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The Neoplastic Transformation Potential of Mammography X Rays and Atomic Bomb Spectrum Radiation

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Considerable controversy currently exists regarding the biological effectiveness of 29 kVp X rays which are used for mammography screening. This issue must be resolved to enable proper evaluation of radiation risks from breast screening. Here a definitive assessment of the biological effectiveness of 29 kVp X rays compared to the quality of radiation to which the atomic bomb survivors were exposed is presented for the first time. The standard radiation sources used were (a) an atomic bomb simulation spectrum and (b) 2.2 MeV electrons from a strontium-90/yttrium-90 (^{90}Sr/^{90}Y) radioactive source. The biological end point used was neoplastic transformation *in vitro* in CGL1 (HeLa × human fibroblast hybrid) cells. No significant difference was observed for the biological effectiveness of the two high-energy sources for neoplastic transformation. A limiting relative biological effectiveness (RBE_M) of 4.42 ± 2.02 was observed for neoplastic transformation by 29 kVp X rays compared to these two sources. This compares with values of 4.67 ± 3.93 calculated from previously published data and 3.58 ± 1.77 when the reference radiation was 200 and 220 kVp X rays. This suggests that the risks associated with mammography screening may be approximately five times higher than previously assumed and that the risk–benefit relationship of mammography exposures may need to be re-examined. © 2004 by Radiation Research Society

INTRODUCTION

Recent studies have shown an increase in the RBE of low-energy (29 kVp) X rays used in mammography breast screening programs compared with higher-energy (200 kVp) X rays (*1, 2*). Some aspects of these data have been challenged, namely the mechanistic interpretation of the dependence on radiation quality (*3*), the culture conditions used (*2*), the quality of the data (*5*), and the choice of standard reference radiation (*5*).

The ICRP (*6*) acknowledges that biophysical considerations and cell studies suggest an RBE of 2–3 for conventional X rays relative to hard X rays, but its recommendation is to ''attribute the same w_R (i.e. 1) for γ rays, X-rays and electrons as a matter of practicability in the absence of definitive information''.

In this paper we present a definitive study of the oncogenicity of mammography X rays compared to high-energy X-ray and high-energy electron sources. The marker for oncogenicity used was neoplastic transformation in the non-tumorigenic HeLa × skin fibroblast cell line, CGL1. The high-energy X-ray spectrum we have used matches that experienced by survivors 1500 m from the epicenter of the Nagasaki atomic bomb. This, in combination with a high-energy ^{90}Sr/^{90}Y electron source, has been used as our standard reference source, in effect matching the energy range upon which the epidemiological evaluations of radiation risks are based.

A clinical mammography X-ray set provided the low-energy X rays (29 kVp) identical to those used in breast cancer screening. Difficulties in recent studies raised by other groups have been addressed, and we present a rigorous dosimetric assessment of each source and a defense of the culture conditions used and demonstrate biological uncertainties lower than those published previously.

MATERIALS AND METHODS

Cell System

This transformation assay used cells of the human hybrid (HeLa × normal human skin fibroblasts) cell line CGL1 (kindly donated by Professor D. Frankenberg, University of Göttingen, Germany). The CGL1 cell line is non-tumorigenic when injected into nude mice (*7*) and is negative for intestinal alkaline phosphatase (IAP) (*8*). The cells are contact inhibited and form a monolayer at confluence. CGL1 cells have been shown to provide a sensitive and reliable assay for the measurement of neoplastic transformation potential (*9*).

Cells were maintained in MEM (medium and supplements from Invitrogen), supplemented with 10% heat-inactivated fetal calf serum (5% heat-inactivated fetal calf serum for subsequent medium changes), 200 m*M* L-glutamine, non-essential amino acids and 50 μg ml^{-1} gentamicin. Cells were incubated at 37°C and grown in a humidified 95% air/5% CO_2 atmosphere. The pH of the growth medium was maintained at 7.2. Under these growth conditions, the cells have a population doubling time of approximately 19 h.

A single batch of fetal calf serum was used for all experiments described here.

