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THE EFFECT OF BLOOD SAMPLE POSITIONS IN A WATER PHANTOM AT THE TIME OF IRRADIATION ON THE DICENTRIC YIELD

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The Effect of Blood Sample Positions in a Water Phantom at the Time of Irradiation on the Dicentric Yield

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Blood samples of man and rabbit, placed at various distances from the surface of a water phantom with a dosimeter were exposed to 250mGy of ^{60}Co γ-rays.

Increases in the dicentric yields in the lymphocytes were observed with increased distances from the surface of the water phantom.

As a variation of the dicentric yield with increasing distance in water was found, in the experiment to obtain calibration curves for biological dosimetry, it is recommended that blood samples should be positioned at a constant distance from the surface of a water phantom at the time of irradiation. ICRU REPORT 23 recommends that the calibration measurement be carried out with an ionization chamber positioned at 5cm depth below the surface of a water phantom for 150 kV-10 MV X rays, and ^{137}Cs and ^{60}Co γ-rays. As the same reasons which determine a 5cm depth in the recommendation, should be applied to this case, it is desirable that the experiment be carried out with blood samples positioned at 5cm distance from the surface of a water phantom.

Keywords : Co-60, Dicentrics, Human, Rabbit, Lymphocyte, Phantom

二動原体染色体発生率に及ぼす照射時の血液標本の水ファントム内の位置の影響

日本原子力研究所東海研究所保健物理部
井上　義教

（1995年1月5日受理）

ヒトおよびウサギの血液試料を測定器と共に水ファントムの前面から種々の距離に置き，^{60}Ｃｏγ線で250mGy照射した。

水深の増加と共にリンパ球中の二動原体染色体の発生率の増加が観察された。

水深に伴う二動原体染色体発生率の変動が見られたので，生物学的線量計測を行う際に必要な校正曲線を求める実験においては，照射時に血液試料を水ファントム内の一定の位置に置くことが推奨される。ＩＣＲＵ報告量23は標準線源として150kV-10Mv Ｘ線，^{137}Ｃｓγ線，あるいは^{60}Ｃｏγ線を用いて校正を行う際，水ファントムの表面下５cmの深さの位置に電離箱を置いて行うように勧告している。勧告において５cmの水深が決められた理由と同じ理由がこの場合にも当てはまるので，実験の際血液試料を水ファントムの水深５cmの位置に置いて照射が行われることが望まれる。

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1. INTRODUCTION

Various dosimeters ,such as the film badge and the thermoluminescence dosimeter, have been used for personnel dosimetry. These dosimeters are generally worn on the chest and the whole body dose is estimated from their readout. But in the case of suspected exposure where no dosimeters are worn and in the case of partial body exposure outside the chest where a dosimeter is worn, it is difficult to estimate the exposure dose by the physical dosimetry. In such case, biological dosimetry can be used to estimate the absorbed dose of the over exposed persons, which is based on the relationship between the absorbed dose and chromosome aberrations(dicentrics) in blood lymphocytes.

It is reported [1,2,3]that the chromosome aberration yield in lymphocytes following a uniform whole body irradiation is similar to that obtained from blood samples irradiated to the same dose level *in vitro*. Therefore it is possible to construct *in vitro* curves relating radiation dose to chromosome aberration yield and to use these curves to estimate dose by analysing blood samples from people accidentally overexposed [2,4]. As the effects of doses of around 50 mGy can be detected by this method[5], it is thought that this method is suitable for dosimetry in the case of accidental exposure.

In oder to obtain the relationships between absorbed dose and dicentric yield, it was planned to irradiate blood samples in a water phantom with ^{60}Co γ-rays. In this case, as LET(linear energy transfer) values[6] of incident radiation varies with distances in water to blood samples, it may be expected that the yield of dicentrics varies with distances from the surface of the phantom to samples, even if blood samples are exposed to the same dose. However, the studies of the variation of dicentric yield with the sample positions in a water have not been yet reported. Therefore, the relationships between dicentric yields and the sample positions were investigated.

2. MATERIALS AND METHODS

Human blood samples(male,20-35 years old) and 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 × 75.2 mm in height × 1 mm thick wall) were placed in a water phantom(30cm in width × 30cm in length and 25cm in height) at 1.5, 5, 10, 15, and 20 cm distances from its wall , respectively (Fig. 1) (Photo.1) and the samples were irradiated 250mGy at each sample position with ^{60}Co source(3.7×10^{12}Bq). The distance from the source to the sample was 1.4 m. The dose rates

1. INTRODUCTION

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ranged from 7.7mGy/min(at 1.5 cm distance) to 3.3mGy/min(at 20 cm distance). As it is well known that dicentric yield depends on the temperature of blood sample at the time of irradiation[7,8], the water temperature of the phantom was kept at 37 ℃ for human blood samples and 38 ℃ for rabbit blood samples. The dose at each sample position was measured using IONEX DOSE/DOSE RATE METER 2400/3 with a 0.6 cm^3 chamber inside a latex tube. The absorbed dose in Gy was obtained by multiplying the exposure in roentgens by 0.00974 (Gy/roentgen conversion factor).

