Chromosome Damage in Liver Cells from Low Dose Rate Alpha, Beta, and Gamma Irradiation: Derivation of RBE

Abstract. *Relative biological effectiveness (RBE) for chromosome damage in liver cells was determined after low dose rate exposures to alpha, beta, or gamma irradiation. Protracted exposures to beta and gamma irradiation were equally effective, whereas low dose rate exposures to alpha emitters were 15 to 20 times more damaging than exposures to beta or gamma irradiation. These data support the use of the quality factor of 10 recommended by the International Commission on Radiological Protection and the National Council on Radiation Protection for estimating the biological hazard from internally deposited alpha emitters. When the dose rates were low, all types of chromosome damage observed were produced by single-hit processes.*

Consideration of the biological effectiveness of different radiation types is important in setting the radiation standards used as guidelines to limit human radiation exposure. The lack of adequate data for high linear energy transfer (LET) radiation delivered at low dose rates creates uncertainties in determining biological effectiveness for such exposure conditions. The LET for ionizing radiation is a measure of the average amount of energy deposited in tissue per unit of distance traveled. Internal deposition of alpha-emitting radioisotopes such as ^{239}Pu results in low dose rate exposure to high LET radiation. The projected inventory of alpha-emitting isotopes related to nuclear power production is very large and creates a potential for incorporation of some of these isotopes into humans and a need to understand the radiobiology of such exposure. The method for determining the relative biological effectiveness (RBE), which is used in estimating hazard, is to divide the dose of a standard irradiation which produces a measured biological effect by the dose of test irradiation required to produce the same biological change. The test irradiation used is usually acute exposure to ^{60}Co gamma rays or 250-kv-peak x-rays (*1*).

We have determined the RBE for exposure to high and low LET radiation delivered at low dose rates, using chromosome damage as the measure of biological change and either brief (*2*) or protracted (*3*) exposure to ^{60}Co as reference irradiation. With protracted ^{60}Co as the test exposure, the RBE values were independent of dose. An RBE of 1 was observed for an internally deposited beta emitter, while values of 15 to 20 were observed for the alpha emitters tested. These RBE's are in general agreement with the quality factors of 1 for beta and 10 for alpha emitters used to estimate hazard by the International Commission on Radiological Protection (ICRP) and the National Council on Radiation Protection (NCRP). When brief exposure to ^{60}Co was used as the reference irradiation, the RBE changed as a function of radiation dose and appeared to be less suitable for estimating hazards from internally deposited alpha-emitting isotopes such as ^{239}Pu. One reason for these different RBE values is that many dose-response curves for brief exposure to x-rays are nonlinear (*4*), while those for high LET radiation are often linear (*5*).

The fact that the frequency of chromosome aberrations produced by high LET radiation increases as a linear function of radiation dose implies a single-hit process (*5*). If protracting the dose of a low LET radiation also results in a linear dose-response relationship, it can be inferred that gamma irradiation can also interact with chromosomes to produce aberrations by a single-hit process. An RBE for protracted exposure to either low or high LET radiation can thus be easily derived by comparing the slopes of the dose-response lines. Since environmental radiation exposure will be protracted, such relationships are important in deriving meaningful quality factors to be used in setting standards for internally deposited alpha-, beta-, or gamma-emitting radionuclides.

For the experiments described in this report metaphase chromosome aberrations in the livers of Chinese hamsters were used as a biological measure of damage, and brief and protracted external ^{60}Co exposures as reference radiation. Radiation doses from intraperitoneally injected ^{144}Ce, ^{239}Pu, ^{241}Am, and ^{252}Cf citrates (*6–8*), all of which localize in the liver, were compared to the reference radiation. Cerium-144 is a beta emitter, whereas ^{241}Am, ^{239}Pu, and ^{252}Cf emit alpha particles. In addition to alpha particles, ^{252}Cf also decays by the emission of neutrons, gamma rays, and fission fragments.

