Effects of Beta-ray Irradiation on Mitosis in *Tradescantia* with Reference to Dosage Estimation¹

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Received October 1, 1959

Introduction

In the last decade, the use of radioisotopes for biological investigations has increased remarkably. There are two ways of utilization, namely, as a tracer application and as a radiation source. With an increase of radioisotope utilization, a new problem arose which concerns the effects on the cell of beta-ray irradiation due to the incorporated beta-emitters. Heretofore, only a few experiments have been performed to test the cytological effects of incorporated beta-ray emitters. Giles and Bolomey (1948) reported that quantitative measurements in terms of counts per minute (cpm) of beta-rays from incorporated P^{32} showed a good linear correlation with the relative frequency of chromosome aberrations in *Tradescantia* pollen grains. In their work, P^{32} was incorporated as an inorganic substance and was thought to have disintegrated in both chromosomes and cytoplasm. The specific incorporation of C^{14} into chromosomes or DNA was carried out by McQuade and his collaborators (1956). Chromosome aberrations found in the roots treated with radioactive thymidine increased with increasing exposure to thymidine- C^{14} . Tanaka *et al.* (1956) treated *Tradescantia* roots with the radioactive hair of a Bikini patient who suffered from the nuclear bomb test explosion in southern Pacific Ocean in 1954, and showed that even so meagre an amount of radiation, mostly of beta-rays, as 0.000356 microcurie per mg hair can augment the aberration frequency by a factor of 2 in 24 hours and up to 13.3 in 144 hours.

In all of these experiments the dosage of beta-ray irradiation was only presented as counts per minute (cpm) or as microcuries (μc), thus making difficult a direct comparison with the effects of X-rays or gamma-rays which are measured in roentgen (r) units.

In microorganisms, on the other hand, inactivation of a single cell caused by the decay of incorporated radioisotopes has been discussed by Hershey *et al.* (1951) and many workers. Strauss (1958) suggested a transmutation effect of P^{32} , that is, in the course of disintegration of P^{32} into S^{32} , a beta-particle

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or an electron yields sufficient recoil energy to be lethal to the cell. Since H^3 , C^{14} , S^{35} and Ca^{45} produce soft beta-radiation, any significant number of disintegrations will lead to a large radiation dose before the emitted beta-particle clears the biological particle. Strauss proposed a theoretical estimate for internal beta-ray irradiation based on the experimental density value of ion pairs.

The most difficult problem in beta-ray experiments is the dosage estimate. The track of beta-particles is short in water and living cells, and the density of ion pair production varies with the kind of nuclide used. The author wishes to discuss this problem on the basis of results obtained from P^{32} irradiated experiments in *Tradescantia*.

Material and methods

1. Experiments on pollen grain mitosis

A. Material and its cultivation

The spiderwort *Tradescantia paludosa*, clone 6, was cultivated in the Boysen-Jensen's nutrient solution, in which the plant continued to grow normally for more than one month without adding any more elements, and without changing the solution when 5 to 10 plants were grown in 1 liter.

B. Concentration of added P^{32}

Radioactive orthophosphate ($H_3P^{32}O_4$) in dilute HCl solution was provided from England through The Japan Radioisotope Association. To remove contaminated metaphosphate the solution was boiled for an hour (Yoshikawa *et al.* 1957), then diluted with carrier solution of KH_2PO_4 , and added to the cultivation solution. The primary concentrations of P^{32} were 0.05 mc/l and 0.1 mc/l.

C. Measurement of P^{32} incorporation into inflorescence

In order to measure the daily change of P^{32} uptake in the *Tradescantia* inflorescence, the buds were kept under a bell type Geiger-Mueller tube (Fig. 1), and covered with cellophane to fix them on the bottom iron plate of a stand which held the tube. The iron plate with buds was placed at the lowest step of the stand so that the error of the cpm was minimized because of the decrease of the geometry efficiency.

