The Effect of 29 kV X Rays on the Dose Response of Chromosome Aberrations in Human Lymphocytes

Author(s): E. Schmid, D. Regulla, H-M. Kramer, and D. Harder
Source: Radiation Research, 158(6):771-777.
Published By: Radiation Research Society
DOI: http://dx.doi.org/10.1667/0033-7587(2002)158[0771:TEOKXR]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1667/0033-7587%282002%29158%5B0771%3ATEOKXR%5D2.0.CO%3B2

RADIATION RESEARCH **158**, 771–777 (2002)
0033-7587/02 $5.00

The Effect of 29 kV X Rays on the Dose Response of Chromosome Aberrations in Human Lymphocytes

E. Schmid,[a,1] D. Regulla,[b] H-M. Kramer[c] and D. Harder[d]

[a] *Institute of Radiation Biology and* [b] *Institute of Radiation Protection, GSF-National Research Center for Environment and Health, 85764 Neuherberg, Germany;* [c] *Physikalisch-Technische Bundesanstalt (PTB), 38116 Braunschweig, Germany; and* [d] *Prof. em. of Medical Physics and Biophysics, Georg-August-Universität, 37073 Göttingen, Germany*

Schmid, E., Regulla, D., Kramer, H-M. and Harder, D. The Effect of 29 kV X Rays on the Dose Response of Chromosome Aberrations in Human Lymphocytes. *Radiat. Res.* 158, 771–777 (2002).

The induction of chromosome aberrations in human lymphocytes irradiated *in vitro* with X rays generated at a tube voltage of 29 kV was examined to assess the maximum low-dose RBE (RBE_M) relative to higher-energy X rays or ^{60}Co γ rays. Since blood was taken from the same male donor whose blood had been used for previous irradiation experiments using widely varying photon energies, the greatest possible accuracy was available for such an estimation of the RBE_M, avoiding the interindividual variations in sensitivity or differences in methodology usually associated with interlaboratory comparisons. The magnitude of the linear coefficient α of the linear-quadratic dose–effect relationship obtained for the production of dicentric chromosomes by 29 kV X rays ($\alpha = 0.0655 \pm 0.0097$ Gy^{-1}) confirms earlier observations of a strong increase in α with decreasing photon energy. Relating this value to previously published values of α for the dose–effect curves for dicentrics obtained in our own laboratory, RBE_M values of 1.6 ± 0.3 in comparison with weakly filtered 220 kV X rays, 3.0 ± 0.7 compared to heavily filtered 220 kV X rays, and 6.1 ± 2.5 compared to ^{60}Co γ rays have been obtained. These data emphasize that the choice of the reference radiation is of fundamental importance for the RBE_M obtained. A special survey of the RBE_M values obtained by different investigators in the narrow quality range from about 30 to 350 kV X rays indicates that the present RBE is in fairly good agreement with previously published findings for the induction of chromosome aberrations or micronuclei in human lymphocytes but differs from recently published findings for neoplastic transformation in a human hybrid cell line. © 2002 by Radiation Research Society

INTRODUCTION

Compared with the extensive published results on the induction of chromosome aberrations in human peripheral lymphocytes by sparsely ionizing radiations with initial energies above 150 kV, relatively few groups have reported on the effects of radiations with lower energies. In experiments covering the range from 0.3 keV carbon K-shell characteristic X rays to 13 MeV electrons (*1*), from 4.8 keV X rays to ^{60}Co γ rays (*2*), from tritium β particles (mean energy 5.7 keV) to 15 MeV electrons (ref. *3*, using data from NRPB, Didcot, UK), and 5.4 keV chromium K_α-shell characteristic X rays to 3 MeV electrons (*4*), significant variations in the initial slopes of the dose–response curves for dicentrics have been reported. Substantial differences in biological effectiveness have also been reported between 14 kV X rays and ^{60}Co γ rays for the induction of micronuclei in human lymphocytes (*5*) and recently in the narrow energy range of 29 kV and 200 kV X rays for neoplastic transformation in cells of the human hybrid cell line CGL1 (*6*). For all these cellular effects, the observed dependence of the biological effectiveness on the energy of sparsely ionizing radiations has been attributed to microdosimetric differences between these radiations.

In consideration of this experimental evidence of a non-negligible increase in the maximum relative biological effectiveness at low doses, RBE_M (equivalent to the ratio of the yield coefficients α of the dose–response curves for the radiation of interest and a reference radiation), for cellular effects by a factor of 5 to 10 with decreasing photon energy, we saw a need for a systematic investigation using the highest possible accuracy, e.g. avoiding the interindividual variations in sensitivity or differences in methodology usually associated with interlaboratory comparisons. The present work forms part of a set of studies of chromosome aberrations with various X-ray and γ-ray spectra in which blood from the same donor has been used, so that the influence of interindividual variations is eliminated. This study was performed with X rays generated at a tube voltage of 29 kV to investigate a quality of X rays commonly used in X-ray diagnostics. The results are compared to the dose–response relationship for ^{60}Co and ^{137}Cs γ rays and for various X-ray spectra, obtained with blood from the same donor. The variation of RBE_M in the narrow energy range from about 30 to 350 kV X rays (*1, 2, 5–7*) is discussed in detail.

