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Interpretation of the Dose and LET Dependence of RBE Values for Lethal Lesions in Yeast Cells

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Survival data on yeast cells proficient or deficient in the repair of DNA double-strand breaks (dsb) and data on the induction of dsb are used to interpret the dose dependence of the RBE value for lethal lesions after irradiation at high dose rate followed by 72-hr liquid holding providing optimum conditions for repair of potentially lethal lesions (RBE_{DP}, DP = delayed plating). The radiations applied are conventional (150 kV), soft (50 kV), and ultrasoft (4 kV) X rays, 30-MeV electrons (or ^{60}Co γ rays), and 3.5-MeV α particles. Analysis shows that the dose dependence of the RBE_{DP} value can be explained by the combination of two dose-independent RBE values, one for the single-particle traversal effect (RBE_{spt}) and the other for the accumulation of dsb (RBE_{dsb}) due to the traversal of more than one particle through the cell nucleus. Furthermore, it is shown that the LET dependence of RBE_{spt} values describing the linear component of the lethal lesions must be considered separately for "electron" and "particle" radiations.

INTRODUCTION

The effectiveness of a given radiation compared to a reference radiation in producing the same level of response is described by the relative biological effectiveness (RBE) and defined as the ratio of absorbed doses of the reference and the test radiation (*1*).

The RBE value may be a function of the absorbed dose depending on the irradiation and postirradiation conditions and the cell system used. Evidence has been presented (*2*) that the capacity of irradiated cells to repair and/or misrepair critical primary lesions may be responsible for the dose dependence of RBE values. It has been shown in yeast cells unable to repair double-strand breaks (e.g., rad52 cells) that DNA double-strand breaks (dsb) seem to be the critical primary lesions induced by ionizing radiation (*3–5*). Therefore one dsb may be regarded as one potentially lethal lesion (PLL) in yeast cells (*6*). Dose-dependent RBE values for reproductive death with either immediate or delayed plating (IP or DP) are observed after irradiation of wild-type yeast cells[1] (*7*) which are proficient in the repair of dsb (*3, 4, 8, 9*) at a high dose rate. For these cells the RBE values are high at low absorbed doses and decrease with increasing absorbed dose. In contrast, the RBE values for reproductive death are dose independent

[1] M. Schäfer, Biophysikalische Untersuchungen der Strahlenwirkungen von 241-Am-Alpha Teilchen an Hefezellen. Thesis, Johann-Wolfgang-Goethe Universität, Frankfurt am Main, FRG, 1973.

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when mutant cells are used which cannot repair dsb (*5*). For these cells there is also no difference observed between the IP and DP survival curves (*10, 11*). At extremely low dose rate and optimum conditions for the repair of dsb during irradiation (*9*) the survival curves of wild-type yeast cells are exponential[1] (*7*). The corresponding RBE values are therefore dose independent.

In the present paper only survival curves of cells irradiated at a high dose rate followed by 72-hr liquid holding providing optimum conditions for the repair of PLL (DP survival curves) were analyzed. The lethality is then exclusively due to the irreparability or misrepair of critical primary lesions (dsb). They are denoted in this paper as lethal lesions. In contrast to DP survival curves, IP survival curves after irradiation at a high dose rate also comprise PLL which become lethal under IP conditions. That this is so is demonstrated by DP experiments which result in a higher survival of irradiated cells.

The RBE values for the induction of dsb are dose independent as demonstrated in several eukaryotic cell systems[2] (*5, 12–14*). However, a dose dependence of the RBE values for the residual dsb is observed when repair of dsb is completed in liquid holding conditions (*15*). After irradiation with ^{60}Co γ rays at low dose rate a virtually linear relationship between residual dsb and dose has been observed (*16*), suggesting that at low dose rate the RBE value for the residual dsb might be dose independent as in the case of survival.

These findings at both the survival and dsb level suggest that the RBE value becomes dose dependent at high dose-rate irradiation when repair and/or misrepair of dsb occurs. In this paper it is shown that the dose dependence of RBE values for lethal lesions in wild-type yeast cells irradiated at high dose rate may be explained by the combination of two dose-independent RBE values: One is correlated to the track structure of a single particle traversing the cell nucleus, the other refers to the interaction of dsb which are accumulated by more than one particle.

