

JAERI-Research
95-039



DICENTRIC YIELDS INDUCED IN RABBIT BLOOD LYMPHOCYTES
AFTER EXPOSURE IN VITRO TO X-RAYS

June 1995

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編集兼発行 日本原子力研究所
印刷 ㈱原子力資料サービス

Dicentric Yields Induced in Rabbit Blood Lymphocytes after Exposure
In Vitro to X-rays

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(Received May 1, 1995)

For the purpose of biological dosimetry, it is essential to establish the relationship between dicentric yields and absorbed doses. The present experiment was carried out to obtain data for rabbit lymphocytes as a reference for this relationship.

As data at low dose level are scanty, rabbit lymphocytes were exposed to various doses, especially below 0.5 Gy, of 150 kVp X-rays and analysed at their first mitotic division for dicentric yields. The yields at high dose level were compared with data reported by other authors. The linear-quadratic equation, which is generally accepted, for the dose-response relationship was obtained by the iteratively reweighted least squares method. However, as the present experiment result showed that the dose-response relationship at low dose-levels was likely to be linear, a dose-response line was calculated by the linear regression analysis. As the result of the chi-square tests, it was found that the dicentric yield was better fitted to the linear model at low doses below 0.5 Gy than the linear quadratic model.

Keywords: Dosimetry, X-rays, Rabbit, Lymphocyte, Dicentrics

X線 in vitro 照射後ウサギリンパ球中に誘発された二動原体染色体発生率

日本原子力研究所東海研究所保健物理部

井上 義教

(1995年5月1日受理)

二動原体染色体発生率に基づく生物学的線量計測を行うには、二動原体染色体発生率と吸収線量との関連を求めておくことが必要である。その参考となるデータを得るため、ウサギのリンパ球を用いて実験を行った。

低線量域でのデータの報告が殆どなされていないので、ウサギのリンパ球を150 kVpX線で照射し、特に0.5 Gy以下の種々の線量における第一有糸分裂中期のリンパ球中の二動原体染色体発生率を調べた。そして他の研究者の高線量域でのデータと比較した。線量—反応関係を表す式として一般に認められている線形—二次(LQ)式を繰り返し重み付き最小二乗法を用いて求めた。しかしながら、本実験結果は低線量領域での線量—反応関係が直線状であることを示したので、直線式を線型回帰分析により求めた。カイ二乗検定の結果、0.5 Gy以下の低線量域においては、二動原体染色体発生率は直線式で最もよく適合できることが分かった。

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1. INTRODUCTION

Many studies¹⁾ have been carried out up to now in order to find end-points which can be used in biological dosimetry to estimate the absorbed dose received by persons; and it is appreciated that dicentric is one of the most sensitive end-points yet demonstrated.

In order to provide a biological dosimetry service (that use dicentric chromosomes as end-points), in vitro curves relating dicentric yields to doses of X-rays, γ -rays and neutrons are needed. However, as the use of calibration curves²⁾ derived from data obtained in other laboratories would not necessarily be valid unless the culture methods and the scoring techniques had been compared, it is essential³⁾ that a competent radiation protection laboratory should generate its own dose-response curves so that this reference data can be used in the estimation of doses in the event of a radiation accident.

The present experiment was carried out to obtain such reference data for biological dosimetry in JAERI.

2. MATERIALS AND METHODS

Rabbit blood samples (male and female, about 1 year old) were used. Rabbit blood was chosen because the karyotype ($2n = 44$) of rabbit is similar to that ($2n = 46$) of man.

Blood samples in polyethylene cylindrical containers (14 mm in diameter \times 75.2 mm in height \times 1 mm thick wall) with the dosimeter (IONEX DOSE/DOSE RATE METER 2400/3) in a 0.6 cm³ chamber inside a latex tube were placed at 5 cm⁴⁾ distance from the surface of a water phantom (30 cm in width \times 30 cm in length \times 25 cm in height) maintained at 38°C, and were exposed to 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5 and 2 Gy of X rays.