The methods used in these experiments, a detailed breakdown of aberration types, and dose-response data for different aberration types have been previously described (*2, 3, 6–8*). Basic information is included in Table 1 to show the scope of the experiment. Briefly, the livers of Chinese hamsters were exposed for varied lengths of time to the different types of radiation. Most of the cells were probably in the G_0 or nonproliferating stage of the cell cycle at the time of exposure. After accumulation of a predetermined dose, 60 percent of the liver was surgically removed to stimulate cell division. Colchicine was injected 50 hours after partial hepatectomy and the hamsters were killed 4 hours later. Cells were scored in the metaphase stage of the cell cycle. All chromosome slides were coded and scored without the scorer knowing the activity or dose level. Aberrations scored were rings, dicentrics, chromosome deletions, and symmetrical chromosome exchanges. All aberrations were given a value of 1 in calculating the number of aberrations per cell for determining dose-response relationships.

Dose to the liver was determined by external dosimetry after ^{60}Co exposure. For the internal emitters, the dose was calculated by using the estimated time-integrated activity, determined by measuring the activity in microcuries per gram of liver at the time of partial hepatectomy and when the animal was killed. The measured activity was extrapolated over the experimental time interval by using previously determined liver retention curves. The time-integrated activity thus derived for each animal was used to calculate a dose, which was related to the individual chromosome response. Details of dose calculations were previously published (*6*).

At each sampling time, dose-response

Table 1. Experimental design for determining the RBE for chromosome damage following low dose rate exposure to alpha, beta, and gamma irradiation. For example, ^{144}Ce citrate injections at six different dose levels were given to one group of animals, which were killed at 6 days, to another group, which were killed at 15 days, and so forth.

Exposure type	Number of activity or dose levels at each exposure time						Total treatments	Number of animals	Number of cells scored
	20 minutes	6 days	15 days	42 days	122 days	362 days			
Brief ^{60}Co	8						8	78	3,570
Protracted ^{60}Co		4	4	4			12	53	1,734
^{144}Ce citrate injected		6	6	6	6	6	30	252	5,024
^{241}Am citrate injected		6	6	6	6	6	30	156	3,141
^{252}Cf citrate injected		5	5	5			15	53	2,196
^{239}Pu citrate injected		1	1	1	1		4	37	1,360
Totals							99	629	17,025

curves were generated. Dose-response relationships were also derived for each activity level sampled at a variety of times. Through 362 days, time or dose rate did not change the dose-response curves. The results reported here represent a best fit of all the data through 362 days and are summarized in Fig. 1, where the aberration frequency in liver cells is related to dose in rads. With the exception of brief ^{60}Co exposure, all dose-response data could be adequately described by linear equations of the general form $Y = a + bD$, where Y = aberrations per cell, a is the intercept, b is the slope of the line in aberrations per cell per rad, and D is the dose in rads. These equations were, for protracted (pro) ^{60}Co and injected (inj) ^{144}Ce, ^{239}Pu, ^{241}Am, and ^{252}Cf,

$$^{60}\text{Co}_{\text{pro}} \quad Y = 0.02 + 3.3 \times 10^{-4}D$$
$$^{144}\text{Ce}_{\text{inj}} \quad Y = 0.02 + 3.1 \times 10^{-4}D$$
$$^{239}\text{Pu}_{\text{inj}} \quad Y = 0.04 + 4.8 \times 10^{-3}D$$
$$^{241}\text{Am}_{\text{inj}} \quad Y = 0.06 + 7.2 \times 10^{-3}D$$
$$^{252}\text{Cf}_{\text{inj}} \quad Y = 0.05 + 3.3 \times 10^{-3}D$$

Dose-response data for brief ^{60}Co exposures were best described by a quadratic equation

$$Y = 0.01 + 1.9 \times 10^{-4}D + 1.8 \times 10^{-6}D^2$$

A nonlinear dose-response curve has been observed for a variety of brief exposures (*4*) and is thought to be related to the mechanism of chromosome aberration production. At low total doses, few chromosomes are broken in any given cell and there is little interaction between chromosomes. As the dose increases, more breaks are present and more interaction occurs. This results in the frequency of aberration production increasing as the square of the radiation dose. Thus, high dose rates produce aberrations by two mechanisms: single events, which increase linearly with dose, and double events, which increase as the square of the dose. The combination of these is reflected in the quadratic dose-response relationship for brief ^{60}Co exposure.