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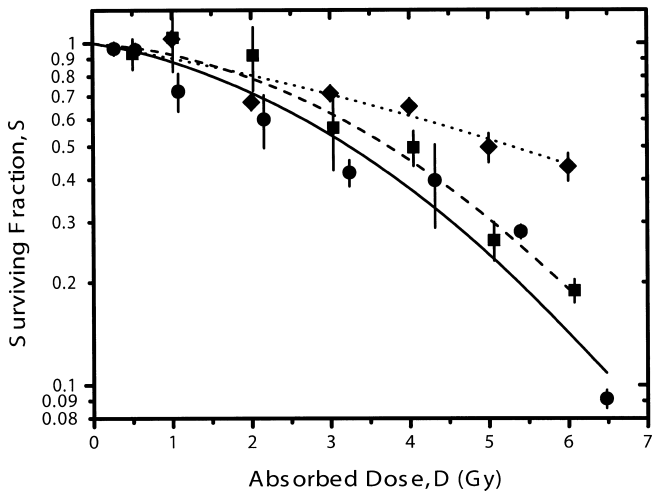

FIG. 1. Surviving fraction, *S*, as a function of absorbed dose, *D*, for CGL1 cells exposed to 29 kVp mammography X rays (●), 2.2 MeV β particles from $^{90}Sr/^{90}Y$ (■), and atomic bomb spectrum radiation (♦). The data were fitted to the linear-quadratic survival curve relationship, $S = e^{-(\alpha D+\beta D^2)}$. Fitted values for the radiation response parameters, α and β are given in Table 1. Uncertainty bars show the standard errors of the means.

TABLE 1
Fitted Radiation Response Parameters for Surviving Fraction (*S*) as a Function of Absorbed Dose (*D*)

Radiation source	Radiation response parameters	
	α (Gy^{-1})	β (Gy^{-2})
29 kVp mammography X rays	0.076 ± 0.027	0.041 ± 0.005
$^{90}Sr/^{90}Y$ 2.2 MeV electrons	0.028 ± 0.065	0.041 ± 0.011
Atomic bomb spectrum	0.092 ± 0.019	0.007 ± 0.005

Notes. Fits have been made using the linear-quadratic relationship $S = e^{-(\alpha D+\beta D^2)}$. Uncertainties are standard errors.

Neoplastic Transformation Assay

Three days prior to irradiation, cells were seeded into either petri dishes (nominal diameter 53 mm, containing 5 ml medium) or 25-cm^2 (T-25) flasks (containing 10 ml medium) depending on the radiation source used. Cell seeding densities (4×10^3 cm^{-2}) were chosen to provide exponentially growing cells at the time of irradiation. After irradiation, the cells were held at 37°C for 4 h prior to subculture. Cell recovery is complete after 3 h and remains largely unchanged after 4 h for low doses (<6 Gy) (*10, 11*). Irradiated cells and unirradiated controls were seeded into T-75 flasks prefilled with 20 ml of 10% heat-inactivated fetal calf serum growth medium to assess neoplastic transformation and cell survival. For the assessment of neoplastic transformation, a cell seeding density of 50 viable cells cm^{-2} was used. A lower cell seeding density (1.5 viable cells cm^{-2}) was used to assess cell survival.

At 13 days postirradiation, the cell survival assay was stained using methylene blue solution (Merck), and the medium for the neoplastic transformation assay was refreshed using growth medium containing 5% heat-inactivated fetal calf serum. A second medium change was carried out on day 18 (again in medium with 5% heat-inactivated fetal calf serum). The same medium change schedule was used for all doses and all radiation sources.

Transformed foci were stained for the surface protein IAP at 21 days postirradiation. IAP has been identified as a marker of neoplastic transformation in this cell line (*8*) and has been shown to be fully expressed at 21 days postirradiation (*12*). Cells were fixed for 20 min with 2% paraformaldehyde in PBS. After fixing and rinsing, 2 ml of Western Blue Reagent (Promega, UK) was added to each T-75 flask. Staining is complete in 15 min. IAP-positive colonies, which appear blue, were scored against a background of colorless IAP-negative (i.e. nontransformed) cells.

Calculation of Transformation Frequency

To assess cell survival, flasks were scored using the criterion that a colony must contain >50 cells. The survival assay was used to calculate the plating efficiency and the surviving fraction.

The neoplastic transformation assay flasks were classed as positive if, by visual inspection, they contained evidence of the blue crystalline product formed from the Western Blue. Flasks were classified as negative if there was no evidence of this product.