Irradiation was carried out with a SHIMAZU HF-420 model C X-ray generator operated at :150 kVp; effective voltage 89.4 keV; tube current 17 mA; 1.49 mm Cu filtration; focus - sample distance 53 cm; 0.1 Gy/min. The absorbed dose in Gy was obtained by multiplying the exposure in roentgens by 0.00955 (Gy/roentgen conversion factor).

For the rabbit blood culture⁵⁻⁸⁾, the supernatant fluid after centrifugal isolation of irradiated blood was added to culture medium containing 5 ml NCTC 135 (GIBCO), 0.1 ml phytohaemagglutinin M (DIFCO) and antibiotics. Bromodeoxyuridine at a final concentration of 6 μ g/ml was added. The cultures were incubated for 45 h at 38 °C in a 95% air/5% CO₂ atmosphere, with colcemid present for the final 3 h at a final concentration of 0.5 μ g/ml. Hypotonic treatment and fixation were performed according to the usual methods. For slide preparation the following simplified modification of the method of Wolff and Perry⁹⁻¹¹⁾ was used to distinguish the first from the later mitotic division. Slides were stained for 15

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minutes with 6 μ g/ml Hoechst 33258 in Soerensen buffer solution, rinsed with distilled water, mounted in the same buffer, and exposed to light from a 400-W mercury lamp* from a distance of 50 cm for 20-30 minutes. Slides were stained with 3% Giemsa(Merck) solution for 10 minutes. The metaphases were analysed for dicentrics.

3. RESULTS AND DISCUSSION

3.1 Dose-response for dicentric yield

Table 1 shows dicentric yields after X-irradiation of rabbit lymphocytes. The data obtained by other authors^{7,12-14)} with the present data are shown in Table 2. For equivalent radiation dose levels, different laboratories have reported different dicentric yields, and this was thought to be due to differences of irradiation conditions, culture methods and numbers of scored cells. The equations of the dose-response relation for the present dicentrics data were calculated by the least squares method and the iteratively reweighted least squares method³⁾. They are

$$Y = 0.042789D + 0.02379D^2 \quad (\chi^2=18.523, \chi_{0.01,9}^2=21.666)$$

$$Y = 0.03921D + 0.02595D^2 \quad (\chi_a^2 = 18.41)$$

where Y is the yield of dicentrics(the number of dicentric chromosomes per cell) and D the absorbed dose in Gy. The critical χ^2 - value for the particular degree of freedom ν at 95%-confidence is given by $\chi_{0.05,\nu}^2$. The statistical technique recommended by IAEA TECHNICAL REPORTS SERIES NO. 260³⁾ for determining the best-fit coefficients is the method of the iteratively reweighted least squares. The report describes that this can be done by maximizing the likelihood of the observations assuming Poisson distributions by the method of the iteratively reweighted least squares. The equation best fitted for the present data is obtained by this method, also.

However, as it has been reported^{15,16)} that the shape of the dose-response curve at low dose-levels (0-0.5 Gy) might be closer to linearity and in Figs. 1 and 2 a plot of the dicentrics against the absorbed dose in the range of 0 to 0.5 Gy seems to be linear, the dose range (0- 2 Gy) was divided into two ranges, the (0-0.5 Gy) range and the (0.5-2 Gy) range; then the equations of the dose-response relation were calculated. For the (0- 0.5 Gy) range, the equations were calculated according to three models, namely; the linear model, $Y = \alpha + \beta D$ where Y is the yield of dicentrics, α and β are constants and D the dose

*Flood light for high intensity discharge lamps(H362S) with mercury lamp(HF-400X) (Iwasaki Electric Company,Ltd.).

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in Gy; the power law model, $Y = c + kD^n$ where c is the background level of dicentric yield, k and n are constants, and the linear-quadratic model, $Y = a + bD + cD^2$ where a , b and c are constants. The constants α and β in the linear equation were 0.000641 and 0.04123, respectively. The values of c , k and n in the power-law model were 0.0006, 0.0369866 and 0.92519, respectively. The values of a , b , and c in the linear-quadratic model were 0.000376, 0.04561 and -0.0089, respectively.

