Introduction to Radiobiology
Lesson 5

Master of Advanced Studies in Medical Physics
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Cell/tissue dynamics

As cells/tissues react to external stimuli like radiation, they change in time, however continuous monitoring is difficult and usually only the **endpoints** are recorded.

The effects are measured with *assays** and the measured results are presented in the form of:

- Cell survival curves.
- Dose response curves.

**Assay: a procedure for measuring the biochemical or immunological activity of a sample*

Three categories of tissue assay are common in radiobiology

- Clonogenic assays measure the reproductive integrity of the clonogenic stem cells in tissue and the measurements result in cell survival curves.

- Functional assays measure functional end points for various tissues and produce dose response curves.

- Lethality assays quantify the number of animal deaths after irradiation of the whole animal or of a specific organ with a given dose. The experiments are usually presented with the LD50 parameter.

Dose-response curves

Plot of a biological effect observed (e.g., tumour induction or tissue response) against the dose given is called a dose-response curve.

Dose-response may refer to:

- Clonogenic end points, i.e., cell survival.
- Functional end points.

Generally, as the dose increases so does the effect.

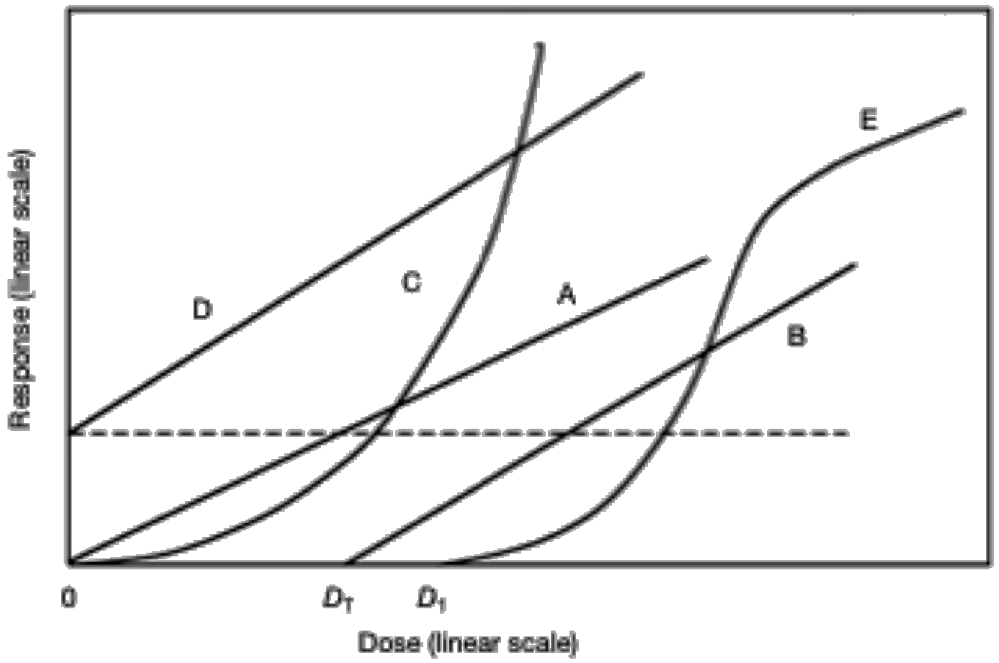
Three types of dose response shapes are common:

- Linear
- Linear-quadratic
- Sigmoid

Dose response curves may or may not have a threshold dose.

Threshold dose is the dose for a particular effect studied below which no such effect will be observed.

All these concepts are important in view of therapy optimization



Dose response curves

(A) Linear relationship with no threshold.

