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The Response of Diploid Yeast to Radiations at Different LET

I. Potentially Lethal and Lethal Damage to Reproductive Capacity

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Stationary populations of diploid yeast (*Saccharomyces cerevisiae*, wild type, strain No. 211) were irradiated with sparsely and densely ionizing radiations at high and in part at low dose rates. Mean LET values ranged from 0.1 to 600 keV/μm. The reproductive capacity of the cells was measured by macrocolony counting. After irradiation at high dose rates, agar-holding reactivation was allowed for 48 hr. Dose–effect curves showed a change from sigmoidal to exponential form with increasing LET and a maximum of the slope of the exponential part at about 70 keV/μm. If the radiation response at higher doses is separated into contributions of potentially lethal and of lethal damage, one finds different dependence on the mean LET. The observed lethal damage production is discussed in terms of single-hit events expected from theoretical considerations.

INTRODUCTION

It is generally accepted that potentially lethal damage (PLD) contributes to the radiation response of living cells irradiated with ionizing radiations at high dose rates and incubated shortly after irradiation in a medium promoting growth. Thus, the dose–effect curves (DEC) measured in such experiments are caused by lethal damage (LD) and that portion of PLD which will become lethal when the cells are forced to divide shortly after irradiation. If, however, the cells are allowed to stay for a certain time in a medium which is free of nutrients or which inhibits growth, then reversion of PLD can occur, and the viability of the cells, measured by macrocolony counting, increases in reference to samples incubated immediately in a medium promoting growth. This phenomenon was observed in bacteria and higher plant cells (*1*, *2*) as well as in mammalian cells (*3*, *4*), although with different time constants which are about 10 times lower for mammalian cells (*5*, *6*). From extensive studies of some physiological parameters which can influence PLD reversion in yeast cells, it was concluded that this repair process is of enzymatic nature (*7*, *8*).

From a survey of published data it seems that uncertainty exists about the

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influence of PLD and the relevance of PLD-repair after low- and high-LET irradiations. For example, Lyman and Haynes reported that the magnitude of liquid-holding reactivation (LHR) is independent of LET (*9*). With another yeast strain, however, the rate and extent of LHR was found to be greater for γ-ray-induced damage than for α-particle-induced damage (*10*). Furthermore, no extensive information is available about the amount of PLD and LD produced by radiations with different LET.

It was of interest, therefore, to carry out a systematic study of PLD and LD produced over a LET range which included the low values, around 0.1 keV/μm, of fast electrons as well as the high values of heavy ions, around 100 keV/μm.

The diploid yeast (*Saccharomyces cerevisiae*) strain No. 211 was chosen as the object of our studies because of the extensive knowledge available about certain physiological parameters which influence its growth and LHR (*8*, *11*, *12*) and because it serves as a simple model system for theoretical studies on the radiation effect (*13*). A further advantage of this cell type is given by the possibility of extracting complete DNA molecules from the cell nucleus, so that investigations bearing on the dose-dependent effects of radiation on this important molecule are possible.

MATERIALS AND METHODS

A. Cells and Survival Assay

Diploid yeast cells (*Saccharomyces cerevisiae*) of the homozygous strain No. 211 were used. Cultures in stationary state, containing about 97% G1-phase cells, were prepared for the irradiation experiments in the following reproducible way: from a liquid culture of these cells a sample with 10^6 cells/ml was distributed homogeneously on solid nutrient agar (20 g/liter glucose, 20 g/liter Bacto agar (Difco), 5 g/liter yeast extract; thickness of agar plate, 5 mm). When incubated at 30°C, cell proliferation ceased due to the reduced glucose flux limited by diffusion (*14*). Maintenance energy metabolism was guaranteed for several weeks without loss of proliferation and of repair capabilities, if the cells were kept at 6°C. Before each experiment the attainment of stationary state was checked by volume spectroscopy of the population with a volume spectrometer (Telefunken–Coulter Electronics). Figure 1a shows a growth curve of yeast. The stationary state with no further growth was reached in about 2 days after inoculation. Usually samples for irradiation were taken after 4 days and the volume distribution of the cells measured (Fig. 1b). The Gaussian-like curves (broken lines) in this figure indicate the subdivisions which correspond to different cell generations in the population. About 55% of these cells are daughter cells with one scar and with a mean volume $\bar{V} = 25\ \mu m^3$.

For irradiation at low doses where one observes a dose-dependent change of radiosensitivity in the DEC, it becomes necessary to use synchronized populations. Therefore, we prepared stationary daughter cells by centrifugation in a linear density gradient consisting of Ficoll 400 (Pharmacia Fine Chemicals). From this, samples with more than 95% daughter cells were obtained (as measured by volume distribution).[1] Due to the fact that densely ionizing particles

[1] M. Nuesse, *Methoden zur Gewinnung synchroner Hefezellen durch Selektion und ihre Grenzen.* Doctoral thesis, University of Frankfurt/Main, 1973.

