JAERI-Research
95-073

DICENTRIC YIELDS INDUCED IN RABBIT BLOOD LYMPHOCYTES BY LOW DOSES OF ^{252}Cf NEUTRONS

November 1995

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編集兼発行　日本原子力研究所
印　　刷　いばらき印刷㈱

JAERI-Research 95-073

Dicentric Yields induced in Rabbit Blood Lymphocytes by Low Doses of ^{252}Cf Neutrons

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(Received October 9, 1995)

Little dose-response data for dicentrics induced in blood lymphocytes exposed in the low dose-range to fast neutrons *in vitro* has been reported. This experiment was carried out to obtain the dose-response equation for dicentrics, in the dose range below 20 mGy, (needed in the event of an accidental exposure to neutrons). Rabbit blood lymphocytes were exposed *in vitro* to ^{252}Cf neutron of mean energy 2.35 MeV. The dose range was 2-20 mGy delivered at dose-rate of 0.145 - 0.31 mGy/min.

The dose-response equation for dicentrics was calculated by the linear regression analysis. The present experimental data was compared with the data, in the low dose range below 50 mGy, reported by other authors. Maximum value of RBE with respect to 150 kV_p X-rays was 40, and it was founded in this experiment that the RBE values were dose dependent, the lower or the higher than about 13 mGy the doses, the smaller RBE values, with the maximum value occurring at about 13 mGy.

Keywords: Dicentric, Neutron, ^{252}Cf, Rabbit, Lymphocyte, Dosimetry, RBE

JAERI-Research 95-073

低線量の^{252}Cf中性子によって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10月9日受理)

速中性子線で*in vitro*照射された末梢血リンパ球中に誘発された二動原体染色体に関する低線量域に於ける線量－反応関係のデータはほとんど発表されていない。そこで、中性子線被爆事故時に必要な20 mGy以下の低線量域における二動原体染色体に関する線量－反応式を求める実験を行った。ウサギの末梢血リンパ球を平均エネルギー2.35 MeVの^{252}Cf中性子線で*in vitro*照射した。照射は2-20 mGyの線量範囲で行い、線量率は0.145 － 0.31 mGy／分であった。二動原体染色体に関する線量－反応式を線型回帰分析により求めた。本実験データと他の研究者が報告している50 mGy以下の低線量域でのデータと比較、検討を行った。

本実験では、150 kV$_p$X線を標準放射線とした場合、ＲＢＥは約13 mGyで最大値40になり、これよりも線量が増大しても、あるいは減少してもＲＢＥ値は減少する傾向を示した。

東海研究所：〒319－11　茨城県那珂郡東海村白方白根２－４

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1. INTRODUCTION

As little dose-response data for dicentrics induced in lymphocytes exposed to fast neutrons *in vitro* has been reported, rabbit blood samples were irradiated with ^{252}Cf to obtain dose-response relationship between the absorbed dose (at lower doses) and the yields of chromosome abberations (dicentrics) induced in peripheral blood lymphocytes. The resulting reference data can be used in the estimation of doses in the event of a radiation accident.

^{252}Cf decays with a half-life of 2.65 years. As a result of its spontaneous fission radioactivity, it emits neutrons at a rate of $2.34\times10^{9}s^{-1}mg^{-1}$ and γ-rays (from both fission events and an equilibrium mixture of fission products) at a rate of about $1.3\times10^{10}s^{-1}mg^{-1}$ [1]. The fission spectrum neutrons have a mean energy of 2.35 MeV, and a modal energy of about 1 MeV[2] as shown in Fig. 1[3]; the associated γ-rays have a mean energy of 0.8 MeV[4] or 1.1 MeV(averaged over the dose deposited)[5]. As the neutron dose rate is about 2.3mGy/h at 1 m per mg and the equilibrium γ-rays dose rate (from an unshielded source) is about 1.39 mGy /h at 1 m per mg (from the test report of Amarsham Company), approximately 62% of the absorbed dose is due to neutrons and 38% to γ-rays. Most of the neutron dose is due to elastic scattering of fast neutrons by hydrogen, with a small contribution from the recoil of carbon, nitrogen, and oxygen nuclei and from the proton released when a thermal neutron is captured by a nitrogen nucleus. In addition, there is a gamma dose due to thermal neutron capture by hydrogen[6].