When the protracted ^{60}Co is used as a reference, the quality factors for effectiveness can be determined by comparing the slopes of the dose-response curves, since they are all linear. The values derived are 1, 15, 20, and 10 for ^{144}Ce, ^{239}Pu, ^{241}Am, and ^{252}Cf, respectively. Since beta particles and gamma rays are both sparsely ionizing and interact similarly with matter, the value for RBE is 1, as would be expected. The high LET alpha particles seem to be 10 to 20 times more effective than either beta particles or gamma rays in producing chromosome aberrations. Californium-252 emits 11.76 Mev per decay, with 6.02 Mev as alpha particles and most of the rest of the energy as fission fragments.

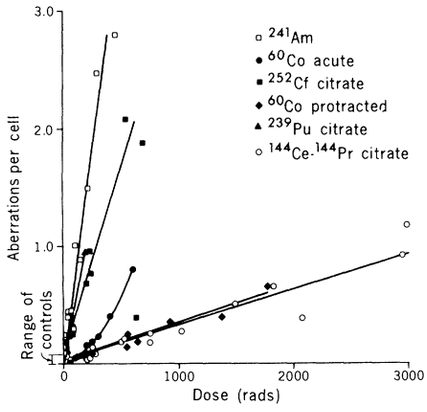

Fig. 1. Dose-response curves for the production of chromosome aberrations in the liver of the Chinese hamster after exposure to alpha, beta, and gamma irradiation.

It is about ten times more effective than the sparsely ionizing radiations in producing chromosome damage. It has been postulated (*8*) that this is due to the ineffectiveness of fission fragments in producing chromosome damage which is scorable at metaphase. If only the dose from the ^{252}Cf alpha particles is related to chromosome aberrations, the quality factor is almost 20. These RBE values for alpha emitters are higher than the quality factor of 10 for alpha emitters used in setting radiation standards and illustrate that for this end point with low dose rate exposure, the quality factor may need to be changed by almost a factor of 2.

Since the dose-response curve for brief ^{60}Co exposure is nonlinear, RBE values for which this curve is used as a reference change as a function of dose. Values of

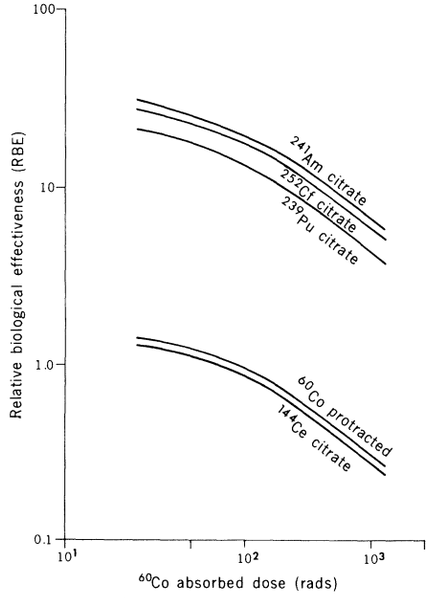

Fig. 2. Relative biological effectiveness of protracted exposure to alpha, beta, and gamma radiation, with brief exposure to ^{60}Co as the reference radiation.

RBE at 50-rad intervals of dose were calculated by forcing all the equations through the same intercept (0, 0.01) and obtaining the dose required to produce an equal chromosome aberration frequency at each interval. These are related to dose of ^{60}Co on a log-log plot in Fig. 2. This type of data presentation has been used by Kellerer and Rossi (*9*) and has the advantage that both coordinates refer to physical quantities: the doses to produce equal effects on the RBE scale and the dose of the reference radiation on the dose scale.