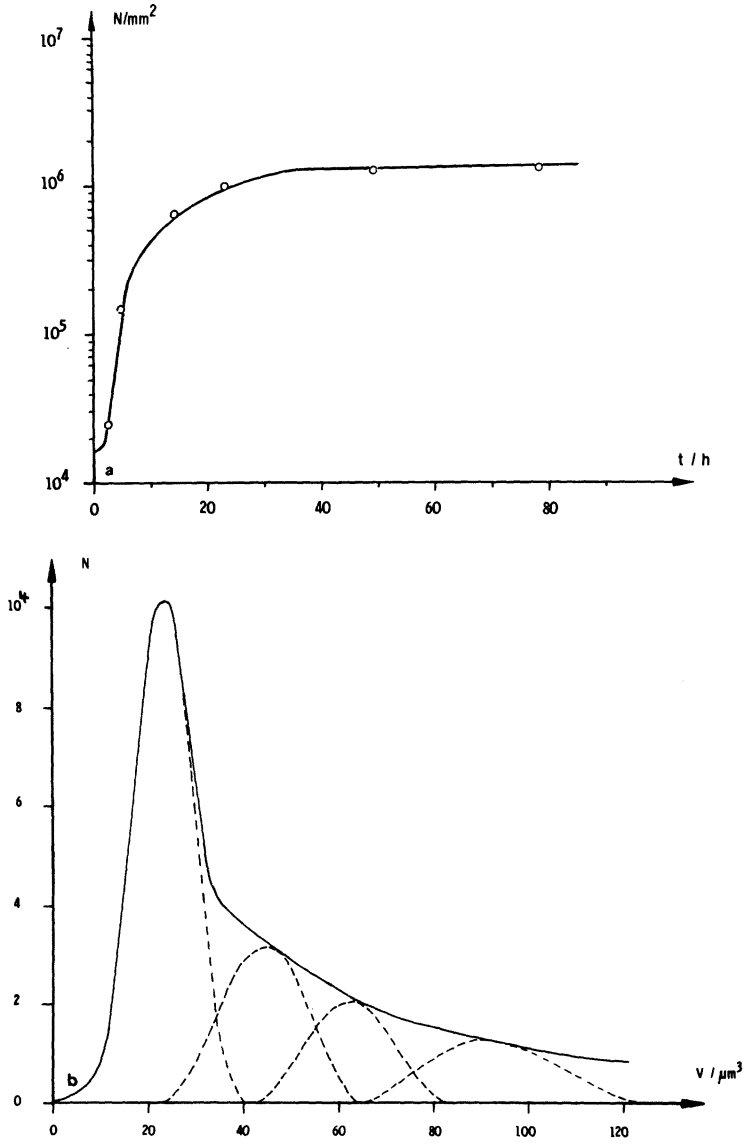

FIG. 1. Growth characteristics of diploid yeast strain No. 211. (a) Number of cells grown on solid agar with nutrients as a function of time. Stationary state is reached 2 days after inoculation. (b) Volume distribution of stationary cells grown on solid nutrient agar, as measured by a volume spectrometer. Broken lines indicate the different generations of cells in the population (daughter cells with one scar, mean volume $\bar{V} = 25\ \mu m^3$; mother cells with two scars, $\bar{V} = 45\ \mu m^3$, etc.).

have relatively short ranges, cell monolayers were used in all experiments. Monolayers were produced by placing a 50-μl sample of suspended cells (3×10^6 cells/ml) on a Millipore filter (type AAWP, 0.45-μm pore size, 20-mm diameter) and allowing the cells to settle. The filters were then placed in target holders containing wet disks which guaranteed sufficient humidity in the layer throughout the irradiation time.

After irradiation, appropriately diluted 1-ml samples were plated on nutrient agar dishes to give about 150 colonies per plate (plating efficiency, PE = 100%). The surviving fraction S of the irradiated sample was determined relative to an unirradiated control sample otherwise handled in the same way.

In order to allow the cell to recover from PLD, the so-called "agar-holding technique" was applied: a 1-ml sample of the irradiated and resuspended cells was plated on each of four agar dishes which contained no nutrients (agar-holding reactivation, AHR). These dishes were incubated at 30°C under aerobic conditions for a certain time interval, t_{rep}, and were then covered with 42°C liquid nutrient agar. The advantage of this technique over liquid holding, in which the cells are suspended in liquid buffer (Liquid-Holding Reactivation, LHR), lies in the fact that uncontrolled cellular multiplication during t_{rep} does not influence the measured value. In most control experiments, however, no difference was found in the extent of repair of cell samples handled by AHR and by LHR techniques.

As can be seen in Fig. 2, there was an increase of the survival S with t_{rep}, until saturation occurred within about 40 hr. Therefore, in most experiments, only the number N of macrocolonies after 48 hr of agar holding was determined. This period guaranteed, even for high doses of high-LET irradiations, a reversion of PLD up to the saturation value.

Irradiations with X rays and low-energy α particles with doses of up to 1500 Gy showed a reproducible exponential DEC beyond the shoulder. DECs were obtained therefore by a visual fitting of the measured S values in the low-dose range and by computerized least square fit to an exponential function in the

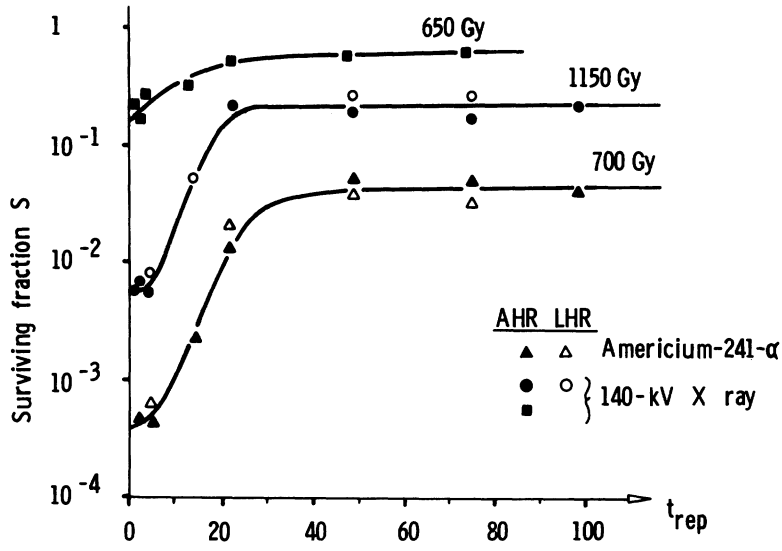

FIG. 2. Surviving fraction of yeast cells as a function of time (in hours), t_{rep}, held on nutrient-free agar (agar-holding reactivation, AHR), respectively, in phosphate buffer (liquid-holding reactivation, LHR) at 30°C, when irradiated with 140-kV X rays and 3-MeV α particles from an americium-241 source. Absorbed dose indicated for each curve.

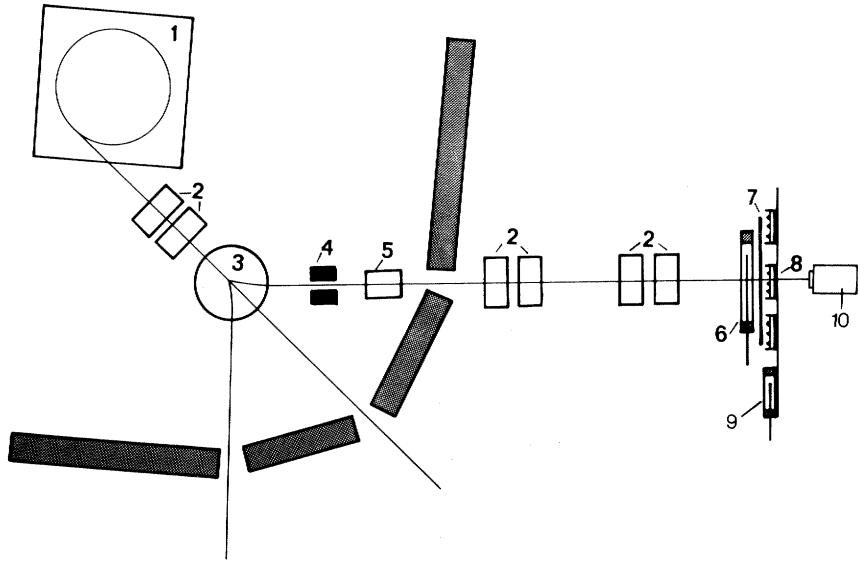

FIG. 3. Schematic drawing of the experimental setup for irradiation with ions at the cyclotron. (1) fixed energy cyclotron, (2) quadrupole lenses, (3) analyzing magnet, (4) beam aperture, (5) beam steerer, (6) transmission type ionization chamber, (7) aluminum foil for energy reduction, (8) remote controlled sample changer, (9) ionization chamber for determination of absorbed dose at target position, and (10) TV camera.