Apparatus used for the measurement of radioactivity was a Tracerlab Geiger-Mueller tube connected with a Model-32 count scaler produced by The Scientific Institute Inc., Tokyo. The effective thickness of the mica window was 1.9 mg/cm^2 .

D. Uptake of P^{32} into the anthers

One of the six anthers of a bud was observed under the microscope

Fig. 1. Geiger-Mueller tube mounting for recording the rate of uptake of P^{32} into the inflorescence of *Tradescantia*.

by the squash method, using either aceto-carmines or aceto-orcin, and when it was in a suitable mitotic stage, the remaining five anthers were weighed one by one and immediately measured for cpm. This process we carried out in haste, taking care to keep them in adequate moisture for living anthers. The uppermost step of the stand about 12 mm from the mica window of the G-M tube was used for the cpm measurements.

E. Detection of chromosome aberrations

Breaks induced by radiation in chromosomes lead to chromosome or chromatid fragments unless they reunite again or are translocated elsewhere; these fragments, in turn, will form micronuclei in the subsequent interphase or prophase. Post meiotic mitoses of pollen grains exhibit a degree of synchronization in *Tradescantia*, and after all the pollen grains have completed mitosis, every cell becomes binucleated uniformly. To obtain reliable statistical results, 4000 to 12000 cells in each experimental section were examined, these having been derived from 3 to 5 anthers. The relative frequency of chromosome aberrations represents a ratio of the number of cells with micronuclei, mostly one, to the total number of cells observed.

2. Experiments on root tip cells

Root tip cells of *Tradescantia paludosa* cultivated in radio-active Boysen-Jensen's solution supplemented with P^{32} were used. For the purpose of providing sufficient oxygen to make the meristematic cells mitoses normally, the nutrient solution was aerated by an air compressor for eight minutes every hour. The starting concentration of P^{32} in the nutrient solution was 0.4 mc per liter, and the decrease of radioactivity, due mainly to the absorption by the plant and partially to the natural decay of P^{32} atom, was calculated from measurements of daily change of the cpm in the solution.

The roots were fixed at 24-hour intervals with Carnoy's alcohol-acetic acid mixture, macerated in 1 N HCl for 6 minutes at 60°C, following which cytological observations were made in aceto-carmines squash preparations.

Results

1. Pollen grain experiments

A. Incorporation of P^{32}

Uptake curves of incorporated P^{32} into inflorescences estimate the dose of P^{32} taken in anthers (Fig. 2). These curves, however, show only relative values of the disintegration of P^{32} in the inflorescence, because the cpm varied with shoot length, root conditions, leaf size, etc. If it is assumed that the incorporation of P^{32} into the anther is proportional to the dose taken in the inflorescence, the daily change of the cpm of the anthers can be estimated from the observed cpm of the anther. The cpm of the anthers may be plotted on the uptake curve according to the concentration of P^{32} and the date of collection; the curve then shows the relative daily change of incorporation

in an anther. The curves shown in Fig. 2 agree in general with those presented by Giles and Bolomey (1948).

B. Chromosome aberrations and cpm of an anther

The lower the dosage given, the lower the frequency of chromosome aberrations; the number of cells observed must be increased in order to obtain accurate results. For this purpose micronuclei determinations are five times more efficient than is the break-analysis method more commonly used in this kind of study. The correlation between radiation dose and micronuclei formation, is shown in Fig. 3. For this experiment the intensity of irradiation was considered to be too low to cause so many chromosome or chromatid exchanges due to two-hit aberrations. Therefore it can be assumed that micronuclei formation is correlated with the formation of chromosome aberrations although the distinction between one-hit and two-hit aberrations, and between chromosome and chromatid breaks, can not be detected.

In this experiment, the anthers were observed on the 3rd, 6th and 9th day after the administration of P^{32} in the nutrient solution. The main results are presented in Table 1.

Fig. 3. Relationship between X-rays dose and frequency of aberrations in *Tradescantia* pollen mitosis. Micronuclei observed after pollen mitosis.

sults of the control experiments indicated that the nutrient solution had no effect on the chromosomes.