In JAERI, a ^{252}Cf source has been used for the calibration of neutron dosemeters for radiation protection. In the present experiment, this source was used for the irradiation of blood samples.

2. MATERIALS AND METHODS

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

Blood samples in polyethylene cylindrical containers (14 mm in diameter × 75.2 mm in height with 1 mm thick walls) were placed in a water phantom (30cm in width × 30cm in length and 25cm in height) maintained at 38℃ at a1.5 cm distances from its wall and the samples were irradiated with 2, 5, 7.5, 10 and 20mGy using a ^{252}Cf source (2.49×10^{8} - 1.14×10^{8} Bq). The distance from the source to the sample was 10cm. The dose rates ranged from 0.145mGy/min to 0.31mGy/min. The dose estimates were obtained from the results of dose assessment with a Neupit badge EF Type-Solid State Track Detector (Chiyoda Safety Appliance Corporation Ltd) and from calculations. Fall-off of neutron dose over a distance of 1.5cm in the phantom was 0.686% [7,8], and that over 8.5cm of air and 1.5cm in the phantom was 0.95%.

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For the rabbit blood culture[9-12], the supernatant fluid after centrifugal isolation of the irradiated blood was added to culture medium containing 5ml NCTC 135 (GIBCO), 0.1 ml phytohaemagglutinin M (DIFCO) and antibiotics. Bromodeoxyuridine at a final concentration of 6μg/ml was added. The cultures were incubated for 45h at 38℃ in a 95% air/5% CO_2 atmosphere, with colcemid present for the final 3 h at a final concentration of 0.5 μg/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 Perry[13-15] was used to distinguish the first from the later mitotic division. Slides were stained for 15 minutes with 6 μg/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 and Fig. 2 show dicentric yields after ^{252}Cf neutrons-irradiation of rabbit lymphocytes. The following equation of the dose-response relation for the present dicentrics data were obtained by the least squares method.

$$Y = (-5.00 \pm 7.96) \mathrm{X} 10^{-4} + (1.672 \pm 0.081) \mathrm{X} 10^{-3} D$$

$$(\chi^2 = 3.18,\ \chi^2_{0.05,5} = 11.07) \qquad (1)$$

where Y is the yield of dicentrics (the number of dicentric chromosomes per cell) and D the absorbed dose in the range of 2-20mGy. When the chi-square tests for the suitability of the equation was calculated, the dicentric yield was fitted to it. The critical χ^2-value for the particular degree of freedom ν at 95%-confidence is given by $\chi^2_{0.05,5}$. In comparison with dicentric yields*1 induced in human peripheral blood lymphocytes exposed *in vitro* to dose in the range of 2-20mGy by a fission neutron spectrum of mean energy 0.4 MeV , reported by Vulpis et al.[16] , dicentric yields observed in the present experiment were higher, (especially at higher doses)

*1 The dose-response equation for the dicentrics was calculated by the author.

$$Y = (2.00 \pm 1.00) \times 10^{-3} + (6.00 \pm 0.43) \times 10^{-4} D \ (\chi^2 = 3.19,\ \chi^2_{0.05,5} = 11.07)$$

where Y is the yield of dicentrics (the number of dicentric chromosomes per cell) and D the absorbed dose in the range of 2-50mGy.

For the rabbit blood culture[9-12], the supernatant fluid after centrifugal isolation of the irradiated blood was added to culture medium containing 5ml NCTC 135 (GIBCO), 0.1 ml phytohaemagglutinin M (DIFCO) and antibiotics. Bromodeoxyuridine at a final concentration of 6μ g/ml was added. The cultures were incubated for 45h at 38℃ in a 95% air/5% CO_2 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 Perry[13-15] was used to distinguish the first from the later mitotic division. Slides were stained for 15 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.

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where Y is the yield of dicentrics (the number of dicentric chromosomes per cell) and D the absorbed dose in the range of 2-50mGy.