When the chi-square tests for the suitability of the four equations were calculated, the dicentric yield was better fitted to the linear model ($\chi_b^2 = 5.099$, $\chi_{0.05,6}^2 = 12.592$) than the power law model ($\chi^2 = 5.472$), the linear-quadratic model ($\chi^2 = 8.308$) or the iteratively reweighted least squares model ($\chi^2 = 11.205$).

For the (0.5-2 Gy) range, the equation was calculated according to the quadratic model, namely, $Y = a + bD + cD^2$. The values of a , b and c were -0.05265, 0.1537 and -0.0218 respectively. As the chi-square tests for the suitability of this equation and the iteratively reweighted least squares model described previously were ($\chi_c^2 = 0.994$, $\chi_{0.05,3}^2 = 7.815$) and ($\chi^2 = 9.348$) respectively, it was thought that the dicentric yield was better fitted to the quadratic model. Furthermore, as $\chi_a^2 (= 18.41) > \chi_b^2 (= 5.099) + \chi_c^2 (= 0.994)$, it was thought that the dicentric yield is better fitted to a set of two different models for the two different dose ranges, {the linear model for the (0-0.5 Gy) range and the quadratic model for the (0.5-2 Gy) range}, rather than a single model, (the iteratively reweighted least squares model) for the full (0-2 Gy) range.

3.2. Distribution of dicentrics among cells

As it has been reported¹⁷⁻¹⁹⁾ that dicentrics follows a Poisson distribution, statistical analysis was attempted to determine how well Poisson statistics describe the distribution of dicentrics among the cells. The observed distribution of dicentrics (tri- and tetracentrics being scored as two and three dicentrics respectively) among the cells for each absorbed dose was compared with the Poisson distribution. A Poisson value is the probability (P_n) that a given cell will have n dicentrics, and its value is determined by the mean number of dicentrics per cell (Y) as given in Eq.(1).

$$P_n = Y^n e^{-Y} / n! \quad (1)$$

The u test was used to evaluate the data according to Poisson probabilities. This method makes use of the fact that for a Poisson distribution the variance equals the mean. Thus, when the two are compared significant differences indicate nonconformation to a Poisson

distribution, while nonsignificant differences suggest a Poisson distribution. In this test the quantity u in Eq.(2) approximates a unit normal deviate.

$$u = \frac{d - (N - 1)}{\sqrt{\text{var}(d)}} \quad (2)$$

In the equation N is the total number of cells scored, d is a coefficient of dispersion defined as $(N - 1)(\sigma^2 / Y)$ (in which σ^2 / Y is relative variance where σ^2 is an estimate of population variance), Y the mean number of dicentrics per cell, and $\text{var}(d)$ is the variance of d given by $2(N - 1)(1 - 1/NY)^{17-19}$.

For a Poisson distribution the variance divided by the mean equals 1, so that $u = 0$. If the distribution is over-dispersed, that is, cells with higher and lower aberration frequencies are more numerous than in a Poisson distribution, then the variance will increase and the relative variance will be greater than 1, resulting in a positive value for u . If on the other hand the distribution is under-dispersed, with cells with aberration frequencies close to the mean more numerous, then the variance will be reduced and the relative variance will be less than 1, producing a negative value for u . If the absolute value of u is greater than 1.96 then the over- or under-dispersion is significant since only a 5% probability exists that the magnitude of u will be greater than this value when the distribution is Poisson.

Table 3 shows the result obtained by using the above mentioned test of the present experimental data, the numbers of cells scored, the numbers of dicentrics observed and their expected values, the numbers of cells containing 0, 1 or 2 dicentrics and their expected values, u values, and relative variance σ^2 / Y for each dose level in Gy. It can be seen in table 3 that the relative variance seems to approximate a value of 1, (except at 1 Gy where the value is 2.95), and this is characteristic of a Poisson distribution. The values of u are negative, except for 1.17 at 0.5 Gy, which indicates under-dispersion and, as the absolute values at 1Gy or below do not exceed 1.96, it would seem clear that dicentric data conforms to Poisson statistics in the range 0 to 1 Gy.