The pulse height distributions for ^{60}Co(3.7×10^7Bq) γ-rays were measured with a 1″ ϕ × 1.5″ NaI(Tl) scintilation detector at 5, 10, 15, 20 cm depth in a water phantom and in air to check the change of the energy spectra of ^{60}Co γ-rays in water. The source to detector distance was 3.2m.

The following procedures were performed for analysing chromosome aberrations(dicentrics) of blood lymphocytes after irradiation.

For the human blood cultures and their slides preparation, a slight modification[9] of the method of Moorhead et al.[10]was used. The cultures were incubated for 50 h at 37 ℃ in a 95% air/5% CO_2 atmosphere, with colcemid present for the final 20 h at a final concentration of 0.03 μg/ml.

For the rabbit blood culture[11-14], the supernatant fluid after centrifugal isolation of irradiated blood was added to culture medium containing 5ml NCTC 135(GIBCO) and 0.1 ml phytohaemaggulutinin M(DIFCO) and antibiotics. Bromodeoxyuridine at a final concentration of 6μg/ml was added. The cultures were incubated for 45 h 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[15-17] was used to distinguish the first from the later mitotic division. Slides were stained for 15 minutes with 6 μg/ml Hoeckst 33258 in Soerensen buffer solution, rinsed, mounted in the same buffer, and exposed to light from 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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*Flood light for high intensity discharge lamps(H362S) with mercury lamp(HF-400X) (Iwasaki Electric Company,Ltd.).

3. RESULTS AND DISCUSSION

Tables 1 and 2 show the data on dicentric yields in human and rabbit lymphocytes at various distances of blood sample positioned in a water phantom after irradiation of 250mGy. The data obtained for rabbit and man are presented in Fig.2. The results show that dicentric yields in the two species increased with distances in water. It is reported[18,19] that ,in terms of the radiosensitivity of chromosomes, rabbit lymphocytes is a little lower than that of human lymphocytes. In this experiment too, the radiosensitivity of rabbit is 0.79 times that of man at the 5 cm distances in water and is 0.89 times at 10 cm distance in water. These values are similar to 0.86[18] observed by L. Fabry and A. Léonard and 0.7[19] observed by M. Rosenthal after 2 Gy irradiation of X-ray . Then the regression lines were calculated from the data on rabbit according to three models, namely, the linear model, $Y = \alpha + \beta D$ where α and β are constants, the power law model, $Y = kD^n$ where k and n are constants, and the exponential law model, $Y = a e^{bx}$ where a and b are constants. The constants α and β in the linear equation were 0.002601 and 0.0007688, respectively. The values of k and n in the power-law model were estimated to be 0.00279 and 0.58705, respectively. The values of a and b in the exponential law model were estimated to be 0.003854 and 0.08293, respectively.

When the chi-square tests for suitability of the three equations were calculated, the dicentric yield was better fitted to the linear model($\chi^2 = 0.4317, \chi^2_{0.05}=9.488$) than the power law model($\chi^2=1.12385, \chi^2_{0.05}=9.488$) or the exponential law model($\chi^2=2.2903$, $\chi^2_{0.05}=9.488$). The least squares linear regression equations relating dicentric yields induced in rabbit and human lymphocytes after 250mGy irradiation to distances in water, $Y = 0.002601 + 0.0007688X$ and $Y = 0.002503 + 0.001048X$ are presented in Fig.2. In these calculations, as the dicentric yield, 0.0101 at 15cm in human data was in the rejection region, it was cut off.

It was examined whether such variation of dicentric yield with distances in water can be explained by the increase of the ratio of the scattered radiation to the γ-rays with the distances in water that incident γ-rays travel, and by the increase of LET values with the increase of the ratio of the scattered radiation to the incident γ-rays.

The pulse height distributions in air and various depths in water are presented in Fig. 3. Fig. 3 shows that the counting rates in the two photopeak regions decreased with depths in water. In Table 3, the counting rates(n_a) in the Compton scatter and backscatter regions, those(n_b) in the photopeak regions, n_a/n_b, and $n_a + n_b$ are presented. Table 3 shows that n_a/n_b increased

with depth, that is, the ratio of the scattered radiation to the primary radiation increased with depth in water.

Using the tissue-air ratio** and the backscatter factor***, the calculated results of the percentage depth doses**** at 1.5, 5, 10, 15 and 20 cm depth were 97.6, 85, 66.1, 51.5 and 38.7, respectively, and are presented in Fig. 4.

The depth dose(the absorbed dose) at each depth point were 104.7, 91.2, 70.9, 55.3, 41.5, respectively. By using these values, the depth doses were separated into the primary doses and the scattered doses[21]. The approximate values of the primary doses were calculated from the equation

$$Y = 100 \times \{(140 - X + 0.5)^2/140^2\}\ e^{-0.0632(X - 0.5)},$$

where Y is the primary dose, X :the depth in water(cm), 0.0632 :the linear attenuation coefficint for water(cm^{-1}), 140: the source to sample distance(cm) and 0.5 :the reference point(cm) for ^{60}Co γ-rays. The scattered doses were calculated by {the depth doses(= the percentage depths X the backscatter factors: 1.073 for 25cm X 25cm field) - the primary doses}. Scatter and primary contributions are plotted against depth as shown in Fig. 5. These curves show that as the depth in water increases the amount of scattered radiation increases initially and reaches a constant value, but that the general shape of the scattered γ-rays spectrum does not change much with depth in water. In contrast, the primary dose decreases exponentially.