2. RTC experiment

Fig. 2. Uptake curves of P^{32} in inflorescence. A. 0.1 mc/l, B. 0.05 mc/l.

In the table, the aberration frequency of control plants cultivated in non-radioactive solution was subtracted from those of treated lots. The range of variation in the weight of anthers was from 0.45 mg to 1.02 mg and for the cpm, corrected for natural background radiation, from 36 to 624 per anther. The data in the table are average values of 4 or 5 anthers; a few, however, are the average of 2 or 3 anthers in the same bud when these anthers were the only ones available. The results of the control experiments indicated that the nutrient solution had no effect on the chromosomes.

Table 1. Relationship between the cpm/mg of anthers and the frequency of aberrations in pollen mitosis in buds kept for 3, 6 and 9 days in nutrient solution supplemented with a certain amount of P^{32}

Period of treatment	P^{32} conc. mc/l	Shoot No.	Total cells	cpm/mg	Aberr. %
3 days	0.05	2	7149	70.5	0.27
	"	3	4782	242.5	0.36
	0.1	5	10230	57.1	0.10
6 days	0.05	1	9000	253.1	0.36
	"	3	8718	731.3	0.63
	"	4	10242	946.5	0.56
	0.1	5	7686	142.0	0.57
	"	7	4800	205.8	0.50
9 days	0.05	2	12000	507.7	0.31
	"	4	9042	1037.0	0.87
	0.1	5	12000	460.0	0.76
	"	7	8790	245.0	0.54

Fig. 4. Calculation of total counts during the treatment by integrating the incorporation curve (see manuscript for more detailed explanation).

Table 2. Frequency of aberrant cells in the root tip of *Tradescantia* treated with radioactive nutrient solution

Period of treatment	Total cells	Aberr. %	Mitotic index %
24 hrs.	7200	3.43	7.02
48	7200	5.38	3.22
72	6600	7.63	0.33
96	6600	10.20	4.97

results presented in Table 2 must be regarded as a preliminary note of this series of experiments.

Because of the absorption of the nutrient solution by the plant, and of the decay of P^{32} atom, the radioactivity of the solution during the period of the experiment declined to about one half of the starting value of beta-rays (Fig. 4). In this experiment both external as well as internal radiation of beta-rays on the root tip cells must be considered. An estimate of the dosage, and conversion into roentgen units will be discussed in the next chapter.

Unfortunately, the air compressor broke down on the third day of the experiment. This accident caused a retardation of cell division. Unfortunately the experiment could not be repeated because of a delay in P^{32} delivery. Hence the re-

Discussion

1. Theoretical view on incorporated P^{32}

One of the purposes of this paper is to discuss the dosage estimate for beta-rays which can be measured only as cpm. The biological effects of radiation are proportional to the energy absorbed in living cells. The yields of energy absorbed can be measured only in "r" units by calculating the amount of ion pair production per 1 ml of H_2O , with the production of 1.79×10^{12} ion pairs being designated as 1 r. The relative biological effectiveness (RBE) for beta-rays is 1.

The first problem to be discussed is a procedural one, that, namely of establishing a relationship between the number of disintegrations and the production of ion pairs. By definition the dose in terms of "r" units, this is directly applicable only for X- and gamma-ray radiation, but an obvious

Table 3. Roentgens absorbed due to a single disintegration for spheres of different radii (Strauss 1958)

Isotope	Radius (μ)			
	0.01	0.1	1	10
H ³	1.5×10^5	1490	22.6	0.023
C ¹⁴ *	2.8×10^4	275	2.75	0.0275
P ³² *	8.0×10^3	80	0.8	0.008
S ³⁵ *	2.8×10^4	275	2.75	0.0275
Ca ⁴⁵ *	2.0×10^4	198	1.98	0.0198

* Average value for several disintegrations, since on the average there is less than one ionization produced in this distance; calculated on the basis of the energy of the average beta-particle: $1r = 1.79 \times 10^{12}$ ion pairs per cc H_2O (ionization energy = 32.5 eV. $1r = 93$ ergs absorbed in water).