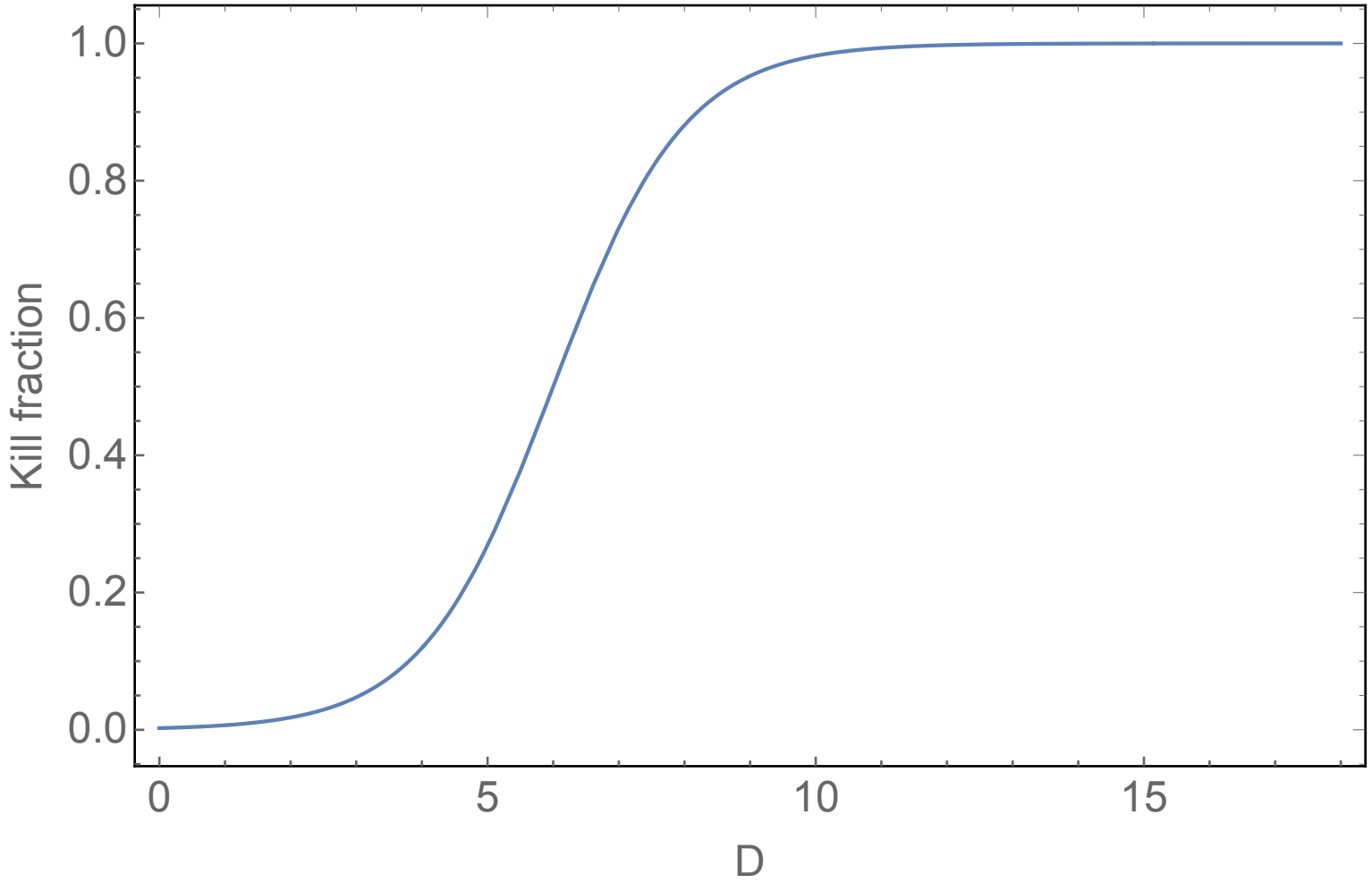
(B) Linear relationship with threshold.

(C) Linear-quadratic relationship with no threshold (stochastic effects such as carcinogenesis).