as shown in Fig.2. However, in general, the increase in mean energy of irradiating neutron results in an increase of dicentric yields[17]. The differences of dicentric yields might presumably be explained by differences in irradiation conditions: for example the irradiation *in vitro* of blood samples at 38℃ rather than room temperature[18,19] or the keeping of blood samples at 37℃ at the time of irradiation. In the literature, the temperature at the time of irradiation was not referred to, but the dose-response equation of x-rays used as the reference radiation, had been calculated from the data of the experiment[20], in which human lymphocytes had been irradiated at room temperature, so it is thought that human blood samples in the 0.4MeV neutron irradiation experiment were also irradiated at room temperature. As the effect of temperature at the time of neutron irradiation *in vitro* on the dicentric yield in peripheral blood lymphocytes has not been reported yet, further study of the temperature effect is expected. Another experiment covering the similar low dose range of 3.55-40.8mGy of 14.8 MeV T-D neutrons was reported by Pohl-Rüling et al.[21,22]. In this experiment, the human blood samples were kept at 37℃ by a heated water jacket. The dose-response equation for the dicentric yield in the dose range of 2-20mGy

$$Y = (2.00 \pm 0.11) \times 10^{-3} + (1.982 \pm 0.052) \times 10^{-4} D \quad (\chi^2 = 3.18,\ \chi^2_{0.05,5} = 11.07) \qquad (2)$$

was calculated by the author. As might be expected from the energy of the irradiating neutrons, the dicentric yield at the same given dose was the lowest of the three experiments, as shown in Fig.2.

As a ratio[5,6] of neutron to γ-ray dose rates at the sample position was1.249, the absorbed doses of γ-rays were in the range of 1.6 -16mGy. The mean energy 1.1 MeV of ^{252}Cf γ-rays averaged over the dose deposited is similar to the corresponding value 1.3 MeV for ^{60}Co γ-rays , so that approximate estimates for the dicentric yields due to the ^{252}Cf γ-rays can be calculated from the dose -response equation ($Y = 1.8 \times 10^{-5} D + 2.9 \times 10^{-8} D^2$) for ^{60}Co γ-rays[5]. The dicentric yields attributable to the ^{252}Cf γ-rays in the dose range of 1.6-16mGy were less than the background value as shown in Table 1.

For the X-rays the existence of a threshold resulting from mechanisms of cellular repair was suggested by Pohl-Rüling et al.[21,22] and for 14.8 MeV neutrons it was also suggested that one could not formally reject the possibility of a threshold. In this context the equation calculated according to the linear-quadratic model was

$$Y = (7.03 \pm 5.91) \times 10^{-4} + (1.20 \pm 1.50) \times 10^{-3} D + (2.2 \pm 0.7) \times 10^{-5} D^2 \quad (\chi^2 = 0.9) \qquad (3)$$

and from the results of chi-square tests, it was found that the dicentric yield was better fitted to the linear-quadratic model (χ^2 = 0.90) than the linear model (χ^2 = 3.18), though it had been assumed that the dose-response curve for dicentrics induced in lymphocytes by neutrons is linear. If this is not attributable to experimental errors, one might be thought to exist of a threshold for neutrons.

3.2 RBE of ^{252}Cf neutron

The relative biological effectiveness (RBE) of a radiation A is defined as the ratio of a dose of standard radiation required for a specific biological effect and the dose of radiation A causing the same effect. A limiting RBE is defined as the ratio of the slopes of the initial portion of both response curves, known or assumed to be linear (i.e., the ratio of the "α terms" of the two dose response curves), which would then reflect, presumably, the maximum RBE value (RBE_M)[23]. Using the dose-response equations[24] for dicentric yields in rabbit lymphocytes following radiation doses in the ranges of 50-2000 mGy by 150kV_p x-rays *in vitro*, and Eq. (1), RBE values were calculated.

$$Y = (6.41 \pm 8.54)\times10^{-4} + (4.123 \pm 0.304)\times10^{-5} D_x \quad (50\text{-}500\text{mGy}) \qquad (4)$$

$$Y = (-5265000 + 15370 D_x - 2.18 D_x^2) \times 10^{-8} \quad (500\text{-}2000\text{mGy}) \qquad (5)$$

RBE values in the range of 2-13.7*2 mGy of ^{252}Cf neutrons were calculated from Eq. (1) and Eq.(4).

$$4123 D_x + 64100 = 167200 D_n - 50000$$

$$D_x/D_n = 40.55 - 27.67/D_n \qquad (6)$$

and RBE values in the range of 13.7-20mGy were calculated from Eq. (1) and Eq. (5).

$$-2.18 D_x^2 + 15370 D_x - 5265000 = 167200 D_n - 50000$$

$$D_x/D_n = (3525.23 - (10035040.19 - 76697.25 D_n)^{1/2})/D_n \qquad (7)$$

The results of calculations are shown in Table 2 and Fig. 3. The maximum RBE value (RBEM)[20] calculated from (α_n / α_x) is $1.672 \times 10^{-3} / 4.123 \times 10^{-5} = 40.6$, with a standard error of ± 5.16. From these results it was found that at least in this experiment the RBE values were

*2 At 13.7mGy of neutron, Eq.7 and Eq.8 give the same value, 38.5.

dose dependent; the lower or the higher than13.7 mGy the doses, the lower RBE values, with the maximum value occurring at13.7 mGy*3 .