extension could be used to describe as 1 r of beta-rays that dose which, in 0.001293 g of air (1 cm³ at 0°C and 760 mm pressure) liberates ions carrying 1 e. s. u. of charge of either sign. It corresponds to the liberation of 2.098×10^9 ion pairs per cm³ of air at 0°C and 760 mm pressure, taking 32.5 eV per ion pair as the mean energy dissipation in air (Lea 1955). Lea presents a table for the energy dissipation in the living tissues corresponding to 1 r of beta-rays defined in the above way. But so little attention was paid to the effect of beta-rays in his time that he listed only a case of 1 MeV beta-rays. Recently Strauss (1958), in his paper on the transmutation experiment, presented a table of roentgens absorbed due to a single disintegration for spheres of different radii (Table 3).

In order to arrive at an estimate of the dosage of beta-rays in roentgen units from the cpm measurements two calculations, based on the physical data, were carried out.

Calculation A

1) By our G-M apparatus, experimentally at the uppermost step of the stand, $1 \mu c = 2.5 \times 10^5$ cpm.

Fig. 5A. gives uptake curves of P^{32} incorporation into the inflorescence; here the cpm on the ordinate gives only a relative amount of P^{32} incorporation. When we get a measure of 1000 as the cpm/mg of an anther on the 9th day in the "0.1 mc" experiment, the value of 1000 is given for the ordinate measure of the curve I at the 9th day, and then the total counts for 9 days, C, can be calculated by integrating the curve I,

$$C = \int_0^{12960 \text{ (9 days, in minutes)}} F(x) dx = 3.0 \times 10^6 \text{ counts/mg}$$

where x is time in minutes (Fig. 5B).

Fig. 5. Decrease curves of cpm of the solution. A. Measured 7 days after the beginning of the treatment. B. Calculated for the time of fixation from curve A. Curve B shows the values of cpm at the time of fixation.

2) Since $1 \mu c = 2.2 \times 10^6$ dpm (disintegrations per minute) when the total counts as calculated above turn out to be 3.0×10^6 /mg provided that the weight of the anther is 0.5 mg, we get the following estimate of the total number of disintegrations per anther:

$$2.2 \times 10^6 \times \frac{3.0 \times 10^6}{2.5 \times 10^5} \times 0.5 = 1.32 \times 10^7 \text{ dis./anther.}$$

3) If we assume that the anther is a sphere consisting of substances with an approximate density of 1.0, the radius of the hypothetical sphere which corresponds to 0.5 mg anther comes to

$$3\sqrt{0.0005 \times 3/4} = 492 \mu.$$

4) From the Table 3, a dosage in r units absorbed in the tissue when a single disintegration occurs in a sphere with a radius of 492μ is calculated as follows:

$$1.32 \times 10^7 \times 0.00008 \times \frac{1}{4.92^2} = 47.6 \text{ r,}$$

where 0.00008 is number of roentgens for a standard sphere with a radius of 100μ . It is reasonable to assume that the proportion of decrease of roentgens for increasing radii, even to 492μ , remains unchanged.

Calculation B

There is another way by which a comparable calculation can be made.

For an average electron energy of 0.70 MeV, there are 6.1 ion pairs produced per mm track in air, and since the reduction of energy to 0.5 MeV changes the value only to 6.4, we can use the 6.1 figure for calculation purposes even though the average electron energy of P^{32} is 0.695 MeV.

1) As the number of ion pairs is proportional to the density, and the density of air at 20°C and 760 mm pressure is 1.20×10^{-3} g/cc, then

$$\frac{6.1}{1.20 \times 10^{-3}} = 5.1 \times 10^3 \text{ ion pairs per mm H}_2\text{O per electron.}$$

2) Assuming a sphere of 0.492 mm radius, a total of 1.32×10^7 disintegrations, an average energy of 32.5 eV released per ion pair and using the conversion factor 1.6×10^{-12} erg/eV, our calculation will be

$$5.1 \times 10^3 \times 4.92 \times 10^{-1} \times 1.32 \times 10^7 \times 32.5 \\ \times 1.6 \times 10^{-12} \times 3/4 = 1.29 \text{ ergs.}$$

The factor 3/4 corrects the figures for uniform disintegrations of isotopes, for in the calculation all disintegrations are assumed to occur at the center of the sphere.