The LET dependence of RBE values for lethal lesions induced by heavy particles has been documented for wild-type yeast cells (*17*). In the present paper the RBE values for lethal lesions induced in these cells by conventional, soft, and ultra-soft X rays are presented. These two sets of data make it possible to compare the LET dependence of RBE values for lethal lesions induced by "electron" and "particle" radiations. Using the restricted track-average LET $\bar{L}_{100}$ to roughly characterize the radiation quality it is shown that the $\bar{L}_{100}$ dependence of RBE values for the lethal lesions induced by "electron" radiations comprises a peak between 2 and 5 keV/μm. In contrast, for "particle" radiations a single peak at several hundred kilo-electron volts per micrometer is observed which agrees with the microdosimetric data. Thus, with respect to the LET dependence of RBE values for lethal lesions, a clear distinction should be made between "electron" radiations depositing energy exclusively by electrons and "particle" radiations depositing energy both in the core and by electrons (δ rays).

[2] D. Blöcher, Strahleninduzierte DNA-Doppelstrangbrüche in Ehrlich Ascites Tumorzellen und ihre mögliche Bedeutung für das Zellüberleben. Thesis, Johann-Wolfgang-Goethe Universität, Frankfurt am Main, FRG 1981.

MATERIALS AND METHODS

Yeast cells. For survival studies a diploid wild-type strain of *Saccharomyces cerevisiae* (211) and a mutant homozygous for the rad52 gene (PR 94) were used. In contrast to the strain 211, the rad52 mutant is not capable of repairing dsb and exhibits a high sensitivity to ionizing radiation (*4*). Double-strand break analyses were performed using the diploid strain 211*B auxotrophic for 2'-deoxythymidine-5'-monophosphate (dTMP), which lacks mitochondrial DNA and whose nuclear DNA can be specifically labeled by [^{3}H]dTMP (*18*). This strain is capable of repairing dsb (*9*).

Survival studies and dsb analyses. For all experiments yeast cells in stationary phase were used. Experimental details of the survival studies with strain 211 and strain PR 94 are given elsewhere (*5, 7*). Delayed plating (DP) experiments were performed by keeping irradiated cells for 72 hr in nongrowth medium at 30°C before plating on nutrient agar. Details of the dsb analyses have been described previously (*5, 19*).

Irradiation conditions at high dose rate. Irradiations were performed with 30-MeV electrons, 150-, 50-, and 4-kV X rays, and 3.5-MeV α particles at high dose rates ranging from 20 to 100 Gy/min. The conditions of irradiation with 30-MeV electrons in the presence of oxygen have been described elsewhere (*7, 19*). For irradiations with 150- or 50-kV X rays an X-ray tube with a beryllium window (Philips) filtered by 0.7-mm Al was used. The cells were suspended in phosphate buffer and stirred in the presence of air. Doses were evaluated by a ferrous sulphate method (*20*). Irradiations with 4-kV X rays in the presence of air were performed on filters in the same way as for the 3.5-MeV α-particle exposures as described elsewhere (*5*). Dosimetry of the 4-kV X irradiations was performed with standard ionization chambers.[3]

Irradiation conditions at low dose rate. Cells of the wild-type strain 211 were irradiated with ^{60}Co γ rays, 160- and 4-kV X rays, and 3.5-MeV α particles at the dose rates of 0.27, 0.25, and 0.14, and 0.17 Gy/min, respectively. These dose rates were low enough to avoid the production of lethal lesions by accumulation and interaction of PLL (see Results). For technical reasons ^{60}Co γ rays were used for the low dose-rate exposures instead of 30-MeV electrons. Cobalt-60 rays and 30-MeV electrons exhibit only a small difference in the distribution of the lineal energy y (*21*) which is due to the secondary electrons of kinetic energies higher than about 20 keV (*22, 23*). Hereby y is defined as the quotient of ϵ divided by $\bar{d}$ where ϵ is the energy imparted in a volume during a single energy deposition event and $\bar{d}$ is the mean chord length in the volume (*1*). This difference in the y distributions may lead to a slightly higher yield of lethal lesions due to single-electron traversals when ^{60}Co γ rays are applied instead of 30-MeV electrons. The yield of electrons with energies around 1 keV ("track ends") is the same for both radiations. For the irradiations with ^{60}Co γ rays under aerated conditions cells were suspended in phosphate buffer at 30°C to provide optimum conditions for repair. Dosimetry was performed by calibrating the exposure time with the ferrous sulphate method (*20*).