[1] Author to whom correspondence should be addressed: GSF-Institute of Radiobiology, P.O. Box 1129, 85764 Neuherberg, Germany; e-mail: eschmid@gsf.de.

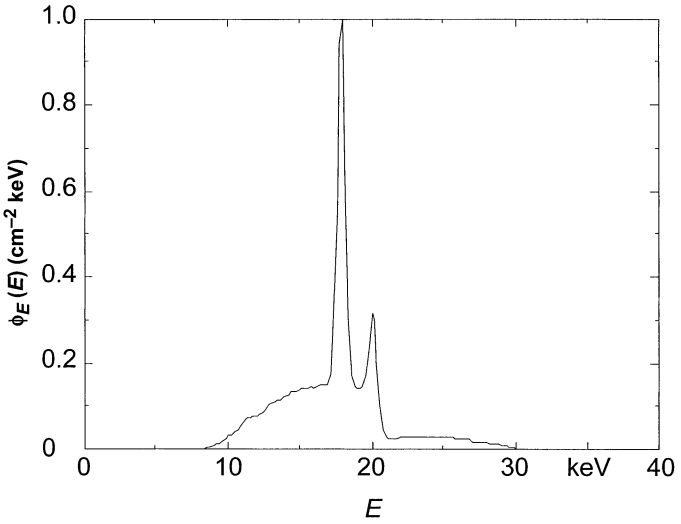

FIG. 1. Photon fluence spectrum for a 30 kV X-ray tube with a quality similar to that of mammography radiation voltage. The measurements were performed at an X-ray tube voltage of 29 kV. The essential difference between a 29 kV spectrum and a 30 kV spectrum is that the high-energy cut-off is at 29 kV instead of 30 kV. The characteristic radiation is almost identical in both cases.

MATERIAL AND METHODS

For the present experiment, peripheral blood was taken from the same healthy male donor whose blood had been used for previous experiments with sparsely ionizing radiations (*4, 7, 8*). Whole blood was exposed in flat ring-shaped plastic (polyvinylcarbazole) chambers, that were sealed with two stretched Mylar foils (polyethylenterephthalate), 20 μm thick. The blood sample inside this chamber was disk-shaped, with a diameter of 23.9 mm and a height of 2 mm.

Irradiation and Dosimetry

The blood samples were irradiated at the X-ray calibration facility of the Physikalisch-Technische Bundesanstalt (PTB), Braunschweig (Germany), with X rays emitted from a molybdenum target. The X-ray tube voltage was 29 kV and the total filtration was 30 μm of molybdenum. The X-ray spectrum obtained is shown in Fig. 1. The most probable photon energy was that of the K_α-shell characteristic X-ray line at 17.4 keV. The corresponding first half-value layer was 0.337 mm aluminum. For irradiations, the beam axis and the axis of the blood disk were made to coincide, and the central plane of the blood disk was positioned at the reference distance of 100 cm from the focal spot. The absorbed doses in the blood samples ranged from 0.115 to 2.194 Gy. To administer the desired absorbed doses within about 15 min, dose rates from 0.009 to 0.140 Gy min^{-1} were used.

The dosimetry consisted of three steps: First, the air kerma was determined at the reference point free in air, i.e. in the absence of both the holder and the blood. To determine the air kerma when the sample was at the reference point, a transmission monitor chamber, positioned about 25 cm from the focal spot, was calibrated using the PTB's primary air kerma standard in terms of air kerma free in air at the reference point. The blood sample was then positioned at the reference point and exposed to preselected values of air kerma free in air, which were given by the product of the monitor chamber reading and the calibration factor determined in step 1.

In the final step, a conversion coefficient from air kerma free in air to the absorbed dose to blood averaged over the blood volume inside the plastic holder was calculated by means of Monte Carlo simulation. The conversion factor from air kerma free in air to the absorbed dose to blood in the sample chamber for the 29 kV X-ray tube voltage was obtained from calculations for 25 kV, 30 kV and 35 kV spectra using a quadratic interpolation to 29 kV. The code used is a homemade photon transport code and takes into account photoelectric interactions and Compton and Rayleigh scattering. After a photoelectric event, either the energy is deposited at the point of interaction or, as an option, a fluorescence photon may be created. Data for the production of fluorescence photons are taken from Hubbell *et al.* (*9*). Compton scattering events are modeled according to the Klein-Nishina differential cross sections modified by incoherent scattering functions (*10*); coherent scattering is modeled according to the differential Thomson scattering cross section modified by atomic form factors (*10*). Numerical values of interaction cross sections are taken from the data contained in the XCOM software developed by Berger and Hubbell (*11*) and mass-energy absorption coefficients from Higgins *et al.* (*12*). For benchmark of the code, see Kramer *et al.* (*13*), Kramer and Grosswendt (*14*) and Baorong *et al.* (*15*). Because of attenuation of the photon fluence, there is a decrease in dose across the blood depth. Normalized to the average absorbed dose to blood in the cylindrical chamber, the absorbed dose at the entrance or exit plane is larger or smaller by about 15%, respectively. Values of the average absorbed dose to blood are given with a relative uncertainty of 2.5% ($k = 2$). The resulting quotient of the average absorbed dose to blood inside the plastic holder and the air kerma free in air in the central plane of the blood sample was 1.02.