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Table 1 Dicentric yields after X-irradiation of rabbit lymphocytes in vitro.

Dose (Gy)	No. of cells scored	No. of dicentrics	Dicentrics per cell \pm SD
0	16000	10	0.0006 \pm 0.0002
0.05	6000	21	0.0035 \pm 0.0008
0.1	6000	24	0.0040 \pm 0.0008
0.2	5000	36	0.0072 \pm 0.0012
0.3	3500	53	0.0151 \pm 0.0021
0.4	3000	53	0.0177 \pm 0.0024
0.5	1674	34	0.0203 \pm 0.0035
1	1179	88	0.0746 \pm 0.0080
1.5	1284	174	0.1355 \pm 0.0103
2	1325	220	0.1660 \pm 0.0112

Table 3 The distributions and expected distributions of dicentrics among the cells at different doses and values of u and relative variance for different doses.

Dose (Gy)	No. of cells scored	Dicentrics and expected values	Distribution and expected values			u values	σ^2/Y
			0	1	2		
0	16000	10	15990	10		-0.05	1
		9.6	15990	9.6			
0.05	6000	21	5979	21		-0.19	1
		20.9	5979	20.9			
0.1	6000	24	5976	24		-0.22	1
		23.9	5976	23.9			
0.2	5000	36	4964	36		-0.36	0.99
		35.7	4964	35.7			
0.3	3500	53	3447	53		-0.51	0.99
		52.1	3447	52.1			
0.4	3000	53	2947	53		-0.75	0.98
		52.2	2947	52.2			
0.5	1674	34	1641	32	1	1.17	1.04
		33.6	1640	33.3	0.3		
1	1179	88	1092	86	1	-1.24	2.95
		84.7	1094	81.6	3.1		
1.5	1284	174	1111	172	1	-3.13	0.88
		162	1121	152	10.3		
2	1325	220	1108	214	3	-3.56	0.86
		201.8	1122	186.3	15.5		

Table 2 Dicentric yields observed in different laboratories in rabbit lymphocytes after exposure to 150-250 kVp X rays in vitro.

Dose (Gy)	Present result	Léonald ¹³⁾ et al.	Faby ¹²⁾ and Léonald	Scott ¹⁴⁾ and Bigger	Bajerska ⁷⁾ and Liniecki
0	0.0006 (10/ 16000)*	0** (0/113)	0 (0/400)	0 (0/200)	
0.5	0.0203 (34/1674)	0.025** (2.8/113)			
0.53					0.0087 (2/230)
1	0.0746 (88/1179)	0.0875** (9.9/113)			
1.06					0.0385 (10/260)
1.5	0.1355 (174/ 1284)				
1.53			0.1333 (16/120)		
1.73					0.1174 (27/230)
1.97					0.1611 (29/180)
2	0.1660 (220/ 1325)	0.2875* (32.5/ 113)	0.275 (110/400)		
2.03				0.2833 (34/120)	

* (10 dicentrics/16000cells)

** The adjusted values^{20,21)} made on the basis of the percentages of cells at first, second and third mitoses being 27, 45 and 28, respectively when the observations were performed at the standardized fixation time of 48 h.