For the irradiations with 160- and 4-kV X rays, and 3.5 MeV α particles in the presence of air cells were monolayered on filters soaked with buffer and kept at 30°C during irradiation to provide optimum repair conditions. For the 160-kV X-ray exposures dosimetry was performed with a special ferrous sulfate system (*20*) and for the 4-kV X-ray and 3.5 MeV α-particle exposures with ionization chambers.

RESULTS

Survival curves of the rad52 mutant. In Fig. 1 are shown the survival curves of the rad52 mutant after irradiation with 30-MeV electrons, 150-, 50-, and 4-kV X rays, and 3.5-MeV α particles at high dose rates. Exponential survival curves are observed for all radiations used as calculated by regression analysis. The D_0 values and the RBE values for the survival of the rad52 mutant relative to 30-MeV electrons, RBE_{rad52}, are given in Table I. These RBE_{rad52} values observed for the dsb repair-deficient rad52 mutant are dose independent.

Survival curves of wild-type cells after irradiation at high dose rate followed by delayed plating (DP survival curves). Wild-type yeast cells were irradiated and then

[3] A. Binder, Diploma work, Johann-Wolfgang-Goethe Universität, Frankfurt am Main, FRG 1979.

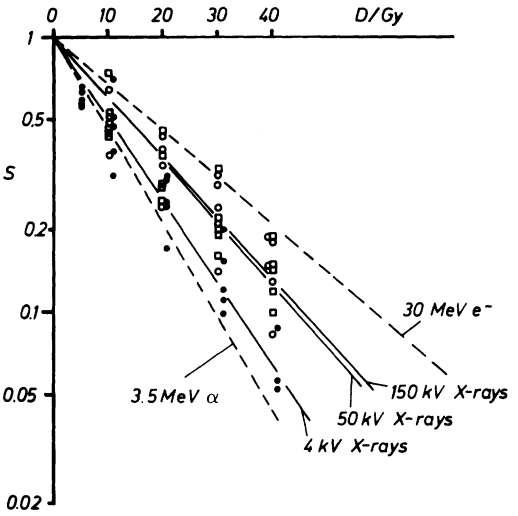

FIG. 1. Survival curves of the rad52 mutant after irradiation with 4- (●), 50- (□), and 150-kV (○) X rays. The best fits for the experimental data were exponential functions with dose (solid lines). For comparison are shown the survival curves after irradiation with 30-MeV electrons and 3.5-MeV α particles (broken lines) as evaluated by regression analysis (5).

allowed to perform maximum repair of PLL (dsb) under nongrowth conditions at 30°C for 72 hr before plating on nutrient agar. The resulting DP survival curves are shown in Fig. 2 for 30-MeV electrons, 150- and 4-kV X rays, and 3.5-MeV α particles. All survival curves are continuously bending. The RBE_{DP} values of 150-kV X rays and 3.5-MeV α particles relative to 30-MeV electrons were found to be dose dependent, whereas a dose-independent RBE_{DP} value was found for 4-kV X rays. The RBE_{DP} values for dsb repair-proficient wild-type cells are presented in Table II for two survival levels, $S_{DP} = 0.9$ and $S_{DP} = 0.1$.

Survival curves of wild-type cells after irradiation at low dose rate. Irradiation of wild-type yeast cells at low dose rates under optimum conditions for repair of PLL (dsb) yields exponential survival curves for ^{60}Co γ rays, 4- and 160-kV X rays, and 3.5-MeV γ particles (Fig. 3). Delayed plating of cells after the low dose-rate exposures did not increase the fraction of surviving cells (results not shown). This demonstrates that all PLL are repaired during these low dose-rate exposures. The RBE values, RBE_{spt}, for the exponential survival curves of wild-type yeast cells after irradiation at low dose rate relative to ^{60}Co γ rays are listed in Table III. They are dose independent.