In the photon energy range of the present experiment (Fig. 1), the kerma to the cell nucleus of a lymphocyte is 10.7% larger than the whole-blood kerma, mainly due to the larger phosphorus content of the nuclear material (*16, 17*). Photoelectron ranges are between about 2 μm (10 keV) and 8 μm (20 keV), so that the energy imparted to the cell nucleus is determined by electrons originating from the blood plasma as well as from the cytoplasm or nucleus of the lymphocyte. Therefore, the absorbed dose to the nucleus lies between the kerma values for whole blood and the nuclear material, approximately 5% over the whole-blood kerma. Since this increment was neglected, the α values determined in this paper (Table 4 and Fig. 4) have a systematic uncertainty of approximately +5%.

Blood Culture and Chromosome Analysis

Immediately after irradiation of a blood chamber with a particular dose, two lymphocyte cultures containing 0.5 ml whole blood, 4.5 ml RPMI 1640 medium supplemented with 15% fetal calf serum, 2.5% phytohemagglutinin, 2.9 μg/ml bromodeoxyuridine (BrdU), and antibiotics were established. During a 6-h transit to the cytogenetics laboratory at the GSF, the cultures were maintained at 37°C in an incubator. After arrival, the cultures were incubated for a further 41 h at 37°C. For the final 3 h, 0.1 μg/ml Colcemid was present.

Chromosome preparation and fluorescence plus Giemsa (FPG) staining were performed according to our standard procedures (*18*). All slides were coded. Chromosome analysis was carried out exclusively in complete first-division metaphases identified by homogeneously stained chromosomes. Dicentrics and centric rings were recorded, and only one fragment (assuming a complete exchange process) was assigned to each exchange. Supernumerary acentric fragments were recorded as acentrics.

RESULTS

Table 1 presents the basic data on the different chromosome aberration types in lymphocytes irradiated with X rays generated at a tube voltage of 29 kV together with the background frequencies from the blood donor, which are not significantly different from the corresponding values obtained from 141 control individuals (total number of cells 92,550) examined in our laboratory (*7*). With increasing dose, the yield of chromosome aberrations increases in the three classes of the aberration types. The ratio of the yields

TABLE 1
The Yield of Chromosome Aberrations after Irradiation with Different Doses of X Rays Generated at a Tube Voltage of 29 kV

Dose (Gy)	Cells scores	Chromosome aberrations per cell		
		Dicentrics	Centric rings	Acentrics
0	92550	0.00041	0.0001	0.0025
0	9200	0.00033	0	0.0025
0.115	2000	0.008	0.001	0.008
0.240	1200	0.018	0.003	0.018
0.509	1000	0.046	0.004	0.056
0.763	700	0.057	0.011	0.057
1.017	500	0.112	0.014	0.094
1.642	400	0.225	0.025	0.143
2.194	300	0.290	0.030	0.230

dicentrics and centric rings is approximately 10 and is independent of dose.

The observed intercellular distributions of dicentrics are given in Table 2 and those of acentrics are given in Table 3 together with the results of the tests for Poisson distributions. The relative variance, σ^2/mean y, approximates unity if the intercellular distribution of the aberrations follows a Poisson distribution. Values of the test quantity u in excess of 1.96 indicate overdispersion at the 5% level of significance (*19*). For dicentrics, a regular distribution was observed at all doses (Table 2), whereas for acentrics, a Poisson distribution was observed at only five of seven doses (Table 3). At two higher doses (1.017 and 1.642 Gy), the intercellular distribution of acentrics is significantly overdispersed compared to Poisson.

A weighted least-squares approximation was used to fit the data for the induction of dicentrics and acentrics with the linear-quadratic function $y = c + \alpha D + \beta D^2$, where $c = (3.3 \pm 1.9) \times 10^{-4}$ and $(2.5 \pm 0.5) \times 10^{-3}$, respectively, given by the controls. Reciprocal variances of the mean (total number of cells analyzed, n, divided by variance, σ^2) were used as weights. The coefficients of the dose–response relationship for dicentrics are $\alpha = (0.0655 \pm 0.0097)$ Gy^{-1} and $\beta = (0.0351 \pm 0.0082)$ Gy^{-2}; for acentrics, they are $\alpha = (0.0721 \pm 0.0099)$ Gy^{-1} and $\beta = (0.0130 \pm 0.0077)$ Gy^{-2}. The stated uncertainties are standard errors of the mean. The resulting dose–response curves are shown in Fig. 2.