3) Therefore the energy absorbed in 0.5 mg or 5×10^{-4} g turns out to be

$$\frac{1.29 \text{ ergs}}{5 \times 10^{-4} \text{ g}} = 2.58 \times 10^3 \text{ ergs/g,}$$

or

$$\frac{2.58 \times 10^3}{93 \text{ ergs/g/rep}} = 27.7 \text{ rep or } 25.8 \text{ rad. (1 rad} = 100 \text{ ergs/g)}$$

The above calculations have neglected the effect of external beta-rays from surrounding tissue on the anthers, obviously an important factor when we consider chromosomal aberrations induced in pollen grain cells. These two calculations are therefore to be regarded as minimal estimates which may range upwards to varying degrees. It was pointed out by McClement (see Strauss 1958) that the measured experimental value for P^{32} is different from the theoretical value which is taken as the first assumption in Calculation B and is somewhat higher than that used here.

The problem how to estimate the dose of external beta ray radiation on the pollen grains is complicated since the surrounding tissue is not so simple or so homogeneous in structure. If the anthers are small compared to the surrounding tissue, which is not the case, then the radiation flux might have been calculated as though there were a uniform disintegration of isotopes in the case of the root tip cells.

2. Accumulated effect of beta-rays on pollen mitosis

The calculations carried out in both methods A and B were based on

the total counts integrated from the uptake curve. By the calculation A, measures in the 5th column (cpm/mg) in Table 1 are converted into r units which are reproduced in Table 4 and Fig. 6.

Table 4. Relationship between frequency of aberration and radiation dosage in r unit

Period of treatment	P ³² conc. (mc/1)	Shoot No.	Total counts integrated	Average weight of anthers	Radius (μ)	Dosage (r/anther)	Freq. of aber. (%)
3 days	0.05	2	1.31×10^5	0.96	612	2.5	0.27
	"	3	4.51	0.81	578	9.5	0.36
	0.1	5	1.24	0.74	561	2.0	0.10
6 days	0.05	1	7.65	0.66	540	12.2	0.36
	"	3	22.12	0.57	514	33.6	0.63
	"	4	28.63	0.51	496	41.8	0.56
	0.1	5	6.13	0.51	496	9.0	0.57
	"	7	8.88	0.69	544	14.6	0.50
9 days	0.05	2	24.34	0.90	599	43.0	0.31
	"	4	49.72	0.69	544	81.6	0.87
	0.1	5	29.81	0.76	566	49.6	0.76
	"	7	15.88	0.73	559	26.1	0.54

The pollen mitosis occurs in general at least 9 days, and at most 14 days, after meiosis. During this period, the pollen grains continue to grow, while their nuclei remain in a resting state.

Fig. 6. Relationship between roentgens calculated from total counts of beta-rays of incorporated P³² and frequency of micronuclei formation in *Tradescantia* pollen grain.

In the present experiments these resting nuclei received beta-ray irradiation continuously. If the dividing nucleus is more sensitive to radiation than the resting, as reported by some authors, the nucleus during the mitotic period will suffer much damage from beta-ray irradiation. If the radiation effect accumulates in spite of partial recovery, it would be expected to increase in proportion to the accumulated dose. The results so obtained, illustrated in Figure 6 and tabulated in Table 4, show a better correlation between radiation

dose and frequency of aberrations than those tabulated in Table 1. A decline in the curve is thought to be caused by a decrease in the relative proportion of the mitotic period during exposure. If the radiation-sensitive period of mitosis is, on the average, three times as sensitive as the resting stage, and if the sensitive period continues for 12 hours, then the dosage rate for 3,

6, and 9 days comes to 4:7:10 when the radiation intensity is unchanged.