DISCUSSION

Interpretation of the Dose Dependence of the RBE Value for Lethal Lesions

Comparison of the survival curves of wild-type yeast cells after irradiation at low dose rate (Fig. 3) and at high dose rate followed by DP (Fig. 2) shows that irradiation at sufficiently low dose rate induces lethal lesions by one-particle traversals, whereas at high dose rate additional lethal lesions are observed which are due to the accu-

TABLES I–V

	I^a		II^b RBE_{DP}		III^c	IV^d	V^e
	D_0/Gy	RBE_{rad52}	$S_{DP} = 0.9$	$S_{DP} = 0.1$	RBE_{spt}	RBE_{acc}	RBE_{dsb}
30-MeV electrons	25.5	1	1	1		1	1
^{60}Co γ rays					1		
150-kV X rays	19.4	1.3	6.0	1.3		1.3	1.3
160-kV X rays					8		
50-kV X rays	18.7	1.35	—	—	—	—	1.35
4-kV X rays	14.0	1.8	2.1	2.1	2	2.1	1.8
3.5-MeV α particles	12.8	2.0	25	3.5	25	3.5	2.6

[a] D_0 values and dose-independent RBE values, RBE_{rad52}, of the exponential survival curves of the rad-52 mutant.

[b] Dose dependence of the RBE values, RBE_{DP}, of the DP survival curves of wild-type yeast cells after irradiation with high dose rate. RBE_{DP} values are given for two surviving fractions (S_{DP} = 0.9 and S_{DP} = 0.1).

[c] Dose-independent RBE values, RBE_{spt}, of the exponential survival curves of wild-type yeast cells after irradiation at low dose rate.

[d] Dose-independent RBE values, RBE_{acc}, for the accumulative component S^* of the DP survival curves of wild-type yeast cells irradiated at high dose rate.

[e] RBE values, RBE_{dsb}, for the induction of dsb by different radiations.

mulation of PLL which interact to yield lethal lesions (7). Therefore, the DP survival curves obtained at high dose rate can be described by

$$S_{DP} = S_{spt} \cdot S^* \tag{1}$$

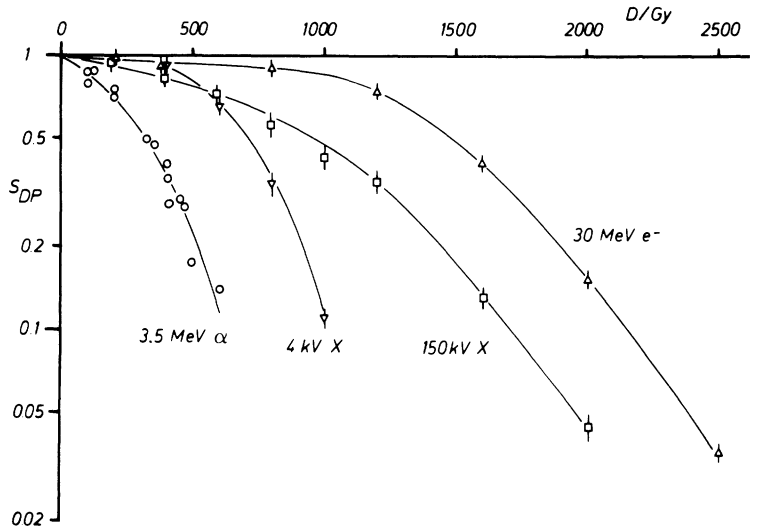

FIG. 2. Survival curves with delayed plating, S_{DP}, for wild-type yeast cells after irradiation with different radiations. Data for 3.5-MeV α particles are from Ref. (17).

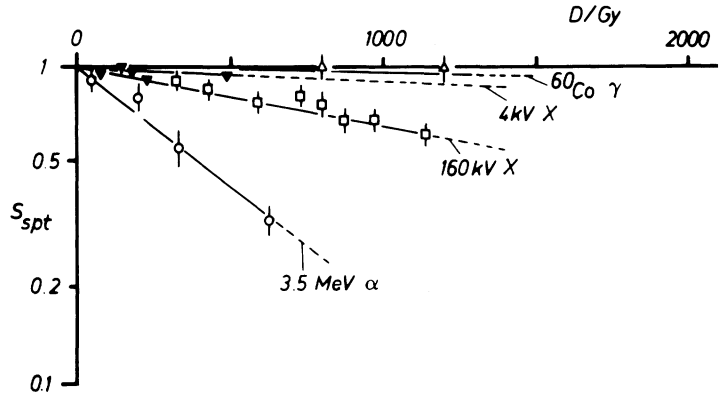

FIG. 3. Exponential survival curves, S_{spt}, of wild-type yeast cells after irradiation at very low dose rate. Data for 160-kV X rays and 3.5-MeV α particles are from Ref. (*17*) and for 4-kV X rays from Binder (private communication).