**The ratio of the absorbed dose at a given point in a phantom to the absorbed dose which would be measured at the same point in free air within a volume of the phantom material just large enough to provide the maximum electronic build-up at the point of reference.

*** The ratio of the absorbed dose at the reference point in a phantom to the absorbed dose which is due to primary photons.

****For gamma rays, the ratio(expressed as a percentage) of the absorbed dose, D_d, at any depth d in a phantom to the absorbed dose, D_m, at a fixed reference point on the beam axis. The reference point is usually at the position of the peak absorbed dose.

Percentage depth dose = $100\ D_d/D_m$

However, in this experiment the source to sample distances, not the source to surface(of a phantom) distance, were kept constant(140cm), so percentage depth dose became slightly smaller(below 4%).

Fig. 4, 5 and Table 3 show that the scattered radiation becomes softer with depth in water and the contribution of the scattered radiation increases with depth in water. That is, the energy of γ-rays becomes lower due to the scatter photon with depth, while the LET value becomes higher.

As it has been reported that the dicentric yield becomes higher with the increase of LET values[22)*****], the author reexamined the relationship between LET values and dicentric yields with the present data and the other authors' data. Table 4 shows absorbed dose-average values of LET for ^{60}Co γ-rays, ^{137}Cs γ-rays and 200kV$_p$ X-rays (since the one-track yield is considerably greater than the two-track yield at low LET in the range of low doses, the appropriate mean is the dose-average LET)[23)]and dicentric yields per 1000 cell per 250mGy. From the LET values(7.9[6)], 8.4[6)] and 9.4[24)]) of ^{60}Co γ-rays, ^{137}Cs γ-rays and 200kV$_p$ X-rays and the corresponding dicentric yields(4.1, 13.5[25)] and 23.22[26)]), assuming that dicentric yields increase approximately proportionally with log LET, the equation Y = -216.102 + 250logX is obtained, where Y is dicentric yield and X is LET value.. When X = 8.3[6)](LET value at 10 cm depth for ^{60}Co γ-rays) is substituted for this equation, Y = 13.67(dicentric yield at 10cm depth) is obtained. This value is in fair agreement with the experimental value(13.67).

LET distributions at 0 and 10cm depths in water phantoms irradiated with ^{60}Co have been calculated by Bruce et al.[6)] with cut-off energies of both 100 eV and 500 eV, and as the variation of the LET spectrum with depth due to increasing scatter is very small, it was concluded that the change is unlikely to be biologically significant[24)]. However, in the present experiment the dicentric yield(0.013) in human lymphocytes at 10cm distance in water was about three times that(0.0041) at 1.5cm distance in water. This fact is in conflict with the above-mentioned conclusion.

While this three times difference could be explained by the difference of LET values on calculation, it might not be explained by the difference of LET values alone. In order to explain this difference, another mechanism should be sought . For example, as it is thought

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*****It is known that the dose-response relation equation between the absorbed dose and dicentric yield is given in the linear-quadratic form,

$$Y = \alpha D + \beta D^2$$

where Y is dicentric yield, D absorbed dose, and α and β are constants. It is also reported[3)] that above 5 kev/μm, α increases approximately proportionally with LET reaching a maximum at about 70 kev/μm and then falling very sharply with increasing LET. The value of β is approximately constant for low LET(less than 10 kev/μm) radiation, falling to zero at high LET(greater than 40 keV/μm).

that the change of the ratio of indirect action to direct action with depth in water might be involved , it seems to be one of the problems that should be investigated hereafter in this regard. This is especially so as at least about 44% of dicentric yield after 1 Gy irradiation of rabbit lymphocytes at a distance of 5 cm in water with X-rays found to be induced correlated with indirect action[27,28].

4. CONCLUSION

As the dicentric yields vary greatly(3 ～ 4 times) with depth in water, where the samples are positined (Table 1 and 2), in experiments for establishing dose-response curves for biological dosimetry, the calibration depth in water(the depth from the surface or the side wall of a water phantom), should be determined.

ICRU REPORT 23[28] recommends that the physical calibration measurement be carried out with an ionization chamber positioned on the central axis of the beam, at a depth, 5cm, below the surface of a water phantom for 150keV -10MV X rays, ^{137}Cs and ^{60}Co gamma rays. The first reason[29] this depth point is decided, is based on the fact that the region of chief clinical interest lies several centimeters below the skin when these radiations are used and the other reason[30] is that it has long been recognized that measurements at an interface between two media are very difficult to make, and that surface or "in-air" measurements may be critically influenced by soft radiations from collimating systems or other material in the radiation beam. As most of these reasons should be considered even in the case of the experiments for biological dosimetry, the blood samples should be positioned at 5cm distance from the surface of a water phantom in experiments for establishing the dose-response curves with these radiations.