For brief ^{60}Co exposures, since the shape of the ^{60}Co curve is the controlling factor, both high LET radiation and protracted low LET radiation produce RBE-dose curves of the same shape. The protracted ^{60}Co and ^{144}Ce exposures start with an RBE of about 1 and decrease to a value of 0.25 by 600 rads. The high LET radiation from ^{241}Am, ^{239}Pu, and ^{252}Cf alpha exposures have initial values of 20 to 30 and decrease to about 5 when compared with the highest brief ^{60}Co dose measured. The slope of the lines observed for these data on a log-log plot reaches a value of about −1 for each exposure schedule.

Kellerer and Rossi (*9*) postulated that the slope of −1 on a log-log plot of RBE against dose helped to prove that brief x-ray irradiation acts by a two-event process, whereas high LET radiation requires only one event to produce a number of different biological changes. This was the basis for their model of two-step interaction of radiation with biological materials. In the model, cells in state one are normal; state two could perhaps be compared with potentially lethal damage reported by Dewey and co-workers (*10*) for both cell mortality and chromosome aberrations. Cells in state two can return to normal or progress to the final state of the abnormal chromosome configuration or cell death, depending on environmental conditions.

Our results with alpha irradiation agree with this concept. The RBE-dose curve decreases with a slope of about −1, implying that the brief gamma exposure produced most of its damage by a two-step process.

The slope of the dose-response curve for protracted ^{60}Co or ^{144}Ce implies that the damage produced by protracted exposure is due to single-event processes. In fact, the slope of the dose-response curves in Fig. 1 for protracted ^{60}Co and injected ^{144}Ce represents a measure of the probability per unit dose for producing lesions of the single-event type with protracted sparsely ionizing radiation.

In recent work chromosome aberrations have been related to cell death (*11*). They have also been related to the total genetic damage and ultimately to the production of cancer (*12*). Thus, chromosome aber-

rations are strongly linked to many late pathological changes.

Low dose rate irradiation of human populations will be produced by internally deposited radioactive materials acquired from the environment. When data obtained from experimental animals are used to estimate human risk, it is beneficial to have low LET reference irradiation delivered at both high and low dose rates. Utilizing a slowly dividing cell system, such as the liver, and recording chromosome damage after a variety of exposure types may have a very real relationship to genetic risk from protracted radiation exposures.

Genetic hazards are currently estimated for high LET radiation by using brief low LET exposures as a reference. Under these conditions, RBE increases as dose and dose rate decrease. The ICRP (*13*) has thus speculated that if protracted low LET radiation has been used as a reference standard for genetic damage, it may be necessary to substantially increase the quality factors now used for protection of the human population from genetic effects of high LET irradiation. The results reported here illustrate that this is not the case. The RBE of alpha emitters, when compared with protracted exposures to beta or gamma emitters, ranges from 15 to 20 even when very low dose rates of beta and gamma are used as a reference irradiation. The quality factor of 10 used by the NCRP and ICRP to estimate risk from high LET irradiation may be low by as much as a factor of 2, but no gross reevaluation seems needed.

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Albugo-Like Oogonia from the American Carboniferous

Abstract. *Fungal oogonia morphologically similar to those in the extant genus* Albugo *have been discovered in the integumental tissues of the fossil gymnosperm ovule* Nucellangium. *Disease symptoms in the fossil ovule are similar to those produced by* Albugo *in living angiosperm hosts.*

Fungal oogonia found in the integumental tissues of a Paleozoic ovule, *Nucellangium*, bear a striking resemblance to those of the extant Phycomycete (Oomycete) genus *Albugo*. Specimens are preserved in coal ball petrifications collected near Oskaloosa, Iowa, from middle Pennsylvanian strata. Of special significance is the fact that the oogonia are found in the so-called proliferated form of *Nucellangium* ovules (*1*). Proliferated ovules are slightly larger than normal ones and are characterized by irregular masses of parenchyma which extend into the locule of the ovule (Fig. 1a), giving the impression of an uncontrolled cancerous-type growth.