high-dose range. From these fitted curves the shoulder width, D_Q (i.e., the intersection of the exponential function with the dose coordinate in a semilogarithmic plot of survival S against absorbed dose D), and the exponential slope, $m = d(\ln S)/dD$, were determined.[2]

B. Irradiations

Six different sources of ionizing radiations were used:

(a) a cobalt-60 γ source (University of Frankfurt) with a dose rate of 0.4 Gy/min at a 60-cm distance from the source as measured by chemical dosimetry (see below);

(b) three X-ray tubes with varying voltages and dose rates: (b1) 70 kV, 700 Gy/min; (b2) 140 kV, 40 Gy/min; (b3) 160 kV, 0.20 Gy/min (Gesellschaft für Strahlen- und Umweltforschung, Frankfurt);

(c) an electron accelerator (Betatron, Gesellschaft für Strahlen- und Umweltforschung, Frankfurt) producing electrons of 30-MeV energy and a dose rate of about 40 Gy/min at a 50-cm distance from the source, as measured by ionization chambers (see below);

(d) a cyclotron (Deutsches Krebsforschungszentrum, Heidelberg) which delivered protons of 21 MeV, deuterons of 11 MeV, helium-3 of 28 MeV, and

[2] When discussing variations in m, the numerical values, $|m|$, are compared; thus, a steeper negative slope is referred to as having a greater m than a shallower one.

helium-4 of 22 MeV energy; the beam currents ranged from 0.3 to 3 μA, after a slit and thus gave dose rates of 10 to 100 Gy/min, as measured by ionization chambers;

(e) two radioactive americium-241 α sources mounted in an irradiation box at a fixed distance from the sample; their activities, mean residual energies at exposure position, and dose rates were: (e1) 1.05 mCi, 3 MeV, 45 Gy/min; (e2) 2.05 μCi, 3.5 MeV, 0.14 Gy/min;

(f) a heavy-ion accelerator (Tandem-Van-de-Graaf, Max-Planck-Institut für Kernphysik, Heidelberg) with maximum energy of 11 MeV per charge unit. Here oxygen-16 ions of 100 MeV energy, delivering a dose rate of about 400 Gy/min, were used.

In the described experiments, oxygen-16 ions of 100-MeV energy, delivering a dose rate of about 400 Gy/min, were used.

The experimental setup for irradiation and measurement of absorbed dose at the cyclotron is shown in Fig. 3 which is in general representive also for the setup at the betatron and heavy ion accelerator where parallel, monoenergetic beams were produced. The beam from the cyclotron [(1) in Fig. 3] was deflected by an analyzing magnet (3) to ensure homogeneity of particle momenta and focused on the target (8) by magnetic quadrupole lenses (2). To get a qualitative impression of the intensity distribution in the beam's cross section, the fluorescent light of a thin foil covered with zincsulfite (ZnS) was observed with a TV camera (10)

TABLE I

Physical Characteristics of the Radiations Used in Irradiation Experiments with Yeast 211[a]

Radiation	E_i (*MeV*)	E_f (*MeV*)	S_m (*MeV* $\times$ *cm*^{2} $\times$ *g*$^{-1}$)	$\bar{L}_{100}$ (*keV* $\times$ μm^{-1})
Electrons	30	30	2.8*	0.1*
Cobalt-60 γ rays	1.2	1.2	—	0.2*
Protons	21	20.2	26	1.6
Protons	21	8.7	51	3.2
X rays	140 kV and 160 kV		—	$\approx$2
X rays	70 kV		—	$\approx$3
Deuterons	11	7.0	105	6.5
Deuterons	11	3.5	180	11
Helium-3	28	24	220	15
Helium-3	28	14	330	21
Helium-4	22	15	390	24
Helium-4	22	6	770	48
Americium-241	5.5	3.5	1100	75
Americium-241	5.5	3.1	1200	80
Americium-241	5.5	2.0	1500	100
Oxygen-16	100	50	6100	380
Oxygen-16	100	20	9500	600

[a] Values E_f, S_m and L_{100} have been calculated with a formula from Bichsel (*12*). Values indicated by an asterisk were taken from ICRU Report No. 16; Linear Energy Transfer. ICRU, Washington, 1970.

at the back of the second ionization chamber (9). However, quantitative measurements were carried out also, using a slit which was moved in the horizontal and vertical direction over the target area. From this it was found that the beam intensity was distributed nearly homogeneously over an area of a 6-mm diameter (variations amounted to about 5%).

A transmission-type ionization chamber in fixed position (6) served for beam monitoring and an identical chamber (9) for determination of absorbed dose D at layer position. The calibration of these chambers was carried out with a standard ionization chamber and with a ferrous sulfate dosimeter (*15*).

The determination of dose rates of the americium-241 α sources was performed with one of these flat ionization chambers.

Dosimetry in X- and γ-ray irradiation experiments was performed with a special ferrous sulfate dosimeter (*16*).

In Table I the initial beam energy, E_i, and the energy, E_f, at target position are noted with mass stopping power S_m and mean restricted LET $\bar{L}_{100}$ (where the index denotes the cutoff level $\Delta = 100$ eV for δ-ray energies) in yeast.

RESULTS AND DISCUSSION

The results of irradiation experiments are given as DECs for immediate and delayed plating. The analysis of DECs was done in two different ways: first, a more formal characterization using D_Q and m values (Materials and Methods) and, second, a semiempirical characterization based on some common assumptions about radiation effects (which are mentioned below).

A. Immediate Plating Effects

In Fig. 4 the surviving fraction S of cells is shown after X and fast-electron irradiation. The shapes of the DECs are similar to those obtained after proton

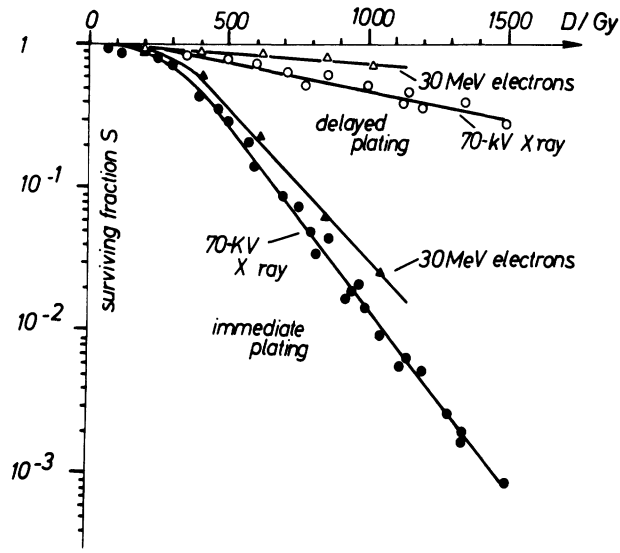

FIG. 4. Dose–effect curves following irradiations of whole populations of yeast in stationary state with 30-MeV electrons and 70-kV X rays at high dose rates (above 10 Gy/min).