where

$$S_{spt} = \exp(-k_{spt} \cdot D) \tag{2}$$

represents the exponential survival curve at low dose rate with k_{spt} as the inactivation coefficient and S^* as the survival curve due to the accumulation of PLL which interact to yield lethal lesions at high dose-rate irradiations. S^* cannot be measured directly but can be calculated using Eq. (1) from the experimentally determined survival curves S_{DP} (Fig. 2) and S_{spt} (Fig. 3). These calculated survival curves S^* ($k_{acc} \cdot D$), are shown in Fig. 4 for different radiations. These S^* curves can be transformed into the S^* curve for 30-MeV electrons (within the experimental error represented by the hatched area in Fig. 4) by multiplying the doses with the dose-independent RBE_{acc} value given in Table IV for the various radiations. The result of this analysis suggests that the dose dependence of RBE values for lethal lesions, RBE_{DP}, may be explained by the combination of two dose-independent RBE values, namely the RBE_{spt} value for lethal lesions induced by single-particle traversals and the RBE_{acc} value for lethal lesions due to the accumulation of PLL induced by more than one particle traversal. The DP survival curve of a radiation T, $S_{DP,T}$, can therefore be derived from the S_{spt} and S^* survival curves of a reference radiation R by multiplying the expression

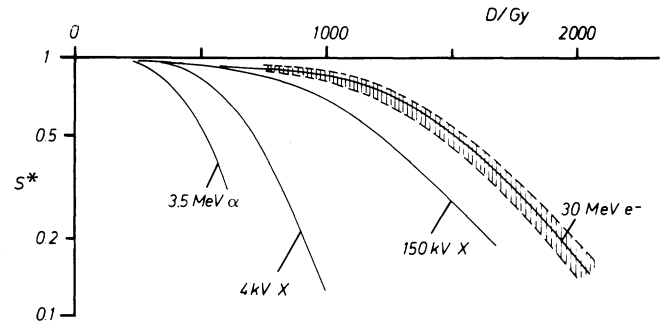

FIG. 4. Calculated survival curves, S^*, which are exclusively due to the accumulation of PLL to form lethal lesions at high dose-rate irradiation. For explanation see discussion.

$k_{spt,R} \cdot D_R$ [see Eq. (2)] and $k_{acc,R} \cdot D_R$ with the dose-independent RBE values $RBE_{spt,T}$ and $RBE_{acc,T}$, respectively.

$$S_{DP,T} = \exp(-RBE_{spt,T} \cdot k_{spt,R} \cdot D_R) \cdot S^*(RBE_{acc,T} \cdot k_{acc,R} \cdot D_R). \quad (3)$$

The product $k_{acc,R} \cdot D_R$ represents the mean number of PLL per cell induced by the absorbed dose D_R. The analytical form of S^* is unknown (*15*).

The induction of dsb in yeast cells is linear with dose up to at least 2400 Gy (*19*). Thus it can be concluded that a dsb is produced by a single particle traversal through the cell nucleus rather than by two-particle traversals. Considering the ionization density or the distances between nearest neighbors of ionizations as a function of electron energy, electrons with kinetic energies between some hundred electron volts and about 1.5 keV produce about 5 to 50 ionizations within a volume of only 10- to 20-nm chord length (*24*) and may therefore be relevant for the induction of dsb. The induction of dsb by a single-particle traversal may be due to one of three possibilities: (a) a direct effect, i.e., single-strand breaks (ssb) in each of the two DNA strands are formed simultaneously by a single traversing electron; (b) a direct induction of one ssb plus a simultaneous formation of a ssb in the opposite strand by a $OH^\bullet$ (indirect effect) when this $OH^\bullet$ is produced by the same traversing electron; (c) the $OH^\bullet$-mediated formation of both ssb in opposing positions of the two DNA strands (indirect effect) when both $OH^\bullet$ are produced by the same traversing electron. The relative contribution of the mechanisms a, b, and c to the induction of dsb is unknown and not important for the considerations presented here.