The estimated coefficients of the linear and quadratic terms of the dose–response relationships for dicentrics and acentrics are shown in Table 4, together with the corresponding values from our earlier experiments with 10 kV X rays (*20*), 60 kV X rays (*8*), weakly filtered (0.5 mm copper) and heavily filtered (3.35 mm copper) 220 kV X rays (*21*), ^{137}Cs γ rays (*22*) and ^{60}Co γ rays (*23*). All these values were calculated by using the same curve-fitting method. For the experiments with 220 kV X rays (*21*) and γ rays (*22, 23*), whole blood was irradiated; for the experiments with 10 kV X rays (*20*) and 60 kV X rays (*8*), separated lymphocytes were irradiated as attached monolayers. The monolayer technique was necessary because of either the strong attenuation of 10 kV X rays or the investigation of dose effects at tissue–metal interfaces. It is worth mentioning that the radiation response of the attached lymphocytes was not different from that of lymphocytes irradiated in whole blood, at least for ^{137}Cs γ rays (*24*). The dose–response curves for dicentrics determined for the low X-ray energies of 10, 29 and 60 kV are compared in Fig. 3.

Table 4 presents the low-dose RBE values for the production of dicentrics or acentrics at various X-ray energies relative to our earlier results for ^{60}Co γ rays (*22*). In the present experiment with 29 kV X rays, RBE_M values of 6.12 ± 2.51 for dicentrics and 2.98 ± 0.56 for acentrics were obtained with ^{60}Co γ rays as the reference. There is a clear increase in the RBE_M with decreasing mean photon energy.

DISCUSSION

The α value of 6.55×10^{-2} Gy^{-1} obtained in the present investigation for the production of dicentric chromosomes in human lymphocytes by 29 kV X rays confirms the observation of a strong increase in α with decreasing photon energy reported earlier (*1–3, 20, 25, 26*). A set of α values obtained with blood from the same donor under constant

TABLE 2
The Distribution of Dicentrics among Cells Irradiated with Different Doses of X Rays Generated at a Tube Voltage of 29 kV

Dose (Gy)	No. of cells analyzed	Dicentrics per cell	Intercellular distribution of dicentrics					σ^2/y	u value
			0	1	2	3	4		
0	9200	0.00033	9197	3	—	—	—	1.00	−0.02
0.115	2000	0.008	1984	16	—	—	—	0.99	−0.26
0.240	1200	0.018	1178	22	—	—	—	0.98	−0.40
0.509	1000	0.046	957	40	3	—	—	1.09	1.94
0.763	700	0.057	662	36	2	—	—	1.05	0.89
1.017	500	0.112	447	51	1	1	—	1.03	0.53
1.642	400	0.225	324	63	12	1	—	1.11	1.56
2.194	300	0.290	226	64	8	1	1	1.10	1.23

TABLE 3
The Distribution of Acentrics among Cells Irradiated with Different Doses of X Rays Generated at a Tube Voltage of 29 kV

Dose (Gy)	No. of cells analyzed	Acentrics per cell	Intercellular distribution of acentrics				σ^2/y	*u* value
			0	1	2	3		
0	9200	0.0025	9177	23	—	—	1.00	−0.17
0.115	2000	0.008	1984	16	—	—	0.99	−0.26
0.240	1200	0.018	1178	22	—	—	0.98	−0.40
0.509	1000	0.056	947	50	3	—	1.05	1.17
0.763	700	0.057	662	36	2	—	1.05	0.89
1.017	500	0.094	458	38	3	1	1.16	2.62
1.642	400	0.143	352	40	7	1	1.21	3.01
2.194	300	0.230	240	51	9	—	1.03	0.42

conditions of cell cycle status and aberration scoring is now available at widely varying photon energies (Table 4, Fig. 4). This large, uniform data set can be used to estimate the RBE_M of 29 kV X rays relative to ^{60}Co γ rays and 220 kV X rays and to compare the results to previous findings for the induction of chromosome aberrations (*1, 2, 25*), micronuclei in human lymphocytes (*5*), and neoplastic transformation in human hybrid CGL1 cells (*6*) (Table 5).

The plot of α as a function of mean energy in Fig. 4 should be regarded as illustrating the variation in α with radiation quality, not as a claim of an intrinsic dependence of α on mean energy. It is well known that the detailed shape of the photon spectrum, including the contribution by characteristic X rays (in the present study, in which an X-ray tube with a molybdenum anode was used, the spectral lines K_α at 17.4 keV and K_β at 19.5 keV), plays a role in addition to mean energy, and that the energy deposition of photons in cellular matter is mediated by photoelectrons and Compton electrons, whose microdosimetric properties are the intrinsic determinants of the aberration yield per unit of dose. In microdosimetric terms, the dose-mean restricted LET, $\bar{L}_{500,D}$ (energy cutoff $\Delta = 500$ eV), increases from 2.82 keV/μm for 13 MeV electrons over 3.37 keV/μm for ^{60}Co γ rays, 6.82 keV/μm for 30 kV X rays (molybdenum target), 12.4 keV/μm for 10 kV X rays (chromium target), and 21.1 keV/μm for 1.5 kV X rays (carbon target) (*27, 28*). Another microdosimetric feature is the electron range (*29*), which can be of special importance for cellular effects involving geometrical features such as chromosome domains.