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Table 1 Dicentric yields after 250mGy irradiation of human lymphocytes at various distances in water with ^{60}Co γ-rays

Distance in water(cm)	No. of cells observed	No. of dicentrics	Dicentrics per cell±SD
1.5	244	1	0.0041±0.0041
5	1830	14	0.0077±0.0021
10	616	8	0.0130±0.0046
15	989	10	0.0101±0.0032
5*	1519	51	0.0336±0.0047

*500mGy exposure (for reference)

Table 2 Dicentric yields after 250mGy irradiation of rabbit lymphocytes at various distances in water with ^{60}Co γ-rays

Distance in water(cm)	No. of cells observed	No. of dicentrics	Dicentrics per cell ± SD
1.5	1927	8	0.0042 ± 0.0015
5	3432	21	0.0061 ± 0.0013
10	1727	20	0.0116 ± 0.0026
15	1756	25	0.0142 ± 0.0029
20	1928	34	0.0176 ± 0.0030

Table 3. The total counting rates in the Compton scatter and backscatter regions(n_a), those in the photopeak regions(n_b), the ratios of n_a to n_b, and $n_a + n_b$

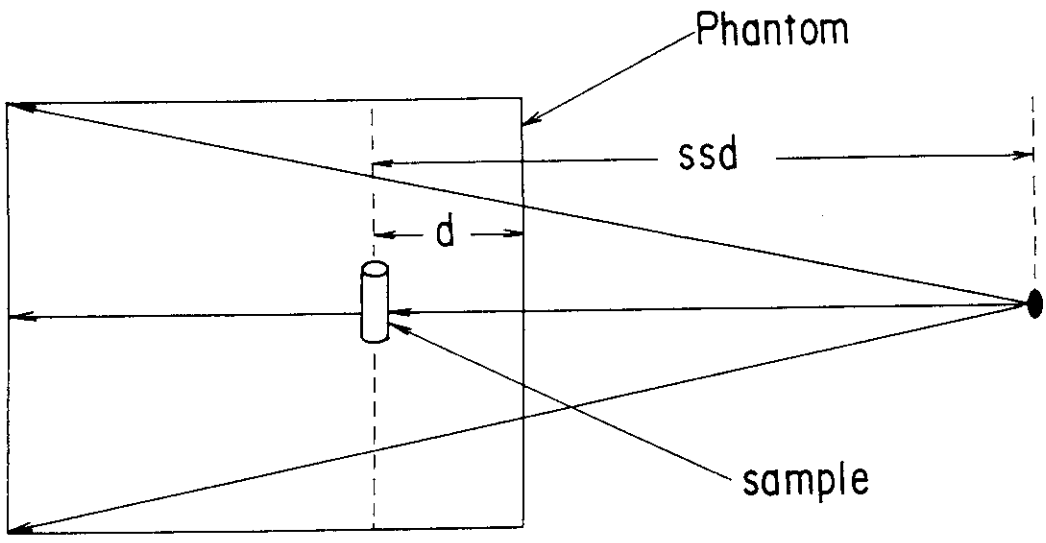
Depth in water(cm)	n_a*	n_b* *	n_a/n_b	$n_a + n_b$
In air	208741	20704	10.082	229445
5	239146	18975	12.603	258121
10	217535	15355	14.167	232890
15	184840	11225	16.467	196065
20	162853	8446	19.282	171299

*The Compton scatter and back scatter region (100 to 900keV)(counts per 300 s)
**The photopeak region (1140 to 1410keV)(counts per 300 s)

Table 4 Absorbed dose-average values of LET with cut-off energy 100eV in water irradiated with ^{60}Co γ-rays, ^{137}Cs γ-rays and 250kV$_p$ X-rays and dicentric yields per 1000 cells per 250mGy

Radiation source	$\overline{L_{100,D}}$ keV/μm	Dicentric yields per 1000 cells per 250mGy
^{60}Co	7.9*[6]	4.1
^{60}Co	8.3**[6]	13(11.6***)
^{137}Cs	8.4*[6]	13.5[25]
250kV$_p$ X-rays	9.4*[24]	23.22[26]

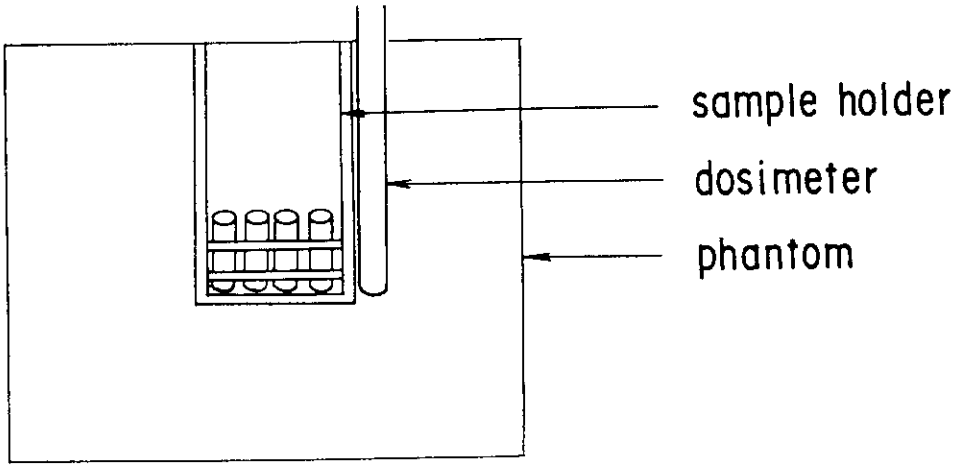
* primary only
** at 10cm depth in water
*** for rabbit