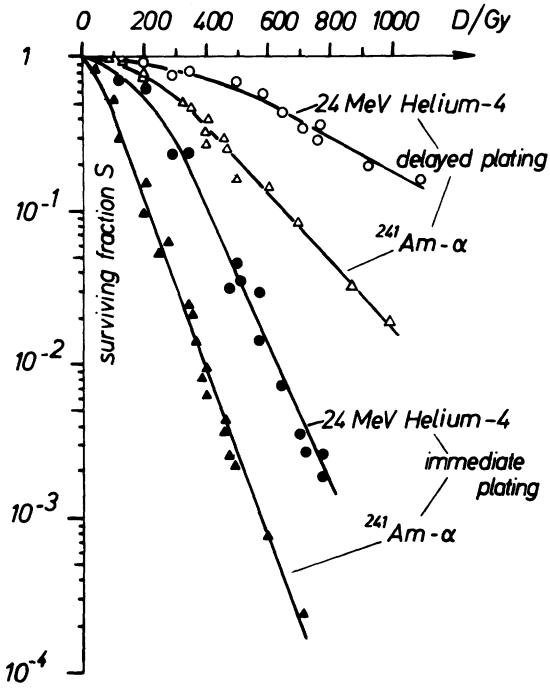

FIG. 5. Dose–effect curves following irradiations with 24-MeV helium-3 ions and 3-MeV americium-241 α particles at high dose rates.

and deuteron irradiations with 10 to 20 MeV energy (*17*). In this LET range, from about 0.1 to 10 keV/μm, the DEC observed for yeast 211 consists of a shoulder with D_Q around 300 Gy, followed by a region with exponential slope of about $m = 5 \times 10^{-3}$ Gy^{-1} (or $D_0 = 1/m = 200$ Gy).

Figure 5 shows the DECs following irradiation with α particles of 24- and 3-MeV energies. The results with other α energies (see Table I) are not plotted here, because they fall at points intermediate between those in Fig. 5. In Fig. 6 the effects of oxygen-16 ion irradiation are given.

Obviously, the parameters D_Q and m of the DEC change within the LET range. At high LET, above 100 keV/μm, the shoulder of the DEC disappeared (Fig. 7), whereas the slope passed a maximum at about 70 keV/μm (Fig. 8). A similar dependence of radiation response on LET was observed in earlier investigations (*18, 19*).

From Figs. 7 and 8, the impression is gained that D_Q and m are related to different reactions in the cell because of the different dependence on LET. Indeed, it has been shown that the shoulder of X-irradiated yeast 211 is due to at least two radiation-induced processes in the cell, one of which is ATP-dependent (*20*), while the other can be influenced by the radical spectrum in the cell (Frankenberg, private communication). However, we found also a change of D_Q by delayed plating. Thus, it seems likely that sublethal damage as well as some sort of potentially lethal damage contributes to the shoulder, and that

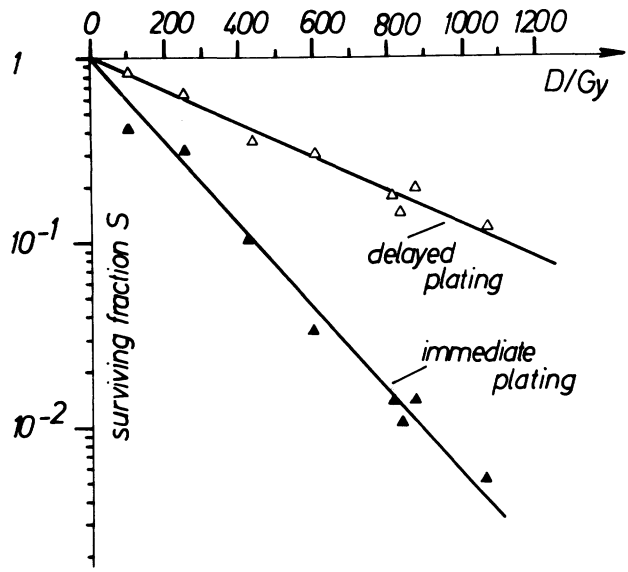

FIG. 6. Dose–effect curves following irradiations with 50-MeV oxygen-16 ions at high dose rates.

different repair mechanisms remove some damage during the "lag time" of immediate plating, whereas the exponential slope is due to PLD and LD alone, as we shall demonstrate later on.

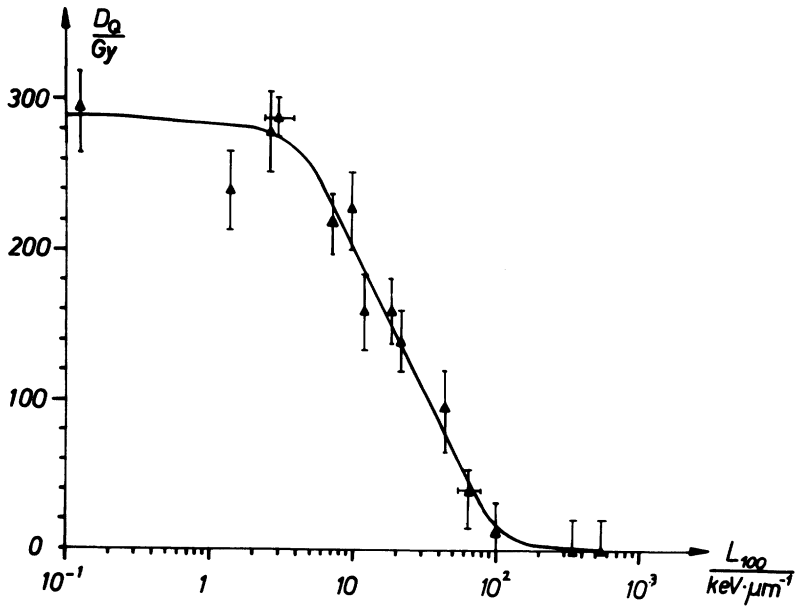

FIG. 7. Shoulder width, D_Q, of dose–effect curves following irradiations at high dose rates and for various LET (immediate plating effect).

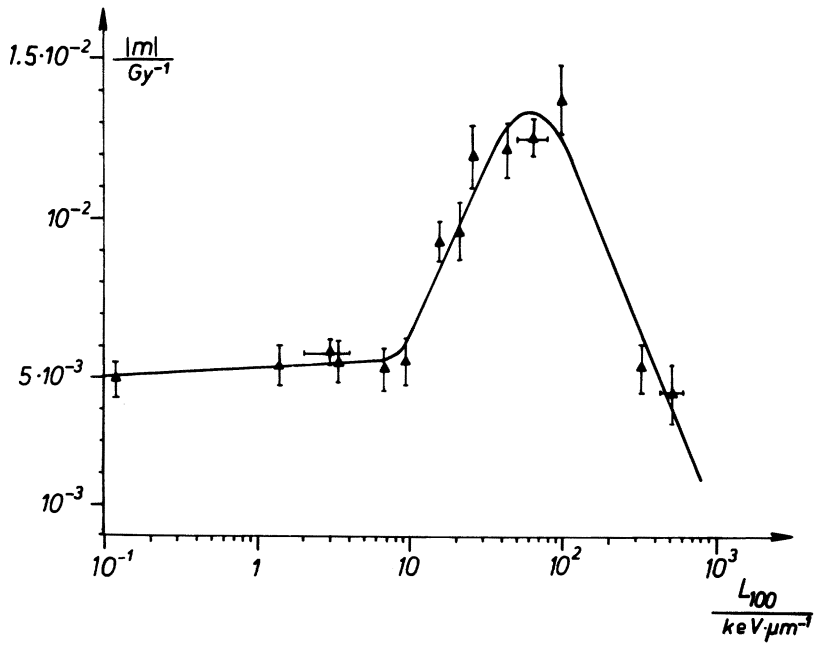

FIG. 8. Slope, m, of the exponential part of dose–effect curve following irradiations at high dose rates and for various LET (immediate plating effect).