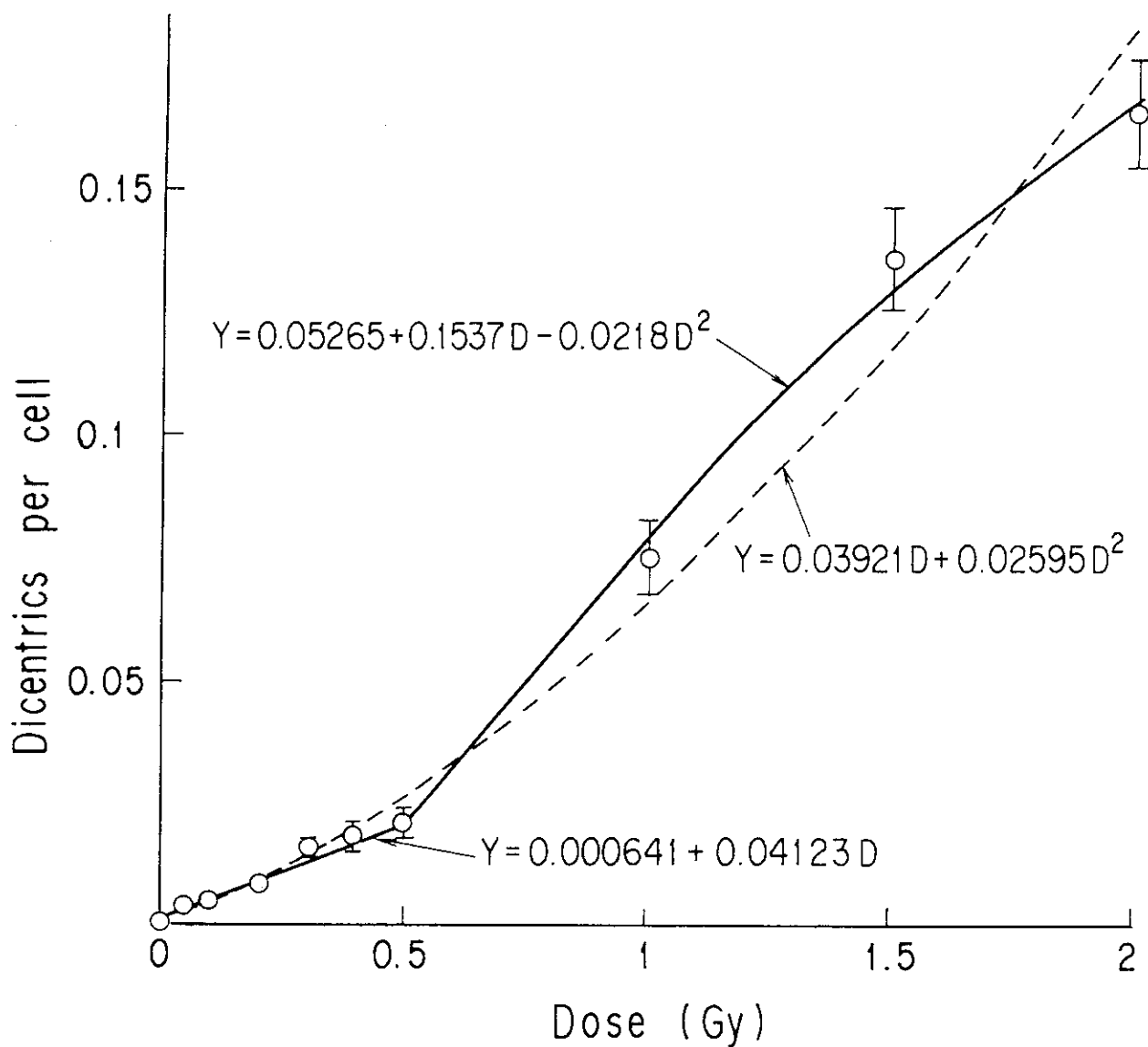


Fig. 1 Dicentric per cell following radiation doses in the ranges of 0.05-2 Gy of 150 kVp X-rays of rabbit lymphocytes in vitro. The solid line represents the linear regression equation (0-0.5 Gy) fitted to data of the experiment and the solid and dashed curves represent the linear-quadratic equation (0.5-2 Gy) and the iteratively reweighted least squares equation (0.05-2 Gy), respectively.