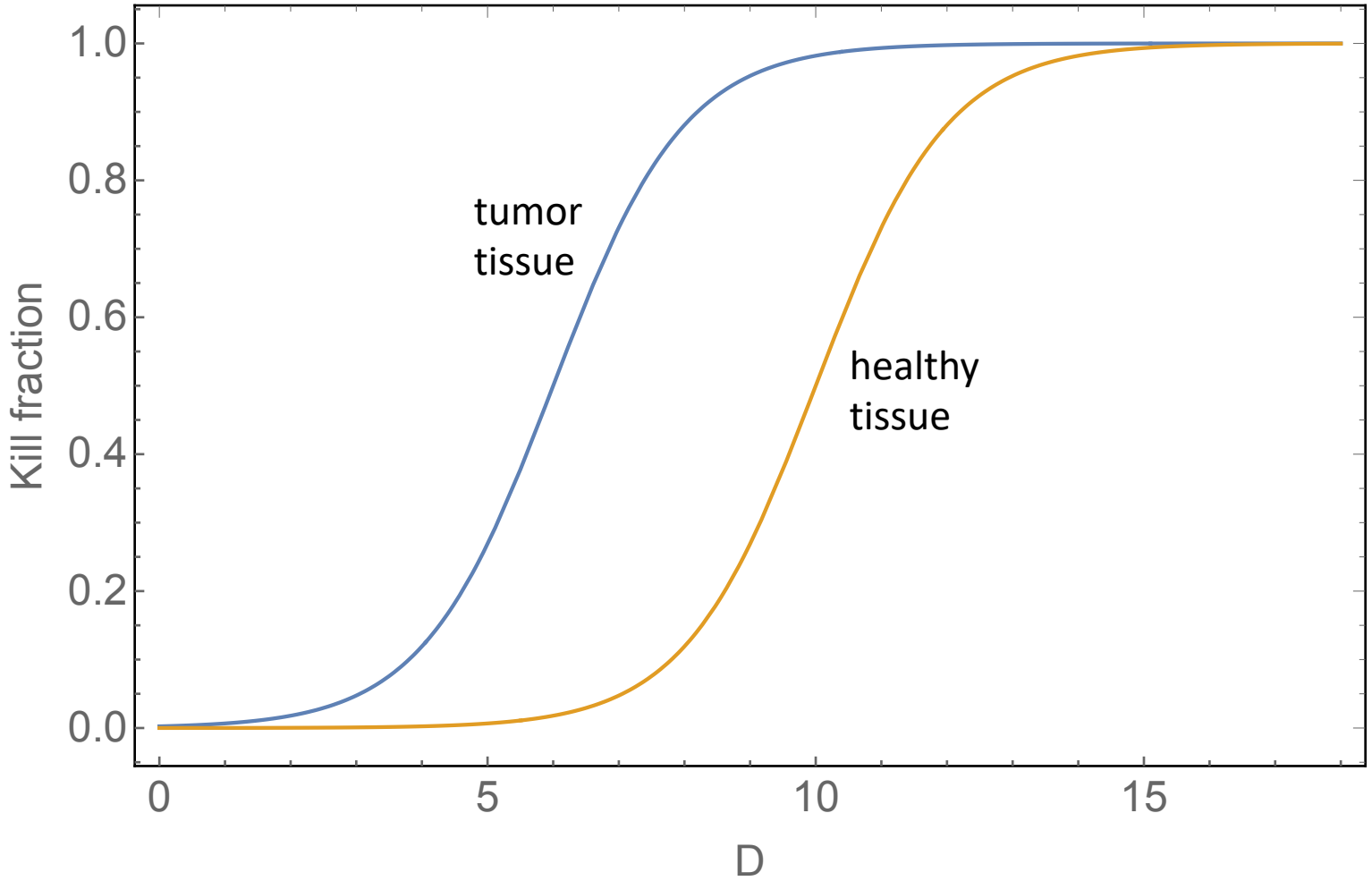
(D) Linear relationship with no threshold and the area under the dashed line representing the natural incidence of the effect.

(E) Sigmoid relationship with threshold D1, as is common for deterministic effects in tissues.