B. Delayed Plating

Measurements of the surviving fraction S after delayed (48 hr) plating on nutrient agar are shown in Figs. 4, 5, and 6 together with the results of immediate plating. Again, in most cases, both a shoulder and an exponential region at higher doses are observed. From these results it becomes apparent that the delayed plating effect was generally not dose-modifying in relation to the effect of immediate plating.

Because delayed plating changes slope values from m to m' with $m > m'$,[2] a factor $F = m/m'$ is introduced which denotes the change in the amount of damage and, thus, the efficiency of agar holding. In practical considerations, however, one usually describes the effect of delayed plating by the quotient of doses at a certain survival level (*9*). This method has the disadvantage of including two effects, the "shoulder effect" and the effect in the exponential region, whose relationship to each other is not well understood.

Figure 9 shows the dependence of F on L_{100}, from which a reduction of the efficiency of AHR with increasing LET becomes obvious.

Presuming enzymatic actions in LHR/AHR, at least three different explanations exist for the observed LET influence: first, inactivation of some important repair enzymes and/or partial inactivation of the genes responsible for the operation of repair systems by radiation; second, a blockage and/or deficiency in energy supply; and third, an increase in the amount of LD with increasing LET. We shall demonstrate, later on, how the third explanation is supported by the analysis of low-dose and low-dose rate irradiation results.

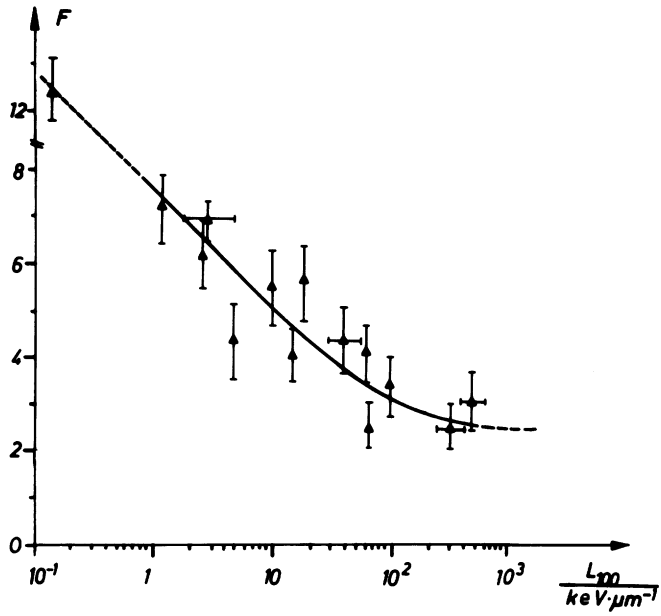

FIG. 9. The quotient $F = m/m'$ of slope values following immediate, m, and delayed plating, m'.

C. Low-Dose-Rate Irradiations

At low dose rates, for diploid yeast in the range up to about 0.5 Gy/min, the radiation response is independent of dose rate, and the DECs are exponential with a lower slope value, m'', in comparison to those values, m, observed at high-dose-rate irradiations (above 10 Gy/min, see Ref. *13*). The change in the response is usually interpreted as resulting from repair of sublethal and potentially lethal damage during the irradiation time, thus leaving only lethal damage (*21*). However, it should be mentioned here that sometimes the dose-rate effect is attributed to the reversion of sublethal damage alone, concluded from the disappearance of the shoulder of the DEC. With this explanation one would expect PLD to contribute to the slope and therefore a change in m'' after delayed plating (which removes PLD). This was not observed in americium-241 α-particle irradiations with doses up to 600 Gy (data not shown here). Therefore it seems that at least for yeast, only LD contributes to the slope m''. The results of irradiations with cobalt-60 γ rays, 160-kV X rays, and americium-241 α particles are shown in Fig. 10. To find out whether the dose rates applied were sufficient to establish a dose rate-independent response, in the case of X irradiation, two different dose rates were applied: 0.15 and 0.30 Gy/min. Both experiments resulted in the same DEC (open circles and squares in Fig. 10). For γ and α irradiations the complete removal of PLD during irradiation time was confirmed by delayed plating. Similar low-dose-rate measurements with other radiation qualities could not be performed because of the long exposure times needed for high doses.

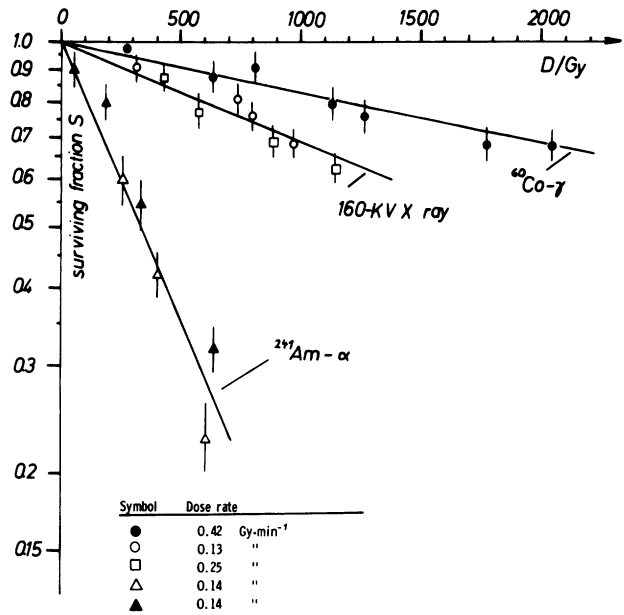

FIG. 10. Dose–effect curves following irradiations with cobalt-60 γ rays, 160-kV X rays, and americium-241 α particles at low dose rates. The effect of α-particle irradiation is given for immediate plating (white triangles) and delayed plating (black triangles) in two different runs.