Studies with 30-MeV electrons under both oxic and anoxic conditions have demonstrated that one to two dsb per cell correspond to a lethal event in the rad52 mutant which is not able to repair dsb (*5*). It can be shown by microdosimetric considerations that for electron radiations it is one dsb rather than two dsb per cell per lethal event (*15*). Since dsb cannot be repaired in the rad52 mutant the RBE_{rad52} values of the X rays used can be considered as the RBE values for the induction of dsb. However, this does not hold true for densely ionizing radiations. For example, the energy which can be deposited by a 3.5-MeV α particle traversing the cell nucleus amounts in the mean up to 80 keV corresponding to 13 Gy per cell nucleus. Since only 7 Gy are needed to induce in the mean one dsb per cell (*5*) more than one dsb is produced by a 3.5-MeV α particle traversing the nucleus. Therefore, the RBE_{rad52} for 3.5-MeV α particles cannot be taken as the RBE value for the induction of dsb. Instead, this RBE value must be determined directly and has been found to be 2.6 (*5*). The RBE values for the induction of dsb (RBE_{dsb}) for the radiations used are listed in Table V. The agreement between the dose-independent RBE_{dsb} values and the dose-independent RBE_{acc} (Table IV) for the accumulative component of the DP survival curves suggests that in addition to those lethal lesions produced by a single-particle traversal (intratrack effect) lethal lesions are formed at high dose rate which are due to the accumulation of dsb induced by more than one particle traversing the cell nucleus (intertrack effect; see Fig. 5c). Since the high dose-rate irradiations are performed at 4°C repair and/or misrepair of dsb during irradiation is unlikely under such irradiation conditions, dsb are accumulated resulting in a high probability for intertrack effects. This is in contrast to low dose-rate irradiations providing optimum dsb repair conditions which result in intratrack effects exclusively. At the moment,

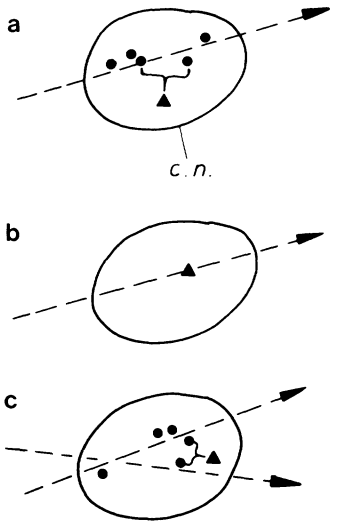

FIG. 5. Induction of lethal lesions. (a) Production of a lethal lesion (▲) by a single ionizing particle (→) traversing the cell nucleus (c.n.) due to the interaction between two dsb (●) produced by this particle (intratrack effect). (b) Production of a lethal lesion by a single traversing particle without interaction of dsb. For details, see Discussion. (c) Occurrence of a lethal lesion because of the interaction between two dsb (●) induced by different particles traversing the cell nucleus (intertrack effect).

for yeast cells no data are available to determine interaction distances between accumulated dsb leading to chromosome aberrations via misrepair (*15*). However, the analysis of split-dose (*25, 26*) or low dose-rate (*27*) irradiations of human lymphocytes suggests that long-range intertrack interactions take hours to be completed and primary lesions (i.e., dsb) initially separated by distances of the order of 100 nm may interact (*28*).

The lethal lesions induced by single-particle traversals through the cell nucleus may be attributed to at least two mechanisms (Fig. 5a,b): (a) Interaction between two dsb produced by a single particle resulting in a lethal lesion by misrepair of dsb. (b) Production of a lethal lesion without interaction between dsb. For example, a chromosome deletion may be produced by a simultaneous massive destruction of the histones at the site of the DNA dsb.