The intensity of beta-rays in the present experiments is very weak compared with the common X-ray experiments. As for point mutation, the frequency does not change with intensity variations, while in the case of chromosome aberrations, a given dose becomes less effective if delivered in a long span of time. When 200 r is given at rate of 50 r and 0.1 r per minute, the latter was one third as effective as the former according to Koller (1952). Although this view seems to hold true to a certain extent, it is apparent that the lower the intensity the less the chromosome aberrations, and the correlation curve simulates an hyperbola.

In an X-ray experiment with high intensity radiation, aberrations increased linearly as shown in Figure 3. When we compare these results with those obtained in exposure experiments by incorporated P^{32} , it becomes clear that the efficiency of P^{32} in inducing aberrations is about one third that of X-rays; this decrease may be explained by the low intensity of radiation from P^{32} .

3. RTC experiment

For calculating the roentgen dose of P^{32} absorbed in RTC tissue, the second method (Calculation B) described for pollen grains may be applicable with a slight modification. The first assumption is that the disintegrations per unit volume in the RTC tissue are substantially equal to those to be produced in the nutrient solution, and therefore the entire system consisting of cells and solution might be considered homogeneous as regards irradiation efficiency. By definition, if a unit of roentgen dose yielding 100 ergs/g is equal to 1 rad, a relationship between the r dose and the frequency of aberrations can be calculated in much the same manner as for pollen grain (Calculation B) (Table 5). The total number of disintegrations can also be computed by integrating the curve in Figure 4.

With 0.4 mc/1 of P^{32} , rads per minute can be calculated as follows:

$$\begin{aligned} &5.1 \times 10^3 \times 1.0 \times 2.2 \times 10^6 \times 0.4 \times 32.5 \\ &\quad \times 1.6 \times 10^{-12} \times 3/4 = 1.74 \times 10^{-1} \text{ ergs} \\ &\frac{1.79 \times 10^{-1}}{4.19 \times 10^{-3}} = \text{ergs/g/min.} = 0.41 \text{ rads per minute,} \end{aligned}$$

where 1.0 is average distance of track of P^{32} beta-particle and 4.19×10^{-3} is the volume of a sphere of 1.0 mm radius.

In X-ray experiments at higher intensity, 100 r yielded aberrations of 2.7 % when the material was fixed 48 hours after irradiation. This result is more than 3 to 4 times as effective when compared to the P^{32} results, and

Table 5. Relationship between aberration frequency and radiation dosage in roentgens in RTCs

Period of treatment	Dosage in r	Frequency of aberrations %
24 hrs	533	3.43
48	976	5.38
72	1341	7.63
96	1640	10.20

agrees well with the results obtained from low intensity experiments by several other workers.

Further information concerning the difference between chronic irradiation with X- or gamma-rays and that of beta-rays are needed. Experiments designed to provide these data are now in progress.

The transmutation effect discovered in microorganisms will probably occur in chromosomes in higher organisms. Though one of the purposes of the present research was to elucidate this question, the author failed to get results sufficiently reliable to provide meaningful answers.

Summary

The dosage of beta-rays could be calculated in terms of r units, with the equations being based on the number of ion pairs produced per disintegration.

Compared with the effect of high intensity X-rays, P^{32} treatment was about $1/3$ or $1/4$ less effective in inducing chromosome aberrations. This was observed not only in the case of pollen mitosis, but also in root tip cell division. The decrease of effectiveness might be caused by the low intensity of irradiation.

Acknowledgement

The author wishes to express his thanks to Dr. N. Tanaka for much valuable advice during the work and for his kindness in reading the original manuscript. To Dr. B. S. Strauss, the Fulbright exchange professor now staying in the University of Osaka, the author is also indebted for the privilege of obtaining permission to reproduce Table 3 and for suggestions regarding the calculations.

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