Another peculiarity of the observed aberration numbers per cell has been the tendency of the relative variance to increase slightly with increasing dose, reaching a significant level of overdispersion for acentrics at two of the higher doses (Table 3). A relative variance increasing with dose occurs regularly as a consequence of dose inhomogeneity inside the irradiated blood sample. In the present case, the attenuation factor for the 15 keV photons in the blood sample 2 mm in thickness is 0.73. This means that the resulting intercellular distribution of the aberrations is the result of

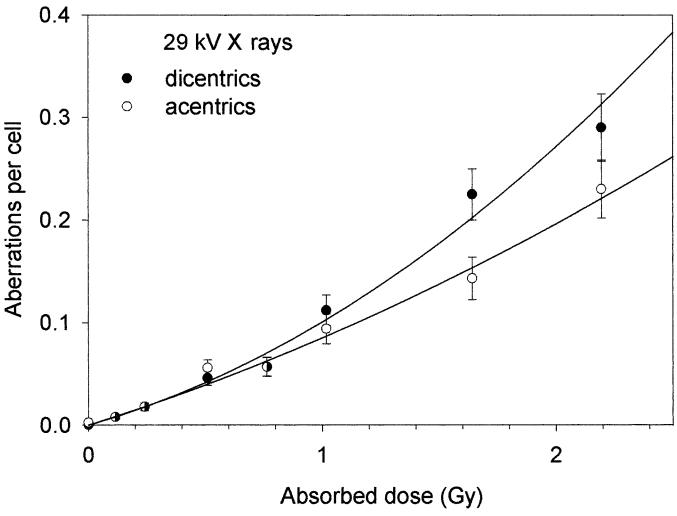

FIG. 2. Dose dependence for the yield of dicentrics and acentrics induced in human lymphocytes by X rays generated at a tube voltage of 29 kV. Standard errors are indicated by vertical bars.

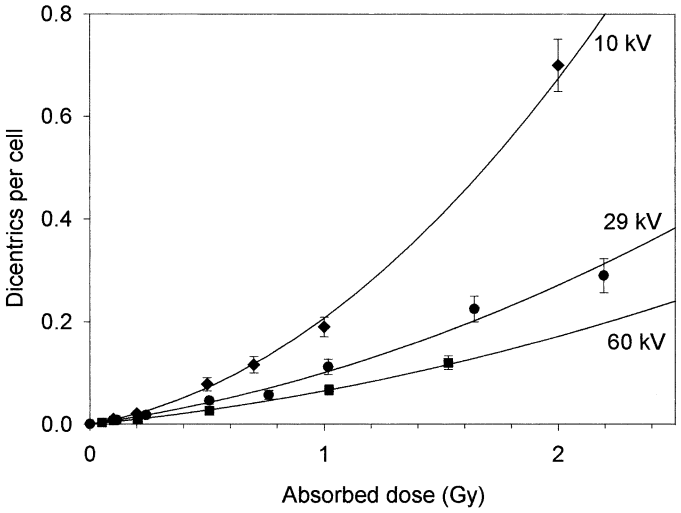

FIG. 3. Dose–response curves for dicentrics induced in human lymphocytes by X rays generated at a tube voltage of 29 kV (present study) together with previously published curves for 10 kV X rays (*20*) and 60 kV X rays (*8*). All studies were performed using blood from the same donor. Standard errors of the mean are indicated by vertical bars.

TABLE 4
Dose–Yield Coefficients α, β and Derived Maximum Low-Dose RBE (RBE_M) for the Induction of Dicentric and Acentrics Determined in Human Lymphocytes of the Same Donor for X- and γ-Ray Exposures with Different Mean Photon Energies

Radiation quality	Mean energy (keV)	Aberration type	Linear coefficient α ± SEM $\times 10^{-2}$ Gy^{-1}	Quadratic coefficient β ± SEM $\times 10^{-2}$ Gy^{-2}	RBE_M ± SEM relative to ^{60}Co γ rays	Reference
10 kV X rays	5.4	Dicentrics	7.70 ± 1.20	13.0 ± 1.50	7.20 ± 2.98	(*20*)
		Acentrics	7.40 ± 1.60	12.1 ± 1.60	3.06 ± 0.77	
29 kV X rays	17.4	Dicentrics	6.55 ± 0.97	3.51 ± 0.82	6.12 ± 2.51	Present
		Acentrics	7.21 ± 0.99	1.30 ± 0.77	2.98 ± 0.56	results
60 kV X rays	48	Dicentrics	4.44 ± 0.68	2.07 ± 0.76	4.15 ± 1.71	(*8*)
		Acentrics	4.50 ± 1.30	3.40 ± 1.40	1.86 ± 0.59	
220 kV X rays	96	Dicentrics	4.00 ± 0.30	5.98 ± 0.17	3.74 ± 1.46	(*21*)
		Acentrics	5.30 ± 0.60	5.15 ± 0.28	2.19 ± 0.37	
220 kV X rays	135	Dicentrics	2.20 ± 0.40	4.36 ± 0.24	2.06 ± 0.87	(*21*)
		Acentrics	6.40 ± 0.60	2.99 ± 0.27	2.64 ± 0.42	
^{137}Cs γ rays	662	Dicentrics	1.50 ± 0.50	4.70 ± 0.30	1.40 ± 0.71	(*22*)
		Acentrics	2.88 ± 0.58	3.31 ± 0.20	1.19 ± 0.37	
^{60}Co γ rays	1250	Dicentrics	1.07 ± 0.41	5.50 ± 0.28	—	(*23*)
		Acentrics	2.42 ± 0.31	4.46 ± 0.18	—	