Interpretation of the LET Dependence of the RBE Value for Lethal Lesions

Electron radiations. In Fig. 6a are presented the RBE_{spt} values of the low dose-rate survival curves after irradiation with 160-kV X rays ($\bar{L}_{100} \approx 2$ keV/μm) and 4-kV X rays ($\bar{L}_{100} \approx 13$ keV/μm) relative to ^{60}Co γ rays ($\bar{L}_{100} \approx 0.2$ keV/μm) as a function of $\bar{L}_{100}$ (track-average LET $\bar{L}_{\Delta}$ with cut-off energy $\Delta = 100$ eV) as a rough measure of radiation quality. $\bar{L}_{100}$ was chosen because LET values of most radiation types are available in terms of $\bar{L}_{100}$. Use of another cut-off energy Δ or use of the dose-average LET, $\bar{L}_{\Delta,D}$, with any cut-off energy Δ would also show the principal feature of the LET dependence of the RBE values for lethal lesions. The $\bar{L}_{100}$ values for ^{60}Co γ rays and 160-kV X rays were taken from (*29*). The $\bar{L}_{100}$ value for 4-kV X rays was determined on the basis of the photon spectrum[3] and mass stopping power values

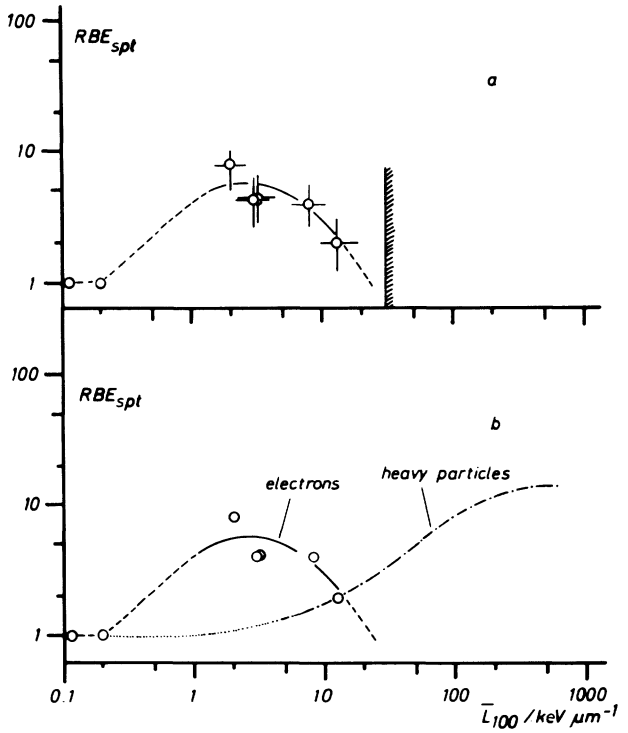

FIG. 6. RBE value for survival as a function of $\bar{L}_{100}$. (a) Electron radiations. Starting with the two reference radiations (30-MeV electrons and ^{60}Co γ rays) the RBE_{spt} values are presented for 160-, 70-, 30-, 10-, and 4-kV X rays. The vertical line at about 30 keV/μm represents the upper limit of $\bar{L}_{100}$ values for electrons. (b) Particle radiations. RBE_{spt} values for heavy particles (· · ·-·-·-·). The dotted line below 5 keV/μm reflects the suggested course of $\bar{L}_{100}$ and for values higher than 5 keV/μm the dashed-dot line suggests the course of $\bar{L}_{100}$—values as evaluated by Ref. (*37*).

(*29*). Also given are the RBE_{spt} values for 70- ($\bar{L}_{100} \approx 3$ keV/μm), 30- ($\bar{L}_{100} \approx 3$ keV/μm), and 10-kV X rays ($\bar{L}_{100} \approx 8$ keV/μm) relative to 30-MeV electrons ($\bar{L}_{100} \approx 0.1$ keV/μm). The $\bar{L}_{100}$ value for 70-kV X rays was obtained by interpolation of the $\bar{L}_{100}$ values for 200- and 50-kV X rays [see Table I in (*29*)]; for 30- and 10-kV X rays the $\bar{L}_{100}$ values were taken from Virsik *et al.* (*28*). These latter RBE_{spt} values are derived from the corresponding DP survival curves (*30*) by modifying Eq. (1) to yield Eq. (4).