From the results in Fig. 10, slope values, m'', have been calculated as follows:

$$m'' \text{ (cobalt-60 } \gamma \text{ rays)} = -(2.0 \pm 0.3) \times 10^{-4}/\text{Gy}^{-1}$$
$$m'' \text{ (160-kV X rays)} = -(4.1 \pm 0.4) \times 10^{-4}/\text{Gy}^{-1}$$
$$m'' \text{ (americium-241 } \alpha \text{ rays)} = -(2.1 \pm 0.3) \times 10^{-3}/\text{Gy}^{-1}.$$

Obviously, m'' increases with increasing LET of the radiation (see below).[2]

D. Low-Dose Irradiations

In Fig. 11 the DECs after immediate plating of synchronized daughter populations are shown for X and α irradiation with high dose rates (broken lines show the results of low-dose-rate irradiations for comparison). In the high-dose range, the results for synchronized cells are nearly identical with that for the whole population.

The low-dose irradiation results serve for determination of the initial slope value m_0, i.e., the value obtained for dose $D \to 0$. As we have seen in the last section, there is a production of LD by radiation, the amount of which is dose-independent per unit dose, or in other words, there is an initial slope $m_0 = m''$. This amount must be present in the DEC's of Fig. 11, and it must be equal to m_0, if our previous considerations about the dose rate response are true.

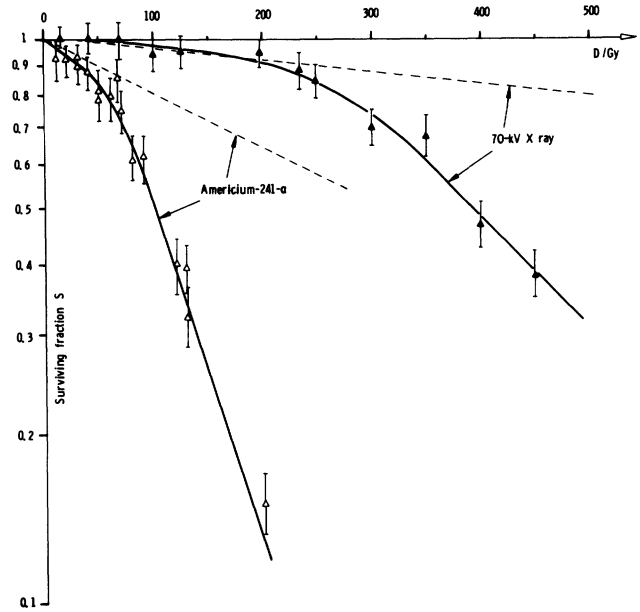

FIG. 11. Dose–effect curves for synchronized daughter cells of yeast in stationary state, irradiated at high dose rates and plated immediately after irradiation (solid lines). Low-dose-rate results are given for comparison (broken lines).

Using f experimental points of survival $S_i(D_i)$ from the low dose irradiations at high dose rates, the slope m_i of the DEC at dose $D_{ik} = (D_i + D_k)/2$ with $k = i + 1, i + 2, \ldots, f - 1, f$, and $D_k > D_i$ was calculated for each set $[S_i(D_i); S_k(D_k)]$ of the series $i = 1, \ldots, f - 2, f = 1$ according to (1):

$$m_i = \frac{\ln S_k - \ln S_i}{D_k - D_i}. \tag{1}$$

Due to the Poisson variance, $\Delta S/S$ (on the order of 5%) and to dose uncertainties, $\Delta D/D$ (on the order of 4%), the uncertainty $\Delta m/m$ becomes large at low doses and amounts up to about 40% (values based on 95% confidence intervals). Therefore, the calculated points, m_i, drawn in a semilogarithmic plot against dose D (see Fig. 12) were not used for the extrapolation of m for $D \rightarrow 0$. Instead, slope values were calculated by computer from equal dose intervals of the fitted DECs in Fig. 11. As can be seen in Fig. 12, where the results of these calculations are given by solid lines for X-ray and α-particle irradiations, they fit well to the points m_i gained from the experiment. By extrapolation to $D = 0$, whereas a "linear" relationship between the slope $d(\ln S)/dD$ and D was assumed (linear in a semilogarithmic plot), the following values were found:

$$m_0 \text{ (70-kV X rays)} = -3.1 \times 10^{-4}/\text{Gy}^{-1}$$

$$m_0 \text{ (americium-241 } \alpha \text{ rays)} = -1.8 \times 10^{-3}/\text{Gy}^{-1}.$$

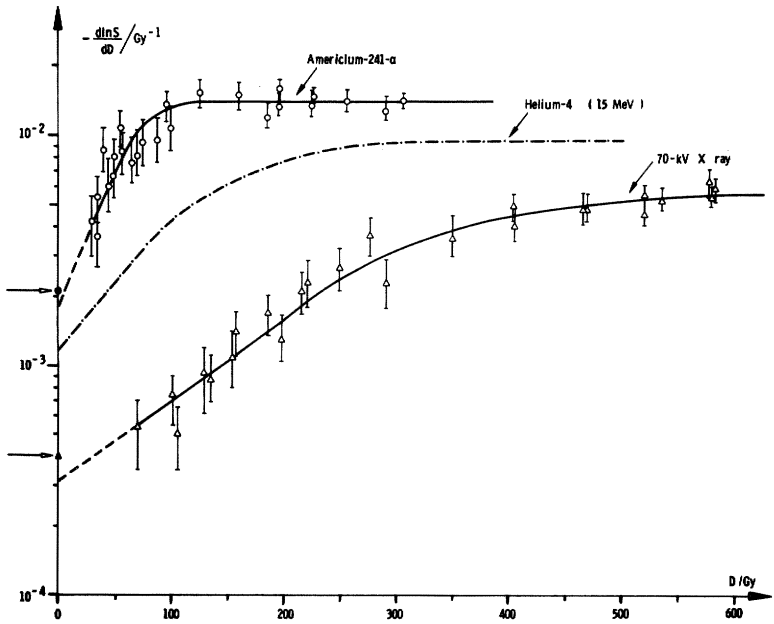

FIG. 12. Slope, $d(\ln S)/dD$, of dose–effect curves (as shown in Fig. 11) as a function of dose. Values, m'', determined by low-dose rate irradiations are indicated by arrows pointing from left to the ordinate. The corresponding slope function for 15-MeV helium-4 irradiation is given for comparison (dash-point line).

A comparison with m'' values gained from low-dose-rate experiments shows good agreement within the uncertainty range (see Fig. 12); therefore, it seems justified to set

$$m_0 = m'' = m_{LD} = \text{constant} \tag{2}$$

for each type of radiation and for doses up to about 600 Gy (which was the limit dose given in low-dose-rate experiments with americium-241 α particles). Of course, there is a limit of determination of m_0 due to the fact that at high LET the shoulder of the DEC disappears (Fig. 6). In these cases LD and PLD are produced together even at small doses, and a separation of these two types of damage becomes impossible by the method shown above.

POTENTIALLY LETHAL AND LETHAL DAMAGE DEPENDENCE ON LET

A. Relationship between PLD and LD

From the previously shown results the assumption can be made:

$$m = m_{LD} + m_{PLD}. \tag{3}$$

This means that the contributions of PLD and LD to the slope, m, of the exponential part of the DEC for immediate plating at high dose rates are additive, or, in other words, that they are produced independently of each other by radiation.