the superposition of multiple Poisson distributions with expected values decreasing with depth inside the blood probe. The degree of overdispersion can easily be calculated for the case of a linear dose–effect relationship, in which the expected value m of the number n of the aberrations per cell can be assumed to decrease with depth x according to $m = \alpha D_0 \exp(-\mu x)$, where D_0 is the surface dose and μ is the attenuation coefficient. If the expected value $\bar{n} = m$ and second moment $\overline{n^2} = m^2 + m$ are averaged over the sample thickness d, the value of the relative variance,

$$\sigma^2/n = (\overline{n^2} - \bar{n}^2)/\bar{n} = 1 + \mu^2 d^2 a D_0/12,$$

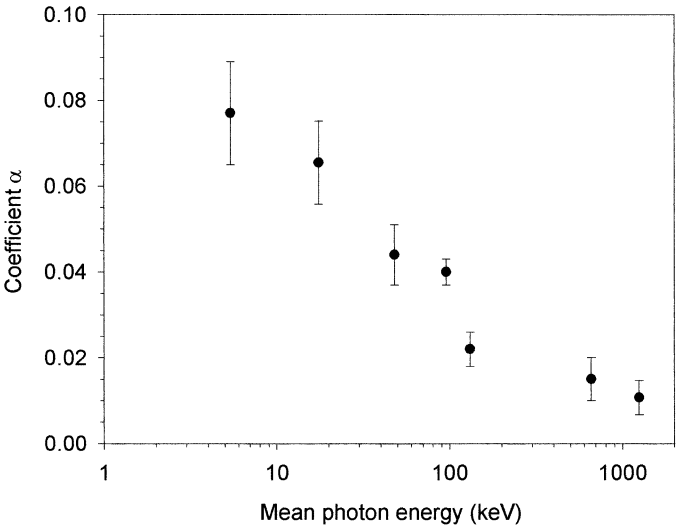

FIG. 4. Induction of dicentrics in human lymphocytes from the same donor by various X and γ radiations *in vitro*. Ordinate: α coefficient of the linear-quadratic dose–response relationship for the induction of dicentrics, from Table 4. Abscissa: Mean photon energy of the X- or γ-ray spectrum.

is obtained in a first approximation. A relative variance increasing linearly with dose D_0 therefore has to be expected.

The other possible cause of overdispersion would be the multiplicity of the aberrations along a single path of an electron through a cell nucleus, e.g. a 15 keV electron that may make about ten local energy deposits of about 200 eV along its approximately 4-μm-long path.[2] However, this kind of overdispersion should be essentially independent of dose since it reflects a property of the single particle traversal (*30*). Moreover, comparison of the observed aberration yield per cell with the calculated number of electron tracks per cell nucleus (1 Gy is equivalent to an absorbed energy of about 6 keV per μm^3) shows that an efficiency per track of the order of 10^{-3} is required to produce an aberration. We therefore interpret the slight, dose-dependent increase in the relative variance of our aberration data (Table 3) as being due to dose inhomogeneity in the blood sample.

Using an α-particle microbeam, it has been observed that non-hit cells contributed significantly to the frequencies of induced mutations and chromosomal changes observed in the total cell population (*31*). If such an effect also exists for whole-blood cultures of human lymphocytes and for low-LET radiations, it would have contributed to the present numerical results. Since the effect can be significantly reduced by inhibiting gap junction-mediated intercellular communication (*31*), it may be important to note that the constant conditions of our whole-blood lymphocyte cultures have been maintained throughout the set of experiments summarized in Table 4. The RBEs we report here

[2] R. Blohm, The passage of electrons through sensitive regions of the cell nucleus. Ph.D. thesis, University of Göttingen, Germany, 1983. [in German]

TABLE 5
Summary for Previous Studies and the Present Experiments of the Linear Dose–Effect α Coefficient and the Maximum Low-Dose RBE (RBE_M) Determined from Comparisons of Soft and Hard X Rays for Various Cellular Effects

Biological end point	X-ray quality (mean photon energy)	Linear coefficient α ± SEM $(Gy)^{-1}$	RBE_M ± SEM relative to higher-energy X rays	Reference
Dicentrics in human lymphocytes	30 kV X rays	0.154 ± 0.053	1.2 ± 0.5	(*25*)
	150 kV X rays	0.127 ± 0.028		
Dicentrics in human lymphocytes	14.6 keV X rays	0.043 ± 0.012	0.8 ± 0.5	(*2*)
	200 kV X rays	0.052 ± 0.027		
	50 kV (8.4 keV) X rays	0.153 ± 0.024	2.9 ± 1.6	
	200 kV X rays	0.052 ± 0.027		
Dicentrics in human lymphocytes	29 kV (17.4 keV) X rays	0.066 ± 0.010	1.6 ± 0.3	Present results
	220 kV (96 keV) X rays	0.040 ± 0.003		
	29 kV (17.4 keV) X rays	0.066 ± 0.010	3.0 ± 0.7	(*21*)
	220 kV (135 keV) X rays	0.022 ± 0.004		
Micronuclei in human lymphocytes	14 kV (10 keV) X rays	0.103 ± 0.024	1.6 ± 0.6	(*5*)
	350 kV (135 keV) X rays	0.064 ± 0.018		
	50 kV (29.6 keV) X rays	0.087 ± 0.024	1.4 ± 0.5	
	350 kV (135 keV) X rays	0.064 ± 0.018		
Transformation in human CGL1 cells	29 kV X rays	0.576 ± 0.935	about 10[a]	(*6*)
	200 kV X rays	0.055 ± 0.268		