ACKNOWLEDGEMENT: The author would like to thank the members of the Radiation Dosimetry Division for dose measurements and irradiations.

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Table 1 Dicentric yields from the neutron dose to blood samples at 10 cm from the californium source. The dose rates were approximately 0.145 mGy min^{-1} - 0.31 mGy min^{-1}.

Neutron dose (mGy)	No. of cells observed	No. of dicentrics	No. of dicentrics per cell ±SD
0	16000	10	0.0006±0.0002
2	10000	35	0.0035±0.0006
5	9300	70	0.0075±0.0009
7.5	4660	47	0.0101±0.0015
10	2477	39	0.0158±0.0025
20	708	24	0.0339±0.0069

Table 2 The RBE for ^{252}Cf neutrons with respect to 150 kV_p X-ray for the dicentric yield.

Neutron dose (mGy)	RBE†	Neutron dose (mGy)	RBE††
2	26.71	13	39.92
3	31.33	14	37.98
4	33.63	15	36.30
5	35,02	16	34.84
6	35.94	17	33.55
7	36.60	18	32.41
8	37.10	19	31.39
9	37.48	20	30.48
10	37.78		
11	38.03		
12	38.24		
13	38.42		

† RBE calculated from equations(1) and (2) in the text.
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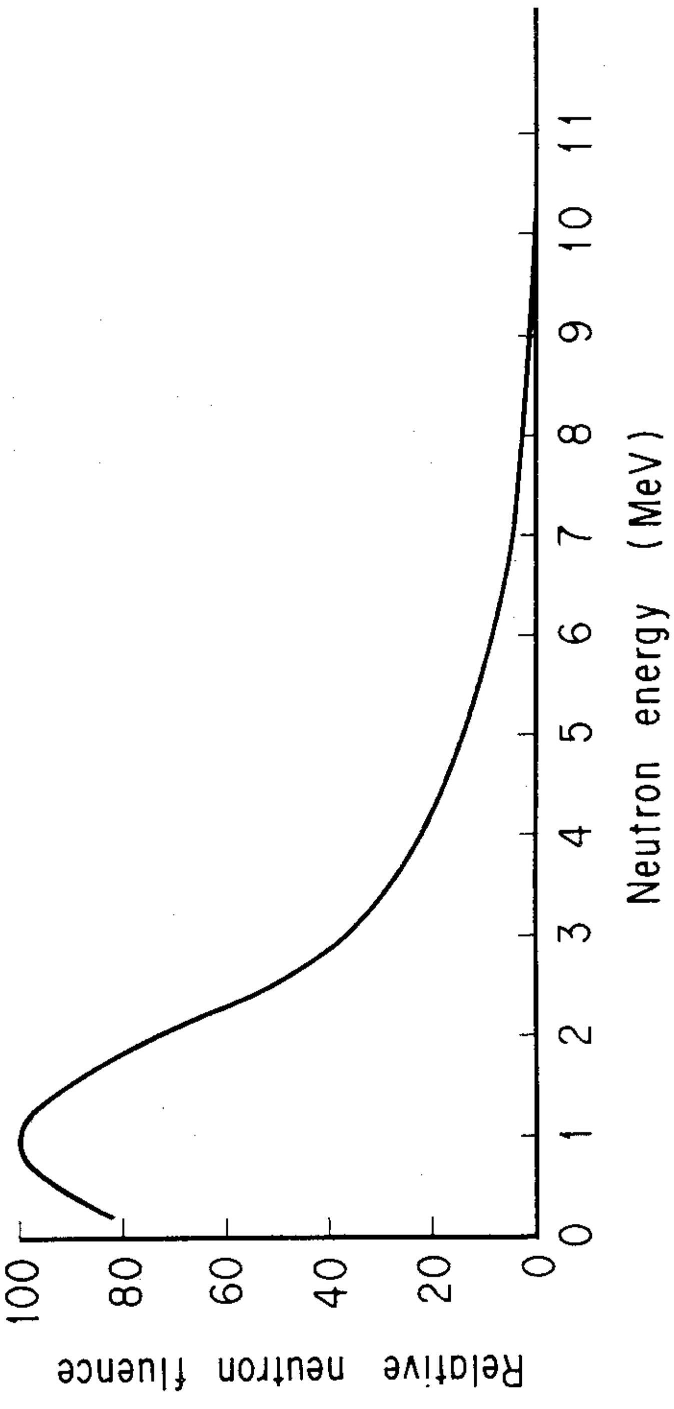