$$-\frac{\ln S_{DP}}{D} = k_{spt} - \frac{\ln S^*}{D}. \tag{4}$$

Extrapolation to zero dose yields the value of k_{spt} from which RBE_{spt} values can be determined. The linear component of lethal effects as expressed by the RBE_{spt} values increases with increasing $\bar{L}_{100}$ up to the $\bar{L}_{100}$ region of standard X rays, forming a peak at $\bar{L}_{100}$ values of about 2 to 5 keV/μm. In a previous paper (*7*) it was shown by microdosimetric analysis that the increase of RBE_{spt} is due to the relative increase of electrons with energies between about 2 and 30 keV which may produce several slow electrons with kinetic energies between several hundred electron volts and about

1.5 keV per traversal through the cell nucleus. Thus more than one dsb may be induced per single-electron traversal which may then interact with each other, resulting in misrepaired DNA molecules (Fig. 5a). The decrease of the RBE_{spt} values despite the further increase of $\bar{L}_{100}$ is caused by the small amount of energy which can be deposited in the cell nucleus by one electron. The RBE_{spt} value approaches zero for the $\bar{L}_{100}$ value of about 30 keV/μm since the corresponding electrons have energies lower than 100 eV and are therefore not able to induce more than one dsb. An increased inactivation of mammalian cells (*31, 32*) and the increased yield of chromosome aberrations (*33–36*) observed after irradiation with conventional X rays relative to ^{60}Co γ rays agrees with the findings presented above.

Particle radiations. The $\bar{L}_{100}$ dependence for the linear component of lethal lesions (α term) observed in DP experiments with wild-type yeast strain 211 has been presented by Bertsche and Lachet (*37*) for various "particle" radiations. The α term shows a peak at about 2 keV/μm and another peak at several hundred kilo-electron volts per micrometer. The peak at 2 keV/μm was found for 15-MeV protons, whereas 9- and 20-MeV protons do not contribute to this peak. This result seems to be incompatible with the radiation physics of protons of energies between 9 and 20 MeV. The core of the proton tracks cannot be responsible for such a peak at 2 keV/μm since if this were the case the α term should increase continuously for protons with decreasing energy from 20 MeV down to 9 MeV because of the *continuous* increase of the corresponding $\bar{L}_{100}$ values from 1.5 to 2.0 to 2.6 keV/μm. Likewise, the δ rays of the 15-MeV protons cannot account for this peak at 2 keV/μm because the fraction of energy which is deposited within the cell nucleus by track ends (i.e., electrons with energies between several hundred electron volts and 1.5 keV) per proton traversal increases *continuously* with decreasing proton energy of 20 MeV down to 9 MeV.

The probability is very low that a proton with an energy in the range of 9 to 20 MeV can produce δ rays with track ends separated by less than 1 μm. Thus such a proton cannot act as a vehicle to transport more than one track end into a cell nucleus with a diameter of 1 μm. On the other hand, "electron" radiations provide electrons with energies between 2 and 30 keV which may act as such a vehicle, resulting in a peak for the production of lethal lesions by a one-particle traversal at $\bar{L}_{100}$ values of about 2 to 5 keV/μm (see Fig. 6a). Therefore, the probability of producing lethal lesions in yeast cells by a traversal of a proton with an energy between 9 and 20 MeV should not differ much from that of ^{60}Co γ rays which deposit less than 5% of the absorbed dose by such vehicle electrons. In contrast, conventional X rays deposit at least 30% of the absorbed dose by those vehicle electrons.

The microdosimetric considerations outlined above have been taken into account to construct the $\bar{L}_{100}$ dependence of RBE_{spt} values for the linear component of lethal effects in yeast cells irradiated with heavy particles (Fig. 6b). The experimental data for the particle irradiations are taken from Ref. (*37*) with the exception of the data for 15-MeV protons. RBE_{spt} values for the various particle radiations are obtained by dividing the linear component (α term) of the linear-quadratic fit [$S_{DP} = \exp(-\alpha D - \beta D^2)$] by the α term for irradiation with 30-MeV electrons. A continuous increase of the RBE_{spt} values of the linear component of lethal effects as a function of $\bar{L}_{100}$ is observed up to a peak at $\bar{L}_{100}$ values of several hundred kilo-electron volts per micrometer. The results presented in Figs. 6a and b show that "electron" radiations which deposit the energy by electrons show a peak for the linear component of lethal

lesions exclusively between 2 and 5 keV/μm, whereas a peak at several hundred kiloelectron volts per micrometer is observed for particle radiations which deposit their energy both within the core of a track and by electrons (δ rays).

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