[a] The authors have stated that the RBE is about 4 at ≤0.5 Gy.

therefore include any possible influence of the bystander effect. It may be prudent to investigate the extent to which the bystander effect together with variations in cell density and culture conditions may be responsible for the well-known interlaboratory variations of the aberration yields (see Table 5).

A survey of the known changes in RBE_M in the energy range from about 30 to 350 kV X rays is given in Table 5. In a comparison between 30 and 150 kV X rays, an RBE_M of 1.2 ± 0.5 was found by Virsik and Harder (*25*). Sasaki *et al.* (*2*) observed an RBE_M of 2.9 ± 1.6 between unfiltered 50 kV X rays and 200 kV X rays. In our present study with 29 kV X rays, RBE_M values of 1.6 ± 0.3 relative to weakly filtered 220 kV X rays (*21*) and of 3.0 ± 0.7 relative to heavily filtered 220 kV X rays (*21*) have been obtained. For micronuclei in human lymphocytes (*5*), RBE_M values of 1.4 ± 0.5 for 50 kV X rays and 1.6 ± 0.6 relative to 350 kV X rays have been found. In the CGL1 neoplastic cell transformation experiment (*6*), stated uncertainties of the α values are very large, and if an RBE_M were calculated, the point estimate would be about 10. The authors conclude that for doses ≤0.5 Gy, the RBE is about 4. RBE_M values of this magnitude have not been observed for the other end points.

ACKNOWLEDGMENTS

The authors thank Dipl. Ing. Ute A. Fill and M. Haney for careful technical assistance. The work was supported financially by the German Federal Office of Radiation Protection under the contract StSch 4278.

Received: March 25, 2002; accepted: August 8, 2002

REFERENCES

1. R. P. Virsik, R. Blohm, K-P. Hermann and D. Harder, Fast, short-ranged and slow, distant-ranged interaction processes involved in chromosome aberration formation. In *Proceedings of the 7th Symposium on Microdosimetry* (J. Booz, H. G. Ebert and H. D. Hartfiel, Eds.), pp. 943–955. EUR 7147, Commission of the European Communities, Brussels, 1980.
2. M. S. Sasaki, K. Kobayashi, K. Hieda, T. Yamada, Y. Ejima, H. Maezawa, Y. Furusawa, T. Ito and S. Okada, Induction of chromosome aberrations in human lymphocytes by monochromatic X-rays of quantum energy between 4.8 and 14.6 keV. *Int. J. Radiat. Biol.* **56**, 975–988 (1989).
3. T. Straume, High-energy gamma rays in Hiroshima and Nagasaki: Implications for risk and w_R. *Health Phys.* **69**, 954–956 (1995).
4. E. Schmid and M. Bauchinger, LET dependence of dicentric yields in human lymphocytes induced by low doses of sparsely ionizing radiations and its implication for risk assessments. *Health Phys.* **74**, 719–721 (1998).
5. F. Verhaegen and A. Vral, Sensitivity of micronucleus induction in human lymphocytes to low LET radiation qualities: RBE and correlation of RBE and LET. *Radiat. Res.* **139**, 208–213 (1994).
6. D. Frankenberg, K. Kelnhofer, K. Bär and M. Frankenberg-Schwager, Enhanced neoplastic transformation by mammography X rays relative to 200 kV_P X rays. Indication for a strong dependence on photon energy of the RBE_M for various end points. *Radiat. Res.* **157**, 99–105 (2002).
7. M. Bauchinger and E. Schmid, LET dependence of yield ratios of radiation-induced intra- and interchromosomal aberrations in human lymphocytes. *Int. J. Radiat. Biol.* **74**, 17–25 (1998).
8. D. Regulla, W. Panzer, E. Schmid, G. Stephan and D. Harder, Detection of elevated RBE in human lymphocytes exposed to secondary electrons released from X-irradiated metal surfaces. *Radiat. Res.* **155**, 744–747 (2001).
9. J. H. Hubbell, P. N. Trehan, N. Singh, B. Chand, D. Mehta, M. L. Garg, R. R. Garg, S. Singh and S. Puri, Review, bibliography, and tabulation of K, L, and higher atomic shell X-ray fluorescence yields. *J. Phys. Chem. Ref. Data* **23**, 339–364 (1994).

10. J. H. Hubbell, W. J. Veigele, E. A. Briggs, R. T. Brown, D. T. Cromer and R. J. Howerton, Atomic form factors, incoherent scattering functions and photon scattering cross sections. *J. Phys. Chem. Ref. Data* **4**, 471–538 (1975).