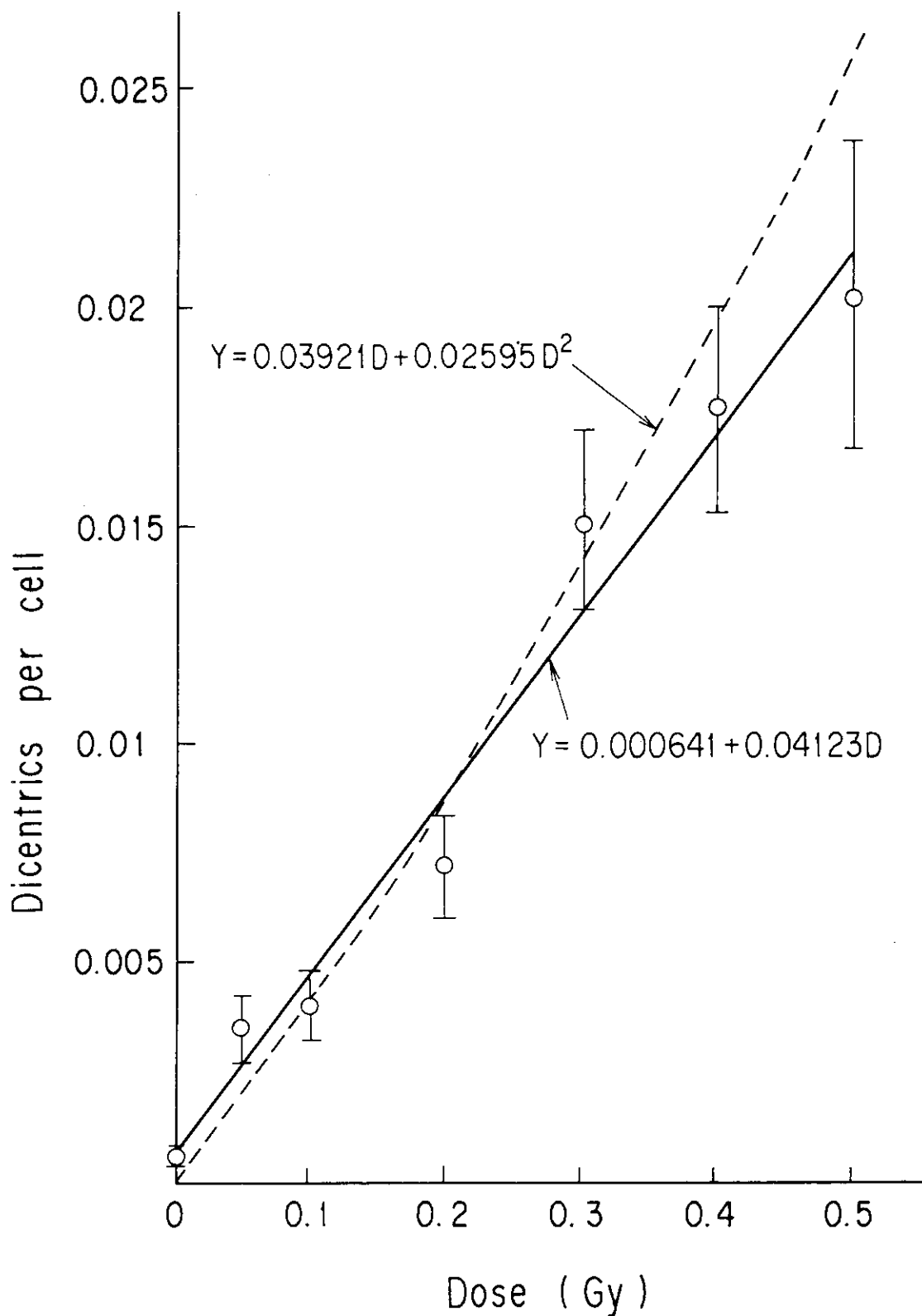


Fig. 2 Dicentric per cell following radiation doses in the ranges of 0.05-0.5 Gy of 150 kVp x-rays of rabbit lymphocytes in vitro. The solid line represents the linear regression equation fitted to data of the experiment and the dashed curve represents the iteratively reweighted least squares equation.