Fig. 1 Neutron spectra from spontaneous fission of ^{252}Cf.

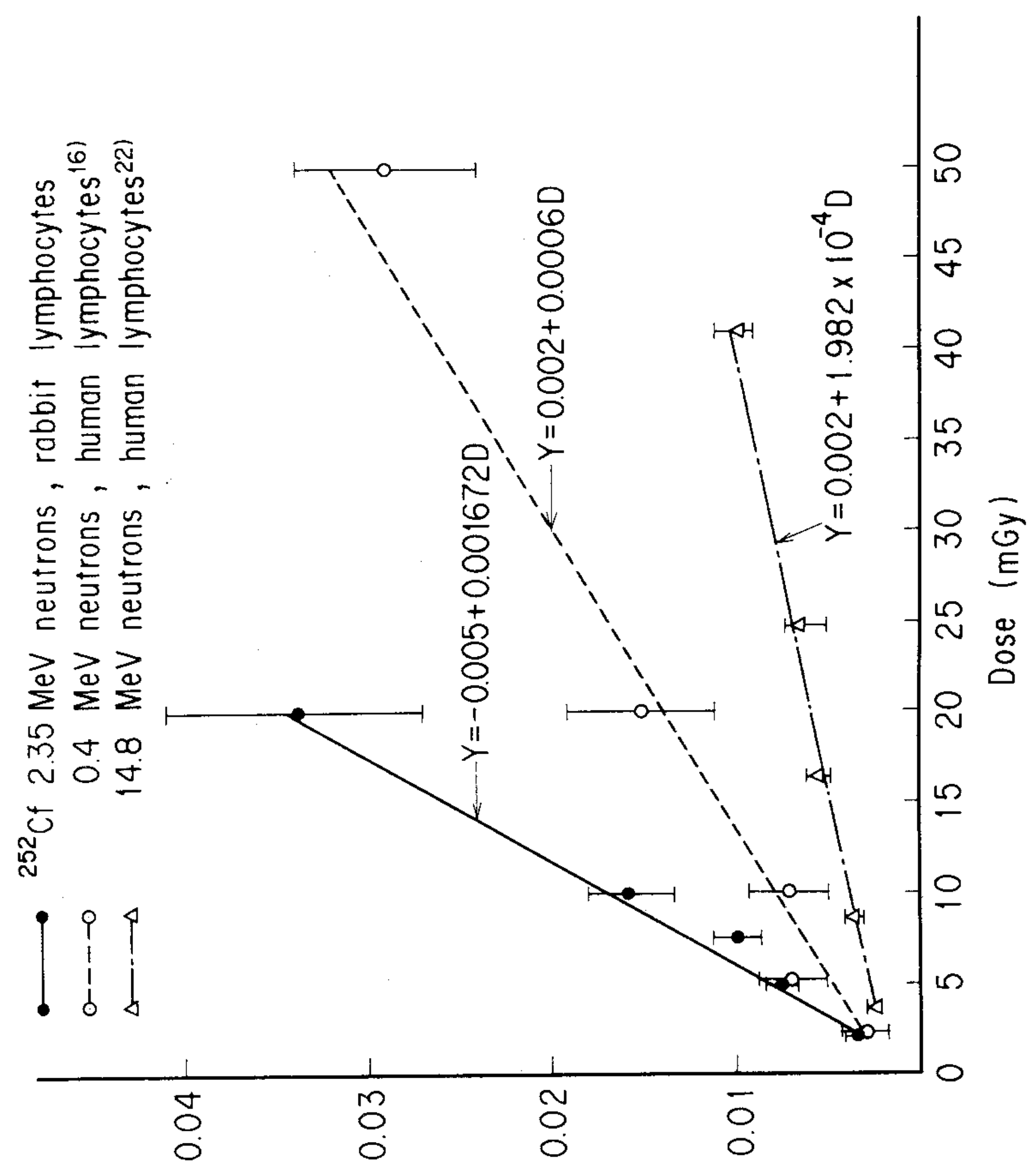

Fig. 2 Dose-response curves for dicentric yield induced by three different energies of neutron radiation