The null method (*13*) was used to calculate the transformation frequency (*T*) expressed as transformants per 10^4 surviving cells. When a desired viable cell density (ρ) of 50 cells cm^{-2} was not observed, the calculated transformation frequency was corrected to this density using published data (*11*). *T*(50) is the transformation frequency corrected to a viable cell density of 50 cells cm^{-2}:

$$T(50) = 0.203\left(\frac{\rho}{cm^{-2}}\right)^{0.407} \times T \text{ (measured)}.$$

Irradiation Protocol

29 kVp mammography source. The cells were irradiated using a clinical mammography unit (GE Medical Systems Senographe 600TS). The tube uses a molybdenum target (12° angle) and molybdenum (0.03 mm) filter. There is an additional inherent 0.8-mm beryllium filter. For the cell irradiations with doses greater than 0.5 Gy, the tube was operated at 29 kVp, 400 mA s. At doses less than 0.5 Gy, 29 kVp, 200 mA s was used.

Mammography dosimetry. Practical dosimetry was performed according to the UK guidelines for X-ray dosimetry (*14*) using a secondary standard parallel plate ion chamber (MDH-Radcal Model 10x5-6M, volume: 6.0 cm^3) free in air. This measurement was confirmed to within ±8% using a theoretical calculation of air kerma using the Institute of Physics and Engineering in Medicine (IPEM) Report 78 (*15*). Both 750 mm air and 1.41 mm Perspex (the flask base thickness) are included in the total beam filtration. The mean photon energy was calculated (*15*) to be 17.0 keV with an air kerma of 88.2 μGy $(mA\ s)^{-1}$ at 750 mm. The first half-value layer was calculated to be 0.346 mm aluminum. An inverse power law relationship was used to calculate the air kerma at the cell layer (185 mm from the X-ray target). A time-averaged dose rate of 1 Gy min^{-1} was observed.

$^{90}Sr/^{90}Y$ high-energy electron source. The cells were irradiated using a $^{90}Sr/^{90}Y$ source (nominal activity 5.18 GBq in 1985) housed in an aluminum jig. The 75-mm-diameter disc of a $^{90}Sr/^{90}Y$ compound is glued onto an aluminum irradiating jig. The compound $^{90}Sr(NO_3)_2$ is incorporated in rolled silver foil with a face thickness of 50 μm (50 mg cm^{-2}). A petri dish is held 6 mm above the source, behind a removable (3-mm-thick) lead shield. Beta particles with a maximum energy of 2.2 MeV are emitted from the ^{90}Sr daughter product (^{90}Y). The source has a track-averaged LET of 0.26 keV μm^{-1} (*16*).

$^{90}Sr/^{90}Y$ dosimetry. To measure the source dose rate, HD-810 radiochromic dye film was exposed to doses between 60 Gy and 180 Gy. A time-averaged dose rate was then calculated. To account for the increase in dose due to backscattered electrons (15% addition to the total dose), the film was exposed in petri dishes containing 5 ml of medium. The hygroscopic film was protected during irradiations using a thin (~15 μm) layer of polyethylene. The dose rate at the cell layer was 1.01 ± 0.03 Gy min^{-1}.

Atomic bomb simulated source. An experimental simulation of an atomic bomb spectrum using a Philips SL15 Linear Accelerator has been

TABLE 2
Neoplastic Transformation of CGL1 Cells for Three Radiation Sources

Dose, D (Gy)	Surviving fraction, S	Total survivors $\times 10^5$	Number of flasks		Transformation frequency per survivor, $T(50) \times 10^{-4}$
			Total, N	Without foci, n	
(a) 29 kVp X rays					
0	1	5.25 ± 0.07	154	139	0.29 ± 0.07
0.27	0.96 ± 0.01	8.41 ± 0.12	148	114	0.54 ± 0.09
0.54	0.96 ± 0.03	4.29 ± 0.12	159	132	0.60 ± 0.11
1.08	0.72 ± 0.09	2.88 ± 0.14	120	92	0.92 ± 0.17
2.16	0.60 ± 0.11	1.42 ± 0.05	62	40	1.56 ± 0.30
3.24	0.42 ± 0.04	0.87 ± 0.06	60	34	2.66 ± 0.46
4.32	0.40 ± 0.11	4.86 ± 0.11	84	18	3.18 ± 0.28
5.40	0.28 ± 0.01	5.31 ± 0.05	90	13	3.94 ± 0.30
(b) ^{90}Sr/^{90}Y β particles					
0	1	4.85 ± 0.10	168	157	0.21 ± 0.06
1.01	1.08 ± 0.25	3.03 ± 0.12	79	66	0.47 ± 0.13
2.03	0.92 ± 0.19	5.22 ± 0.21	119	75	1.12 ± 0.16
3.04	0.56 ± 0.17	4.68 ± 0.15	120	76	1.19 ± 0.17
4.05	0.50 ± 0.06	6.99 ± 0.11	124	60	1.52 ± 0.16
5.06	0.27 ± 0.05	3.72 ± 0.14	59	14	2.82 ± 0.33
6.08	0.20 ± 0.01	4.89 ± 0.18	143	34	4.05 ± 0.32
(c) Atomic bomb spectrum					
0	1	6.45 ± 0.06	117	99	0.36 ± 0.08
1.00	1.03 ± 0.03	6.86 ± 0.08	111	87	0.48 ± 0.09
2.00	0.67 ± 0.03	6.43 ± 0.13	158	99	1.19 ± 0.14
3.00	0.71 ± 0.05	5.16 ± 0.10	79	36	1.51 ± 0.19
4.00	0.65 ± 0.03	5.46 ± 0.11	89	33	1.98 ± 0.21
5.00	0.50 ± 0.05	4.78 ± 0.07	90	20	3.26 ± 0.29
6.00	0.44 ± 0.04	3.31 ± 0.05	58	8	4.12 ± 0.39