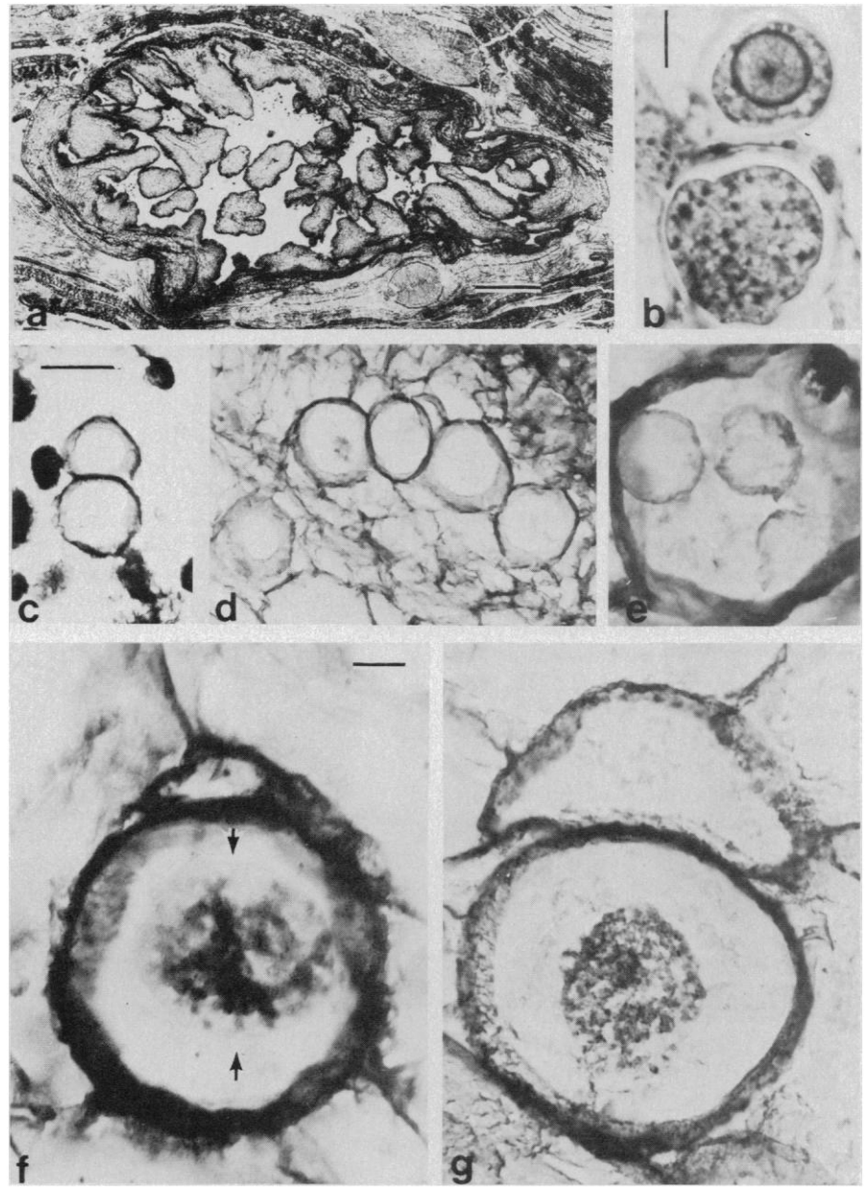

Fig. 1. (a) Section of proliferated ovule showing fingerlike extensions of integument into locule; scale bar, 2 mm. (b) Two *Albugo* oogonia, the top one containing oosphere within surrounding periplasm; scale bar, 20 μm. (c to g) Fossil oogonia. (c) Pair of oogonia free in locule of ovule; scale bar, 100 μm. (d) Oogonia in ovule integument; same scale as (c). (e) Oogonium with two visible spherical inclusions; same scale as (f). (f) Oogonium with membrane-bounded structure (arrows indicate membrane) interpreted as an oosphere; scale bar, 20 μm. (g) Oogonium with antheridium "hat cell" at top; same scale as (f).