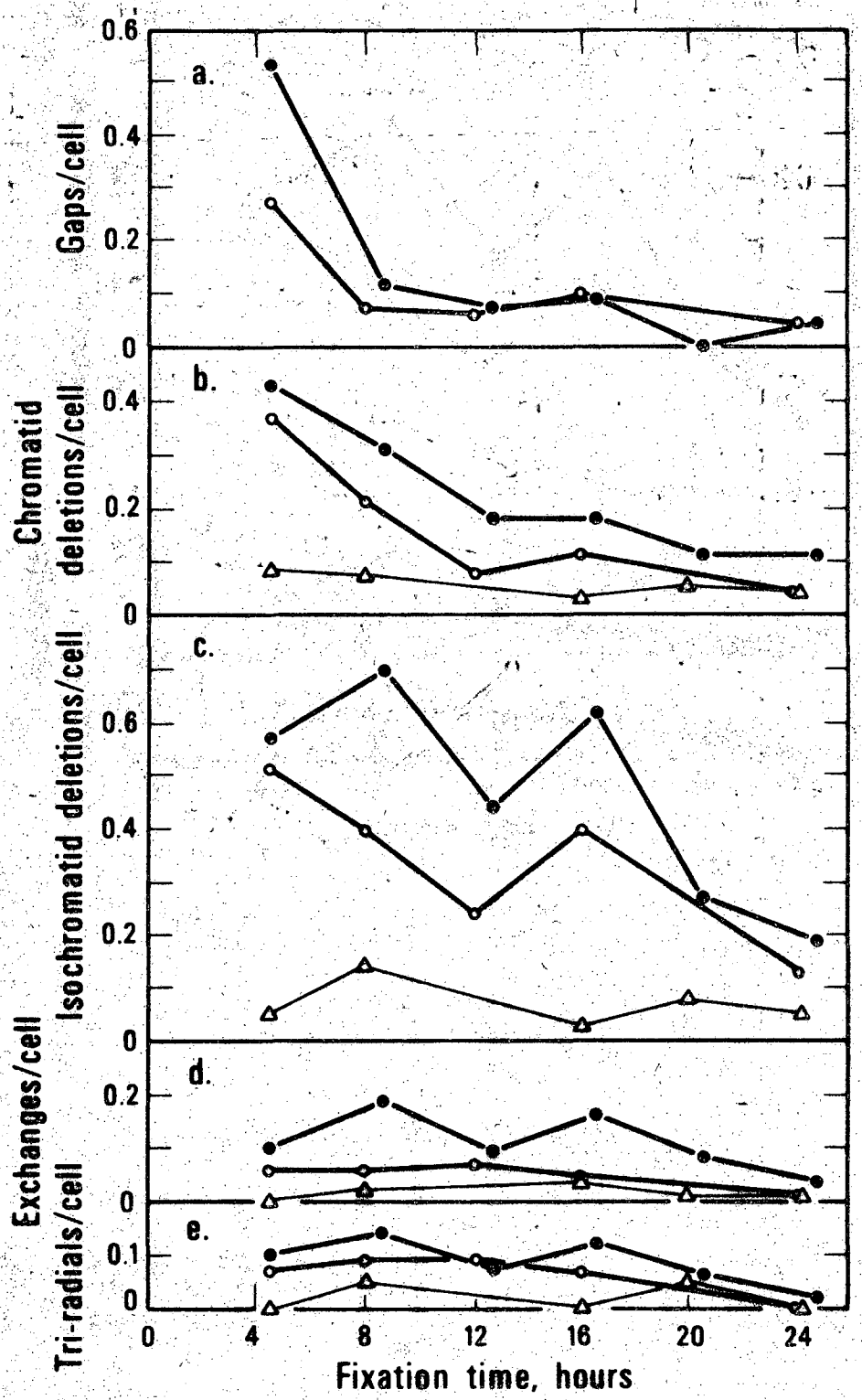

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Fig. 1

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