Notes. Uncertainties are standard errors. N is the total number of flasks, and n is the total number of flasks without foci. $T(50)$ is the transformation frequency corrected to a cell density of 50 viable cells per cm^2.

published (*17*). We used a slightly modified adaptation of this technique, where a four-beam solution was used to match the electron spectrum through the colon for atomic bomb survivors of Nagasaki, 1500 m from the hypocenter. The solution used a 6 MV photon beam and three high-energy electron beams (10 MeV, 12 MeV, 14 MeV). The photon component to the spectrum contributed almost 82% of the total dose. The accelerator was operated to give a dose rate to the cell layer (averaged over the total irradiation time) of 1 Gy min^{-1} (Dr. R. P. Hugtenburg is acknowledged for his assistance in generating this modified method). Details of this modification are to be published elsewhere (manuscript in preparation).

Atomic bomb simulation dosimetry. The linear accelerator dosimetry is checked monthly, according to the established codes of practices for high-energy photon dosimetry (*18*) and the Institute of Physics and Engineering in Medicine code for electron dosimetry (*19*). The photon beam that forms part of the four-modality solution is not used under reference conditions, and a correction factor is applied for the source-to-detector distance and field size.

RESULTS

Cell surviving fraction (S) as a function of absorbed dose (D) for the three sources is shown in Fig. 1. A linear-quadratic fit has been made to the data,

$$S = \exp(-\alpha D - \beta D^2),$$

with the values for fitted linear and quadratic radiation response parameters, α and β, given in Table 1. The observed differences in survival and transformation for the three sources may be due to different target sizes for the two end points. While a clear difference between the radiations is evident at high doses (>3 Gy), there are no significant differences at lower doses.

The results for a total of ~2400 transformation assay flasks, however, show a clear increase at all doses (>1 Gy) in the potential of low-energy X rays to produce a neoplastic transformation, when compared to higher-energy radiation sources (^{90}Sr/^{90}Y 2.2 MeV electrons and atomic bomb spectrum radiation).

The mean spontaneous transformation frequency (corrected to a viable cell density of 50 cells cm^{-2}) was found to be $0.281 \pm 0.041 \times 10^{-4}$. This measurement compares well to that reported by Redpath *et al.* (*20*) ($0.294 \pm 0.03 \times 10^{-4}$) and is similar to those reported by Frankenberg (0.18×10^{-4}) (*1*) and Göggelmann ($0.16 \pm 0.08 \times 10^{-4}$) (*2*).

Measured transformation frequencies for each radiation source are given in Table 2, and plots of transformation frequency (corrected for spontaneous transformation) as a function of absorbed dose are shown in Fig. 2. A linear-quadratic fit has been made to the data:

$$T(50) = aD + bD^2,$$

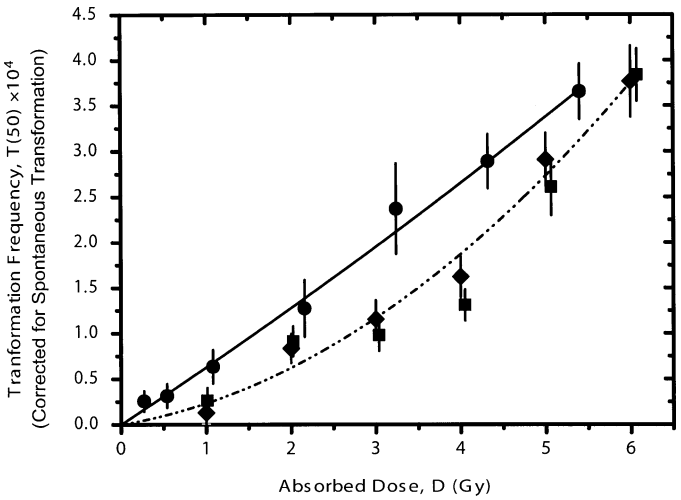

FIG. 2. Transformation frequency, $T(50)$, as a function of absorbed dose, D for CGL1 cells exposed to 29 kVp mammography X rays (●), 2.2 MeV β particles from $^{90}Sr/^{90}Y$ (■), and atomic bomb spectrum radiation (◆). $T(50)$ is the transformation frequency corrected to a cell density of 50 viable cells per cm². A linear-quadratic fit using the relationship $T(50) = aD + bD^2$ has been made to the data for 29 kVp (———) and to the combined date for high-energy X rays (—- - -—). All data points have been corrected for the spontaneous transformation frequency (= 0.281×10^{-4}). Uncertainty bars show the standard errors of the means.

where $T(50)$ is the transformation frequency (corrected for a spontaneous transformation of 0.281×10^{-4} and to a viable cell density of 50 cells cm^{-2}) and a and b are respectively the linear and quadratic radiation response parameters, given in Table 3.

As is evident from Fig. 2, the transformation frequencies for the high-energy electron and atomic bomb simulation source are not significantly different. The a and b parameters for the linear-quadratic relationships are also not significantly different for the two high-energy sources (see Table 3). This is not an unexpected finding, given the low mean LETs of the two radiations. These data may be considered of equal effectiveness, and they have therefore been combined for the purpose of further analysis of the transformation data. The limiting relative biological effectiveness (RBE_M) was calculated using the ratio of the linear fitting parameters (a) for the sources.

TABLE 4
RBE Dependence on Transformation Frequency, $T(50)$

Transformation frequency per survivor, $T(50) \times 10^{-4}$	RBE
0.5	2.27
1.0	1.80
2.0	1.41
3.0	1.22
4.0	1.10
5.0	1.02

Notes. $T(50)$ is the transformation frequency corrected to a cell density of 50 viable cells per cm². The RBE is calculated as the ratio of the dose from the high-energy sources to the dose from the low-energy mammography source required to produce the same transformation frequency.

Table 3 shows the RBE_M for the different radiation qualities. The increased potential of low-energy X rays to induce neoplastic transformation is clearly seen. Mammography X rays are 4.4 ± 2.0 (SE) times more effective at inducing neoplastic transformations of CGL1 cells *in vitro* than high-energy electrons for low absorbed doses. The RBEs at different transformation frequencies are given in Table 4.

DISCUSSION

Our results are the third set of published data on the relative biological effectiveness of 29 kVp X rays for neoplastic transformation in CGL1 cells (*1, 2*). The larger linear component for 29 kVp X rays indicates a greater efficiency at low doses for 29 kVp X rays to induce neoplastic transformation compared with the higher-energy sources by a factor of about 4.5. Although this result is in broad agreement with both previous sets of data, the earlier publications resulted in considerable controversy. Because of the importance of these low-energy X rays in mammography and the relevance of such data in the interpretation of biophysical events, it is clearly imperative that any controversies are quickly resolved.

TABLE 3
Fitted Radiation Response Parameters for Transformation Frequency, $T(50)$, as a Function of Absorbed Dose (D)

Radiation	Radiation response parameters		RBE_M of 29 kVp X rays for this reference source
	a (Gy^{-1})	b (Gy^{-2})	
29kVp mammography X rays	0.614 ± 0.125	0.012 ± 0.028	—
$^{90}Sr/^{90}Y$ 2.2 MeV electrons	0.119 ± 0.077	0.075 ± 0.017	5.16 ± 3.50
Atomic bomb spectrum	0.154 ± 0.083	0.079 ± 0.019	3.99 ± 2.30
Combined high-energy sources	0.139 ± 0.057	0.075 ± 0.013	4.42 ± 2.02

Notes. $T(50)$ is the transformation frequency corrected to a cell density of 50 viable cells per cm². Fits were made using the linear-quadratic relationship of $T(50) = \exp(aD + bD^2)$. The limiting relative biological effectiveness (RBE_M) shown is the ratio of the linear (a) component of the fit to the mammography data, to the linear components for the other radiation sources. Uncertainties are standard errors.