From a comparison between DECs after delayed plating (Figs. 4–6) and DECs of low-dose-rate irradiations (Fig. 10), one finds that m' is always greater than m''. This seems to be in contradiction to the previous analysis: delayed plating would be expected to remove all PLD. However, one may visualize the possibility that LD is also produced by PLD events at high doses (for example, if PLD events interact together to produce LD events). If this explanation holds true, the presumption of independent production of LD and PLD fails, and one has to correct Eq. (3) by an additive contribution to m which is a function of m_{PLD}. From comparison of m' and m'' we find that this contribution is about as large as m_{LD} itself.

A second explanation for $m' > m''$ arises from the fact that all DECs observed after delayed plating in the medium LET range show a shoulder. Preliminary results indicate that the initial slope values, m_0', of these curves are not larger than the corresponding values, m_0, of the immediate plating response. This could be interpreted as a dose dependency of AHR leading to reduced repair capabilities for PLD events at higher doses. It is of interest to note here that such a decrease of AHR efficiency with LET was observed, as shown in Fig. 9.

B. Separation of m_{PLD} and m_{LD}

In those experiments where m_{LD} was determined by low-dose rate results, i.e., by m'', and the parameter m by high-dose rate results, a separation of m_{LD} and m_{PLD} on the basis of Eq. (3) is possible. A comparison between these radiation constants, given in Table II, reveals a large difference in m_{PLD} and m_{LD} for low-LET response. Thus, at least for low and medium LET, a correction of Eq. (3) (as mentioned above) can be neglected, because it would be smaller than the uncertainty of m_{PLD} itself. For high-LET response, however, the value of m_{LD} amounts to about 10 to 20% of m_{PLD}. To use (3) further on, the uncertainty range for m_{LD} values determined from (3) has to be enlarged correspondingly.

In all other irradiation experiments, where no low-dose-rate results were

TABLE II

Slope Values of the Exponential Parts of Dose–Effect Curves and Radiation Constants m_{PLD} and m_{LD}

	Cobalt-60 γ *rays*	*30-MeV electrons*	*70-kV X rays*	*160-kV X rays*	*Americium-241* α *particles*
$-m/\mathrm{Gy}^{-1}$	—	0.0050	0.0057	—	0.0132
$-m'/\mathrm{Gy}^{-1}$	—	0.0004	0.0010	—	0.0058
$-m''/\mathrm{Gy}^{-1}$	0.0002	—	—	0.0004	0.0021[a]
$-m_{LD}/\mathrm{Gy}^{-1}$	0.0002	0.0002	0.0004	0.0004	0.0021
$-m_{PLD}/\mathrm{Gy}^{-1}$	0.0048	0.0048	0.0053	0.0053	0.0118
m_{PLD}/m_{LD}	24	24	13.3	13.3	5.6

[a] Determined from the americium-241 α source with low activity (see Materials and Methods and Fig. 10).

available, the determination of m_{LD} is possible only from the delayed plating slope, m'. If we set

$$m' = k \cdot m'' \tag{4}$$

to determine m_{LD} by $m_{LD} = m'/k$, we must be aware that k is slightly dependent on LET, as can be deduced from Table II: k increases from 2.0 at low LET to 2.8 at high LET. Thus, for each type of radiation up to 100 keV/μm the factor k was determined by interpolation, and for LET values over 100 keV/μm k was set to 2.8 because of the expected saturation in this high-LET range.

In Fig. 13 the values m_{PLD} and m_{LD} calculated from Eqs. (3) and (4) are shown as open circles, and the experimental values for m_{LD} as full circles. Within the uncertainty an agreement is found, for example, between the calculated value, $m_{LD} = -10^{-3}/\text{Gy}^{-1}$, and the value of the initial slope, $m_0 = -1.2 \times 10^{-3}/\text{Gy}^{-1}$, of the DEC for 15-MeV helium-4 irradiation.

From the data in Fig. 13 it becomes obvious that the ratio m_{PLD}/m_{LD} decreases with increasing LET: from 24 at very low LET to 5.6 at high LET (see also Table II). Thus, the decrease is governed by a factor 24/5.6 = 4.3. The decrease of AHR efficiency, F, is similar (Fig. 9): from 12.5 to 2.3, i.e., by the factor 5.4. Therefore it seems that the decline of F with LET is caused mainly by an increase of LD relative to PLD in the low- and medium-LET range.

The dependences of m_{PLD} and m_{LD} on LET need to be explained further. It has been supposed that the low-dose rate response, for which m_{LD} is represen-

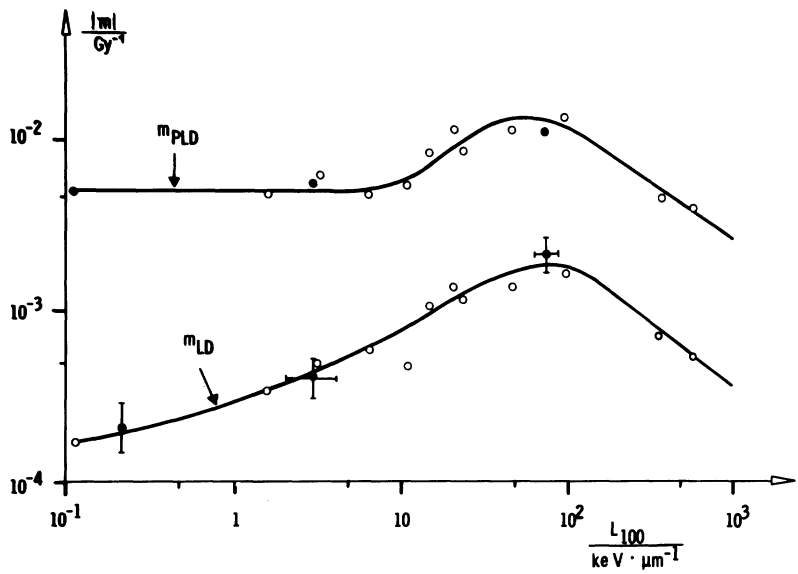

FIG. 13. Radiation constants of dose-effect curves of whole yeast populations in stationary state for production of potentially lethal damage, m_{PLD}, and lethal damage, m_{LD}, as a function of the LET. Full circles with error bars indicate empirical values whereas open circles are values determined from Eqs. (3) and (4) (see text).

tive, is due to single-hit events in the intracellular target responsible for maintenance of cellular proliferation (*22*). According to target theory, single-hit yields, as observed in our experiments, cannot be explained because of the increase of LD with LET (*23*). If one assumes that the LD is produced by exchange of two targets which have been hit by the same ionizing particle, as it was proposed by Neary in his theory of chromosome aberrations (*24*), one finds a function of the yield per unit dose which is nearly proportional to the total rate of energy loss ($-dE/dx$ or L_∞), in a large LET range. This is, however, not the case for the observed LD response of yeast: the slope of the m_{LD} curve changes continuously with LET but remains always less than 1. Either the exchange theory of LD production fails to explain the measurement, or, assuming that it holds true at least for high-LET response, a different response must be considered after low-LET irradiation. Experiments with fast protons are planned to establish whether a maximum of LD yield occurs around 1 keV/μm which is "smeared out" when radiations with broad LET distributions such as X rays and electrons are used.