ssd : source to sample distance

d : distance in water

Side view



Front view

Fig.1 Diagram illustrating the meaning of the distance in water from the side wall of water phantom where samples are positioned.

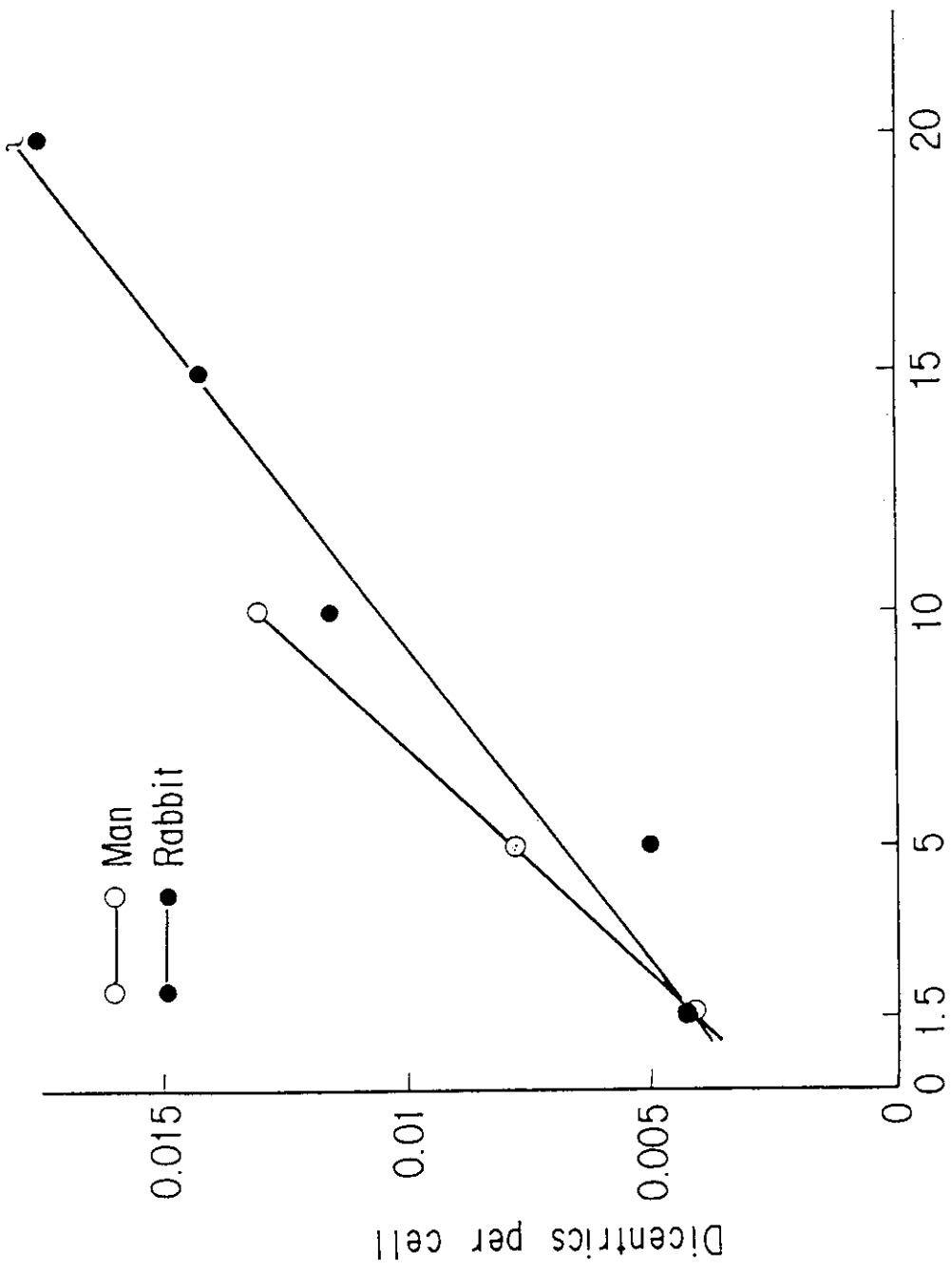


Fig.2 Dicentric yields after 250mGy irradiation of human and rabbit lymphocytes at various distances in water from the side wall of a water phantom with ^{60}Co γ-rays.