TABLE 5
Comparison of Published Culture Conditions for Mammography X-Ray Experiments using CGL1 Cells

Publication	Calf serum concentration		Medium changes	
	Setup of experiment	Medium change	Number	On days
This publication	10%	5%	2	13, 18
Göggelmann *et al.* (*2*)	5%	5%	3	11, 14, 18
Frankenberg *et al.* (*1*)	10%	10%	1	11

Neoplastic Transformation at 29 kVp

Frankenberg and coworkers (*1*) first reported on the biological effectiveness of mammography X rays. They found that 29 kVp X rays (generated using a tungsten target and rhodium filter) are about eight times more effective than high-energy γ rays and about four times more effective than 200 kVp X rays (*21*). These data were rapidly criticized, first by Kellerer (*3*), who dismissed the findings, suggesting that the biological data should be viewed with great caution, and subsequently by Schmid (*4*), who stated the uncertainties are ''too large to permit meaningful conclusions to be made''. In other work, Göggelmann *et al.* (*2*) were more specific and implied that Frankenberg's results were an artefact arising from inadequate culture conditions. Schmid (*4*) also highlighted potential difficulties arising from the choice of the standard reference radiation used by Frankenberg *et al.* (*1*). While the uncertainties reported by Frankenberg *et al.* (*1*) are larger than those reported here, and while their choice of beam filtration may not have been ideal, there is no basis for suggesting that the culture conditions were inadequate. The ensuing discussion addresses each of these three points in turn.

1. Culture conditions

Göggelmann *et al.* (*2*) criticized Frankenberg *et al.* (*1*) for using one, rather than three, medium changes during the culture of CGL1 cells (Table 5). They suggested that the subsequent results are unreliable. They supported this with data presented in Table 5 of their paper, where they compared their culture conditions with those used by Frankenberg *et al.* (*1*). The results are rather surprising, however, since they suggest that a culture protocol involving one medium change would result in transformation frequencies which would be barely detectable—at odds with both the data presented here in Table 6 and by Frankenberg *et al.* (*1*).

It should be noted that the culture protocols adopted by Göggelmann *et al.* (*2*) and Frankenberg *et al.* (*1*) differ not only in the number of medium changes but also in the calf serum concentration used for the cultures, Göggelmann *et al.* (*2*) using 5% rather than the 10% used by Frankenberg *et al.* (*1*). The number of medium changes is just one relevant factor; other factors include the ''quality'' of the particular serum batch, the concentration of serum used, and other local culture conditions that may be difficult to define but that are normally constant within a particular laboratory.

In the experiments described in this paper we have used a protocol that differs from those of both Göggelmann *et al.* (*2*) and Frankenberg *et al.* (*1*); a comparison of the three protocols is given in Table 5. Our choice of protocol was based on the results of preliminary experiments aimed at optimizing our protocols, the results of which are given in Table 6. Two medium changes produce the highest transformation frequency, with either 10% or 5% calf serum, while the transformation frequencies recorded for both one and three changes are reduced, but not to the extremely low values suggested by Göggelmann *et al.* (*2*). One explanation for the surprising reduction observed for three changes is that disturbing the cultures so early in their expression of transformation may result in the removal of a small number of (loosely attached) transformed cells before the foci become sufficiently large that the loss of a few cells became insignificant.

TABLE 6
Effect of Number of Medium Changes and of Serum Concentration used at Medium Changes on the Transformation Frequency of CGL1 Cells

Culture conditions	Dose (Gy)	Total flasks	$T(50) \times 10^4$
One medium change (10% serum)	0	34	0.07 ± 0.07
	6	45	2.98 ± 0.39
Two medium changes (10% serum)	0	64	0.13 ± 0.07
	6	202	5.43 ± 0.27
Two medium changes (5% serum)	0	20	0.29 ± 0.20
	6	49	5.64 ± 0.53
Three medium changes (10% serum)	0	18	0.10 ± 0.10
	6	47	3.43 ± 0.40
Two and three medium changes (all data pooled)	0	82	0.13 ± 0.06
	6	249	4.93 ± 0.23

Notes. The cells were irradiated with 6 Gy of 2.2 MeV electrons from a $^{90}Sr/^{90}Y$ source. The serum concentration of the medium prior to medium changes was 10%. $T(50)$ is the transformation frequency corrected to a cell density of 50 viable cells per cm^2. Uncertainties are standard errors.