11. M. J. Berger and J. H. Hubbell, XCOM: *Photon Cross Sections on A Personal Computer*, pp. 1–10. Report NBSIR 3597, National Bureau of Standards, Gaithersburg, MD, 1987. [see also: http://physics.nist.gov/PhysRefData/Xcom/Text/version.shtml]

12. P. D. Higgins, F. H. Attix, J. H. Hubbell, S. M. Seltzer, M. J. Berger and C. H. Sibata, *Mass Energy-Transfer and Mass Energy-Absorption Coefficients, Including In-Flight Positron Annihilation for Photon Energies 1 keV to 100 MeV*. Report NISTIR 4812, National Institute of Standards and Technology, Gaithersburg, MD, 1992.

13. H-M. Kramer, D. Hoeschen and H. Seguin, Radiological properties of an automatic exposure control system. In *Technical and Physical Parameters for Quality Assurance in Medical Diagnostic Radiology* (B. M. Moores, F. E. Stieve, H. Eriskat and H. Schibilla, Eds.), pp. 51–53. Report 18, British Institute of Radiology, London, 1989.

14. H-M. Kramer and B. Grosswendt, The role of de-excitation electrons in measurements of graphite extrapolation chambers. *Phys. Med. Biol.* **47**, 801–822 (2002).

15. Y. Baorong, H-M. Kramer, H-J. Selbach and B. Lange, Experimental determination of practical peak voltage. *Br. J. Radiol.* **73**, 641–649 (2000).

16. ICRU, *Stopping Powers for Electrons and Positrons*, Table 5.4. Report 37, International Commission on Radiation Units and Measurements, Bethesda, MD, 1984.

17. J. H. Hubbell and S. M. Seltzer, *Tables of X-Ray Mass Attenuation Coefficients and Mass Energy-Absorption Coefficients*, pp. 1–111. National Institute of Standards and Technology, Report NISTIR 5632, Gaithersburg, MD, 1995.

18. R. Wagner, E. Schmid and M. Bauchinger, Application of conventional and FPG-staining for the analysis of chromosome aberrations induced by low levels of dose in human lymphocytes. *Mutat. Res.* **109**, 65–71 (1983).

19. C. R. Rao and I. M. Chakravarti, Some small sample test of significance for a Poisson distribution. *Biometrics* **12**, 264–282 (1956).

20. H. Roos and E. Schmid, Analysis of chromosome aberrations in human peripheral lymphocytes induced by 5.4 keV X-rays. *Radiat. Environ. Biophys.* **36**, 251–254 (1998).

21. E. Schmid, M. Bauchinger, S. Streng and U. Nahrstedt, The effect of 220 kV_p X-rays with different spectra on the dose response of chromosome aberrations in human lymphocytes. *Radiat. Environ. Biophys.* **23**, 305–309 (1984).

22. E. Schmid, H. Braselmann and U. Nahrstedt, Comparison of γ-ray induced dicentric yields in human lymphocytes measured by conventional analysis and FISH. *Mutat. Res.* **348**, 125–130 (1995).

23. M. Bauchinger, E. Schmid, S. Streng and J. Dresp, Quantitative analysis of the chromosome damage at first division of human lymphocytes after ^{60}Co γ-irradiation. *Radiat. Environ. Biophys.* **22**, 225–229 (1983).

24. E. Schmid, L. Hieber, U. Heinzmann, H. Roos and A. M. Kellerer, Analysis of chromosome aberrations in human peripheral lymphocytes induced by *in vitro* α-particle irradiation. *Radiat. Environ. Biophys.* **35**, 179–184 (1996).

25. R. P. Virsik and D. Harder, Chromosome aberrations in human lymphocytes induced by photon and electron radiations, and the sublesion interaction model. In *6th Symposium on Microdosimetry* (J. Booz and H. G. Ebert, Eds.), pp. 869–881. Harwood Academic Publishers, London, 1978.

26. D. C. Lloyd and A. A. Edwards, Chromosome aberrations in human lymphocytes: Effect of radiation quality, dose, and dose rate. In *Radiation-induced Chromosome Damage in Man* (T. Ishihara and M. S. Sasaki, Eds.), pp. 23–49. Alan R. Liss, New York, 1983.

27. E. R. Bartels and D. Harder, The microdosimetric regularities of nanometer regions. *Radiat. Prot. Dosim.* **31**, 211–215 (1990).

28. D. Harder, R. Greinert and D. Frankenberg, Characteristics of electron tracks in matter. In *Radiation Research*, Vol. 2 (M. Moriarty, C. Mothersill, C. Seymour, M. Edington, J. F. Ward and R. J. M. Fry, Eds.), pp. 111–114. Allen Press, Lawrence, KS, 2000.

29. R. P. Virsik and D. Harder, Statistical interpretation of the overdispersed distribution of radiation-induced dicentric chromosome aberrations at high LET. *Radiat. Res.* **85**, 13–23 (1981).

30. H. Zhou, M. Suzuki, G. Randers-Pehrson, D. Vannais, G. Chen, J. E. Trosko, C. A. Waldren and T. K. Hei, Radiation risk to low fluences of alpha-particles may be greater than we thought. *Proc. Natl. Acad. Sci. USA* **98**, 14410–14415 (2001).