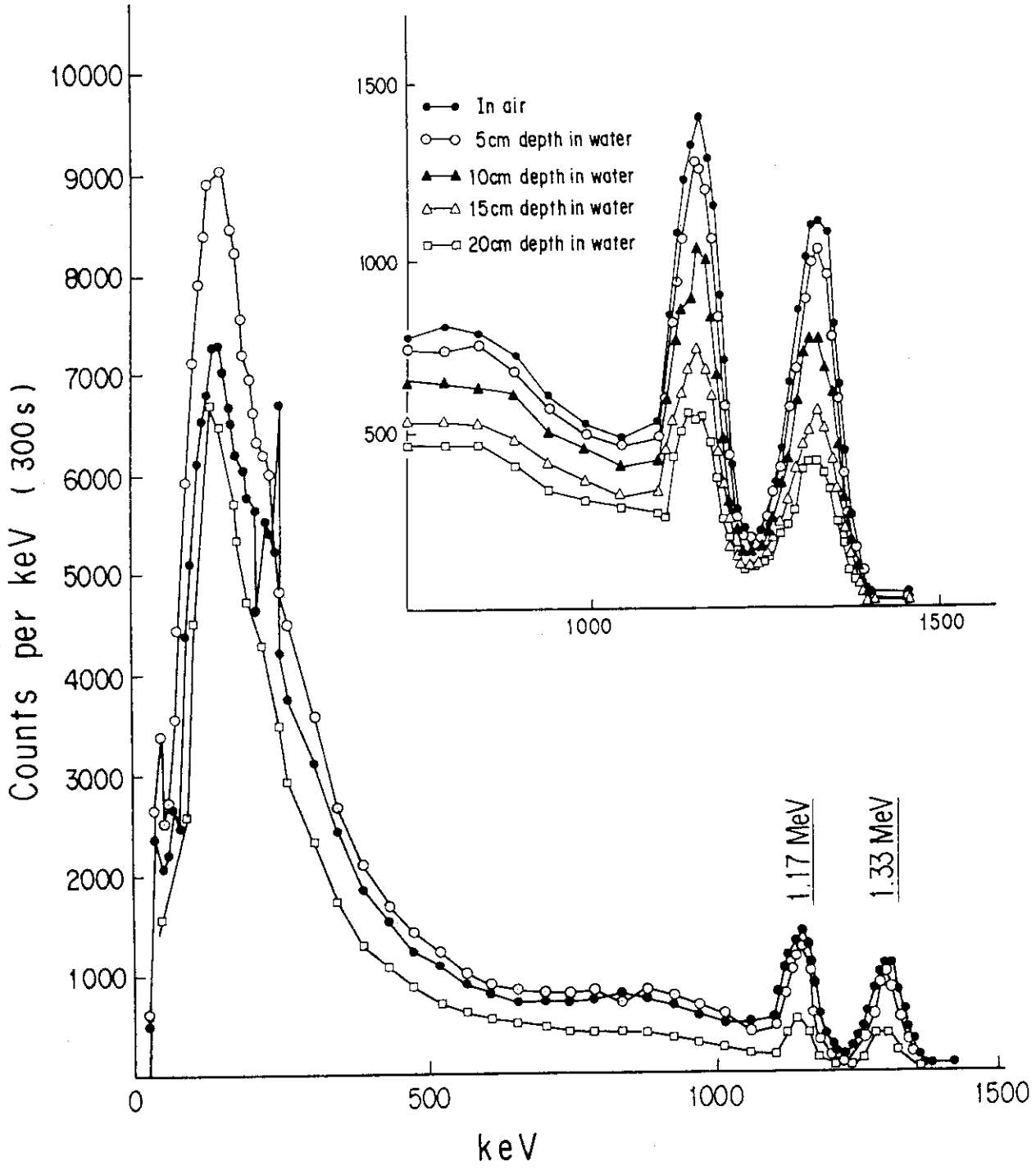


Fig.3 Pulse heights distributions for ^{60}Co γ-rays at various depths in a water phantom and in air.