TABLE 7
Comparison of the Linear (*a*) and Quadratic (*b*) Coefficients of Fits made to the Relationship of Neoplastic Transformation Frequency to Dose for CGL1 Human Hybrid Cells Exposed *In Vitro*

Publication	Reference radiation	Radiation response parameters				Limiting RBE, RBE_M
		29 kVp X rays		Reference		
		a (Gy^{-1})	*b* (Gy^{-2})	*a* (Gy^{-1})	*b* (Gy^{-2})	
Frankenberg *et al.* (*1*)	200 kVp X rays	0.518 ± 0.254	0.071 ± 0.06	0.111 ± 0.076	0.026 ± 0.018	4.67 ± 3.93
Göggelmann *et al.* (*2*)	220 kVp X rays	1.410 ± 0.266	−0.012 ± 0.074	0.394 ± 0.180	0.151 ± 0.049	3.58 ± 1.77
This work	A-bomb spectrum	0.614 ± 0.125	0.012 ± 0.028	0.139 ± 0.057	0.075 ± 0.013	4.42 ± 2.02

Notes. Uncertainties are standard errors. It should be noted that we have used the corrected values for the parameters from Frankenberg *et al.* as published in their addendum (*1*) to calculate the limiting RBE.

It is apparent that the criticism of the experimental protocol of Frankenberg *et al.* (*1*) by Göggelmann *et al.* (*2*) is unfounded and that there is no scientific basis on which these results should be dismissed.

2. Dosimetry and source considerations

Schmid *et al.* highlighted the importance of rigorous dosimetry and the careful choice of beam filtration (*5*). They also highlighted a potential difficulty in the choice of beam filtration used by Frankenberg *et al.* (*1*) for a 200 kVp X-ray source. This was queried by Schmid (*4*).

In the work described here, we have used high-energy electron and photon sources, closely matching the spectrum of the radiation to which the atomic bomb survivors were exposed, as the reference radiation. This provides a unique reference radiation.

We have used a clinical mammography unit to expose the cells *in vitro.* The dosimetry for this unit has been carried out using the same criteria as is used for the routine testing of mammography X-ray sources, upon which measurements and calculations of mean glandular dose are made by the UK National Health Service Breast Screening Programme.

3. Uncertainty considerations

A comparison of the radiation response parameters for both 29 kVp and reference radiations for transformation by ourselves, Frankenberg *et al.* (*1*), and Göggelmann *et al.* (*2*) is presented in Table 7. In all cases the quadratic component (*b*) of the fit to a plot of neoplastic transformation frequency as a function of dose is negligible. In addition, the linear coefficients (*a*) for mammography X rays in this work and that of Frankenberg are close in value, as are the linear components for the reference radiations. Those of Göggelmann *et al.* (*2*) are somewhat higher, although this is partially explained by their normalization of transformation frequency to 30 cells cm^{-2}, rather than 50 cells cm^{-2} as used by ourselves, Frankenberg *et al.* (*1*) and others (*22*). As pointed out by Schmid (*4*), the uncertainties associated with the data of Frankenberg are relatively high, while those for both our data set and that from Göggelmann *et al.* (*2*) are much smaller. In all cases uncertainties associated with the 29 kVp X rays are less than those for the reference radiation, a reflection of the significant quadratic component for the latter.

In our case, the reduction in uncertainty is a result of using a large data set, with the experiments for each dose repeated several times. We observed only small variations between the calculated transformation frequencies at each dose. We also measured transformation frequencies above background for mammography X rays at doses lower than did other workers. Since the linear component of the fit dominates at low doses, using lower-dose data points further reduces the associated uncertainty of the linear part of the fit. We have measured a transformation frequency above background for doses greater than 0.27 Gy. Additionally we have used a combination of two high-energy sources for our reference radiation and have shown these sources to be equivalent in terms of neoplastic transformation potential.

Despite the differences in the values of the radiation response parameters and the different reference radiations used, none of the RBE_M values are significantly different from each other, as is evident from Table 7. A weighted (inversely by the variance) mean of these three data sets gives a value that represents the best estimate available for the RBE_M for neoplastic transformation in CGL1 cells of 4.02 ± 0.72 for 29 kVp X rays compared to high-energy electrons and higher-energy X rays.