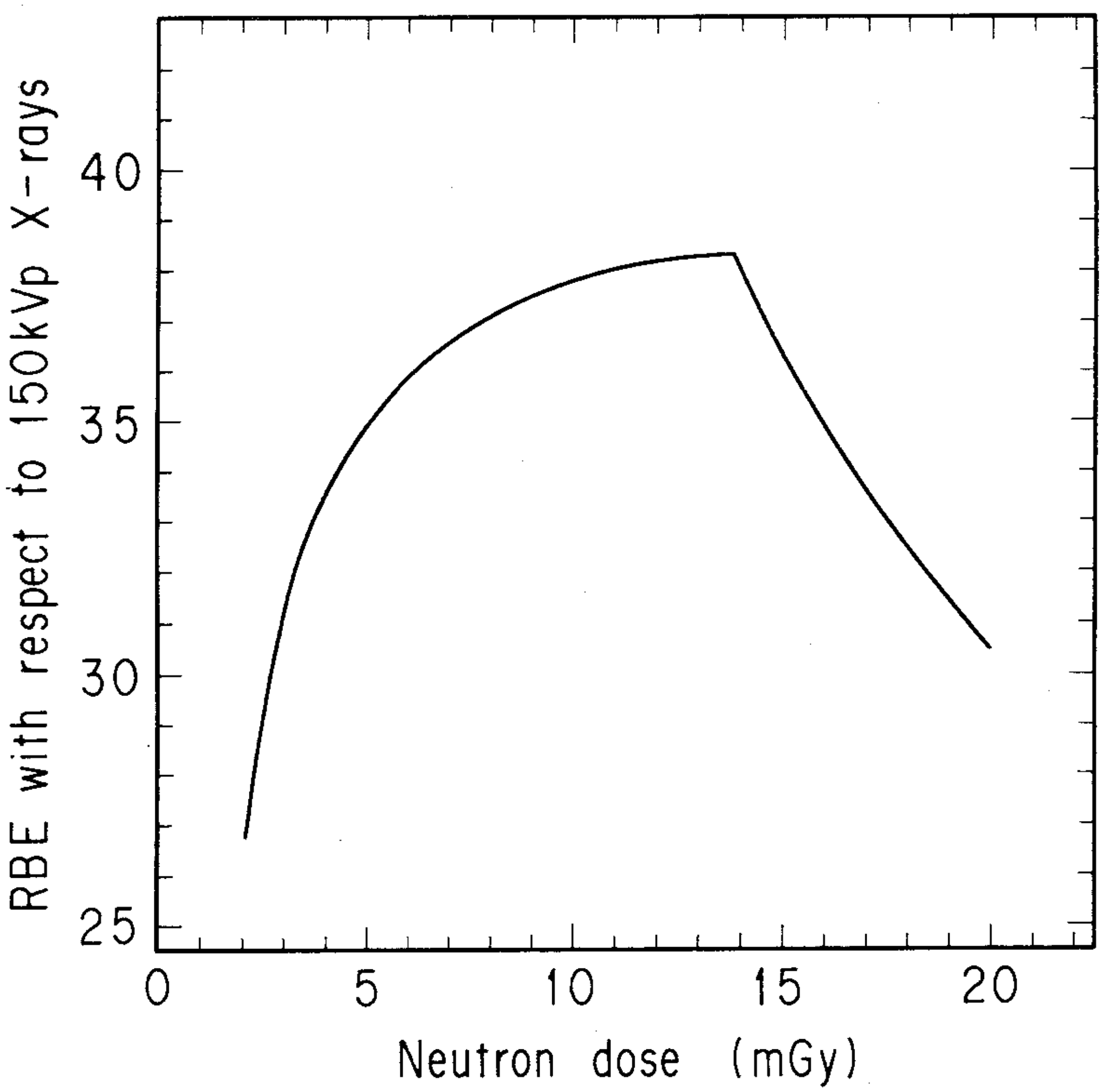

Fig. 3 The variation of RBE for ^{252}Cf neutrons with respect to 150 kV$_p$ X-rays for the dicentric yield.