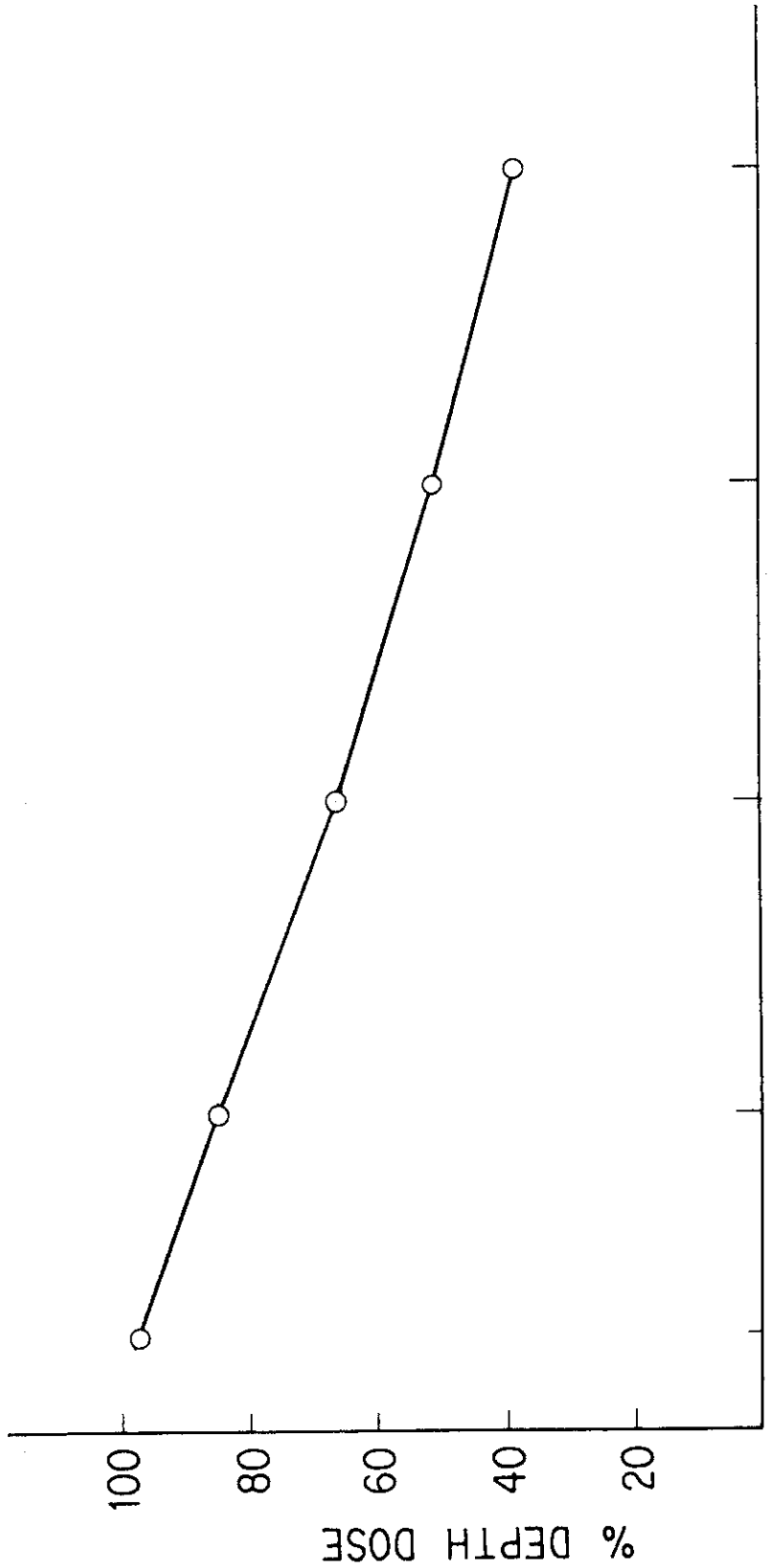


Fig.4 The percentage depth doses at 1.5, 5, 10. 15 and 20cm depth, obtained by calculation from the tissue-air ratio and the backscatter factor.