Other End Points Considered

Schmid *et al.* compared the potential of monochromatic 17.4 keV X rays to induce chromosome aberrations in human blood lymphocytes with that from a mammography source (*5*). The RBE_M for 200 kVp X rays as the reference radiation for the monochromatic source is approximately half that calculated for the 29 kVp mammography source. This reflects the fact that a mammography source produces a complex spectrum, which, although it peaked at 17.4 keV, does contain a significant proportion of X-ray energies lower than this (*15*). These results seem to support the theory that lower-energy X rays produce clusters of low-energy electrons that have an increased biological effect.

Recently, Slonina *et al.* used micronucleus induction in

human fibroblasts as an end point to measure the RBE of 25 kVp X rays compared to a 200 kVp X-ray source (*23*) and derived an RBE of about 1.3. This is somewhat lower than any of the values reported for neoplastic transformation or chromosome aberrations. There are a number of possible reasons for this observation. First, for the 25 kVp X irradiations, the X-ray beam was filtered with 0.3 mm aluminum and 1.4 mm medium (plus a small thickness of plastic). The resulting X-ray spectrum is quite different from that produced by a clinical mammography unit, with the low-energy components (and thus possibly the more biological effective energies) being removed by this relatively high level of filtration. Second, due to the process of their formation by both LET-sensitive and LET-insensitive mechanisms, micronucleus induction is not necessarily a very sensitive measure of radiation quality. Third, being primary cells, the results may not be comparable to those quoted in this paper.

Two other recent studies have also reported lower RBE values for mammography X rays or for X rays of similar energies. Brenner *et al.* (*24*) reported a comparison of 15.2 keV monoenergetic X rays with ^{137}Cs γ rays for oncogenic transformation in C3H 10T½ mouse fibroblasts. Their preliminary data (these were work-in-progress results) suggest a low-dose RBE of 2.0 ± 0.8. However, based on the data of Schmid *et al.* (*5*) discussed earlier, it seems that the low-energy components present in mammography X rays as opposed to monoenergetic X rays in the energy range 15–18 keV may be responsible for the higher RBEs of mammography X rays. Frankenberg-Schwager *et al.* (*25*) used both mammography X rays (tungsten anode and rhodium filter) and low-filtered 30 kVp X rays to measure mutations in human SV-40 transformed fibroblasts and hamster A_L cells. Responses for mutation data were linearly related to dose for all radiations, and they obtained RBE values of 2.4 ± 0.2 for 30 kVp X rays compared with 200 kVp X rays and 2.7 ± 0.2 for mammography X rays. For cell killing, the RBE values were close to unity. Both these sets of results for mutation are lower than reported by the same group (*1*) for neoplastic transformation in CGL1 cells as discussed above. However, based on a possible RBE of 1.5–2 for 200 kVp X rays compared with radiations with lower LET values, these data may not be different from those reported in this paper.

One significant difference between the low-energy radiations in the reports discussed above and those reported in this paper is that we used a clinical mammography unit having a molybdenum target and molybdenum filter, a radiation source not used in the other studies.

Accurate determination of a value for RBE_M for the survival data presented in this paper is difficult due to the uncertainties involved with measuring cell survival at low doses. Since the relevance of cell survival data to carcinogenesis is questionable, the determination of accurate RBE values for cell survival was not considered as a main objective of this work.

CONCLUSIONS

Neoplastic transformation has been shown to be a reliable and accurate end point to assess the neoplastic potential of both high- and low-energy X rays. We have observed a limiting RBE of 4.42 ± 2.02 when low-energy mammography X rays are compared to high-energy radiation sources, such as that to which survivors of the Nagasaki atomic bomb were exposed. The use of an atomic bomb spectrum simulation as the reference source overcomes difficulties in extrapolating an RBE calculated using a mid-energy X-ray source (e.g. 200 kVp) to a higher-energy source. When pooled with previously published data, a best estimate of the RBE_M for 29 kVp X rays compared with higher-energy X rays and electrons is 4.0 ± 0.7.

This result suggests a need to re-evaluate the risks associated with mammography breast screening. On the basis of the best estimate for RBE_M, the 95% confidence intervals for the increased risks for mammography are 2.7 to 5.3 times that previously assumed. Attributing the same w_R for γ and X rays of all energies should be viewed with caution due to the increasing amount of evidence suggesting a value significantly greater than unity for low-energy X rays.

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