Up to now no explanation exists for the observed response of PLD on LET (see Fig. 13). Also, in this case it seems that this response results from a superposition of functions different for low and for high LET.

CONCLUDING REMARKS

Beside other parameters, like depth-dose characteristics, shape of survival curves, etc., values of the relative biological effectiveness (RBE) are considered in discussions of the advantages or disadvantages of certain radiations for tumor therapy. However, RBE values as usually derived from the DECs after high dose rates, are dependent on the definition, for example, on the surviving fraction chosen for comparison. This is because the RBE includes different responses of the cell to irradiation. Thus, if one takes D_{37} values to compare the effect of americium-241 α particle to 70-kV X irradiation, one finds from our measurements $RBE_{37} = 3.8$; however, from the ratio of m_{LD} values, $RBE_{LD} = 6.0$. This large difference demonstrates clearly the need for differentiation into the main contributions of cellular response to ionizing radiation.

In this context it should be noted that the principles laid out here are similar to those of a theoretical model for radiation action which assumes three different states of the cell after irradiation (*13*). However, our investigations have been interpreted in a model-independent way.

In a second paper (Bertsche and Lachet; in preparation), the experimental data on yeast 211 will be analyzed and discussed in the light of several theories of radiation action with two and more parameters. Furthermore, the principles laid out here will be used in similar investigations with stationary Ehrlich ascites tumor cells. Preliminary data suggested a different LET-dependence of m_{LD} and m_{PLD} compared to that of yeast, although RBE functions from high-dose-rate DECs are quite similar. This again is an indication for the relevance of differentiation of survival curves in basic research as well as in radiotherapy.

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REFERENCES

1. T. ALPER and N. E. GILLIES, Restoration of *Escherichia coli* strain B after irradiation: Its dependence on suboptimal growth conditions. *J. Gen. Microbiol.* **18**, 461–472 (1958).
2. V. I. KOROGODIN and T. S. MALUMINA, Recovery of viability of irradiated yeast cells. *Priroda (Moscow)* **48**, 82–85 (1959).
3. R. A. PHILLIPS and L. J. TOLMACH, Repair of potentially lethal damage in X-irradiated HeLa cells. *Radiat. Res.* **29**, 413–432 (1966).
4. J. B. LITTLE, Repair of sublethal and potentially lethal radiation damage in plateau phase cultures of human cells. *Nature (London)* **224**, 804–806 (1969).
5. F. W. HETZEL, J. KRUUV, and H. E. FREY, Repair of potentially lethal damage in X-irradiated V79 cells. *Radiat. Res.* **68**, 308–319 (1976).
6. M. R. RAJU, J. P. FRANK, E. BAIN, T. T. TRUJILLO, and R. A. TOBEY, Repair of potentially lethal damage in Chinese Hamster cells after X and α irradiation. *Radiat. Res.* **71**, 614–621 (1977).
7. M. H. PATRICK and R. H. HAYNES, Dark recovery phenomena in yeast. II. Conditions that modify the recovery process. *Radiat. Res.* **23**, 564–579 (1964).
8. V. K. JAIN and W. POHLIT, Influence of energy metabolism on the repair of X-ray damage in living cells. I. Effect of respiratory inhibitors and glucose on the liquid-holding reactivation in yeast. *Biophysik* **8**, 254–263 (1972).
9. J. T. LYMAN and R. H. HAYNES, Recovery of yeast after exposure to densely ionizing radiation. *Radiat. Res. Suppl.* **7**, 222–230 (1967).
10. N. M. S. REDDY, B. S. RAO, and M. S. S. MURTHY, Liquid holding recovery in stationary and log phase cultures of diploid yeast, exposed to gamma and alpha radiations. *Radiat. Environ. Biophys.* **13**, 167–175 (1976).
11. W. POHLIT and A. DOMKE, Kybernetische Untersuchungen zur Strahlenwirkung auf Hefezellen. I. Vermehrung ideal homogener und synchronisierter Zellpopulationen. *Biophysik* **3**, 44–64 (1966).
12. W. POHLIT and I. R. HEYDER, Growth of cells on solid culture medium. II. Cell physiological data of stationary yeast cells and the initiation of cell cycle in nutrient free buffer solution. *Radiat. Environ. Biophys.* **14**, 213–230 (1977).
13. A. KAPPOS and W. POHLIT, A cybernetic model for radiation reactions in living cells. I. Sparsely ionizing radiations; stationary cells. *Int. J. Radiat. Biol.* **22**, 51–65 (1972).
14. J. MUELLER-ONTJES, Growth of cells on solid culture medium. I. Growth of a homogeneous cell layer on a solid culture medium. *Biophysik* **10**, 143–155 (1973).
15. H. LIESEM, G-value determination for 20 MeV protons. *Phys. Med. Biol.* **22**, 522–524 (1977).
16. D. FRANKENBERG, A ferrous sulphate dosemeter independent of photon energy in the range from 25 keV up to 50 MeV. *Phys. Med. Biol.* **14**, 597–605 (1969).
17. U. BERTSCHE and H. LIESEM, Survival of yeast after heavy ion irradiation. In *Radiation and Cellular Control Processes* (J. Kiefer, Ed.), pp. 105–110. Springer-Verlag, Berlin, 1976.

18. G. W. BARENDSEN, Relative effectiveness of ionizing radiations in relation to LET and the influence of oxygen. In *Biophysical Aspects of Radiation Quality* (IAEA Technical Reports Series No. 58), pp. 44–57. IAEA, Vienna, 1966.

19. P. TODD, Heavy ion irradiation of cultured human cells. *Radiat. Res. Suppl.* **7**, 196–207 (1967).

20. R. REINHARD and W. POHLIT, Influence of intracellular adenosinetriphosphate concentrations on survival of yeast cells following x irradiation. In *Radiation and Cellular Control Processes* (J. Kiefer, Ed.), pp. 117–123. Springer-Verlag, Berlin, 1976.

21. E. J. HALL, Radiation dose rate: A factor of importance in radiobiology and radiotherapy. *Br. J. Radiol.* **45**, 81–97 (1972).

22. J. F. FOWLER, Fundamental aspects of LET in radiobiology. In *Radiation Research—Biomedical, Chemical, and Physical Perspectives* (O. F. Nygaard, H. I. Adler, and W. K. Sinclair, Eds.), pp. 1040–1052. Academic Press, New York, 1975.

23. D. E. LEA, *Actions of Radiations on Living Cells.* 2nd ed. Cambridge University Press, 1962.

24. G. J. NEARY, Chromosome aberrations and the theory of RBE. 1. General considerations. *Int. J. Radiat. Biol.* **9**, 477–502 (1965).