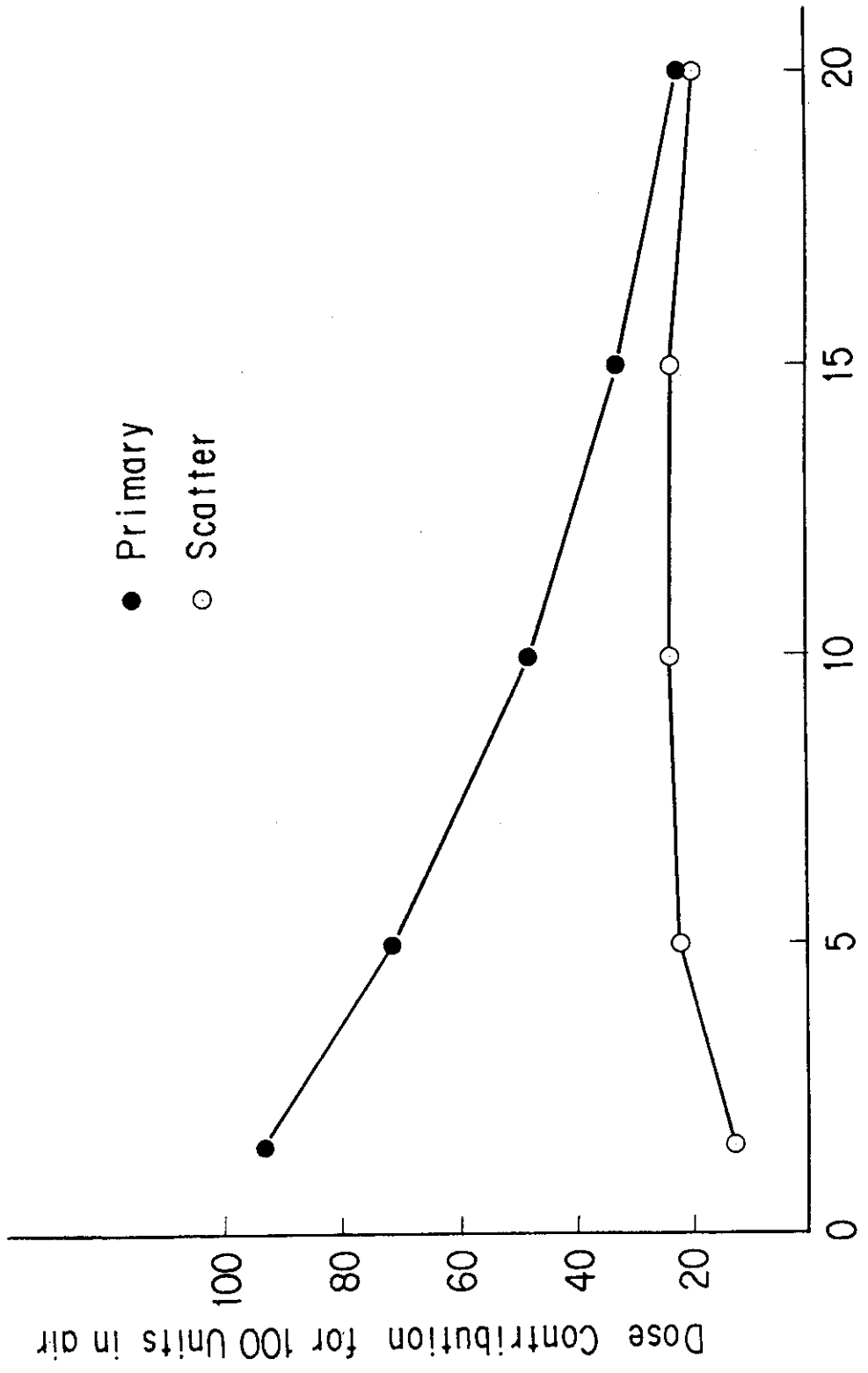


Fig.5 Scatter and primary contributions as a function of depth for a primary absorbed dose of 100units. ^{60}Co, the source to the sample distance 140cm.

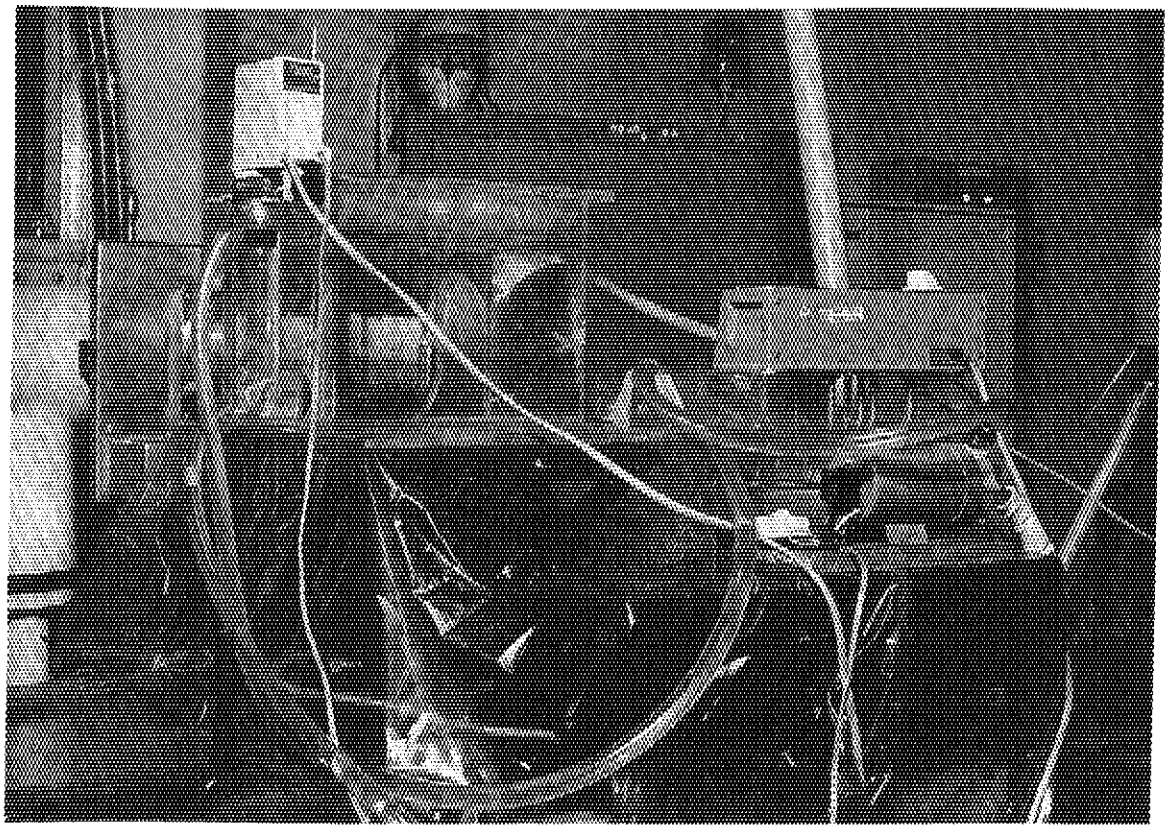


Photo 1 A water phantom