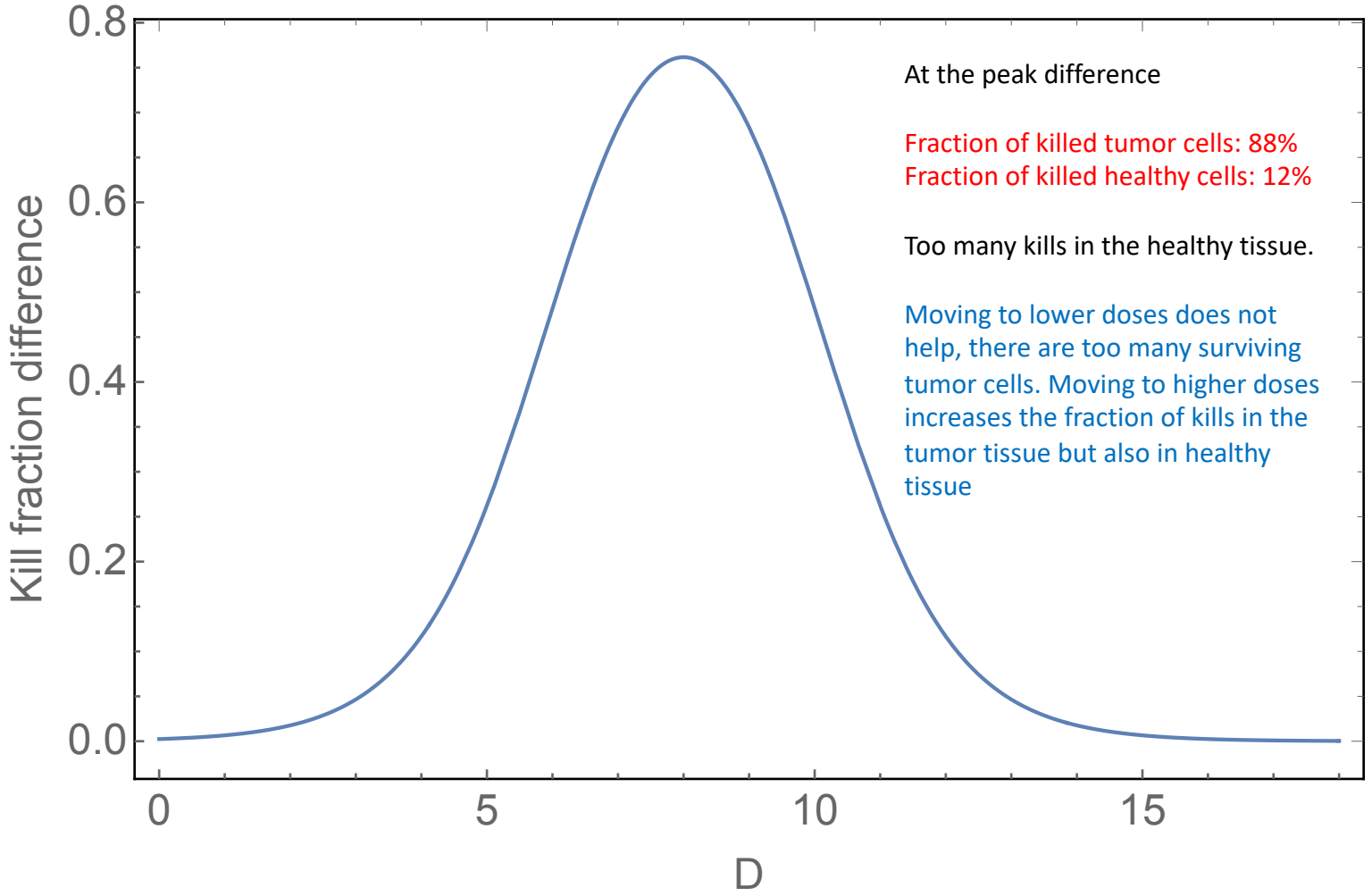
Example of simple dose-response curve and therapy optimization



Dose response for tumor tissue and healthy tissue



Dose response for tumor tissue and healthy tissue



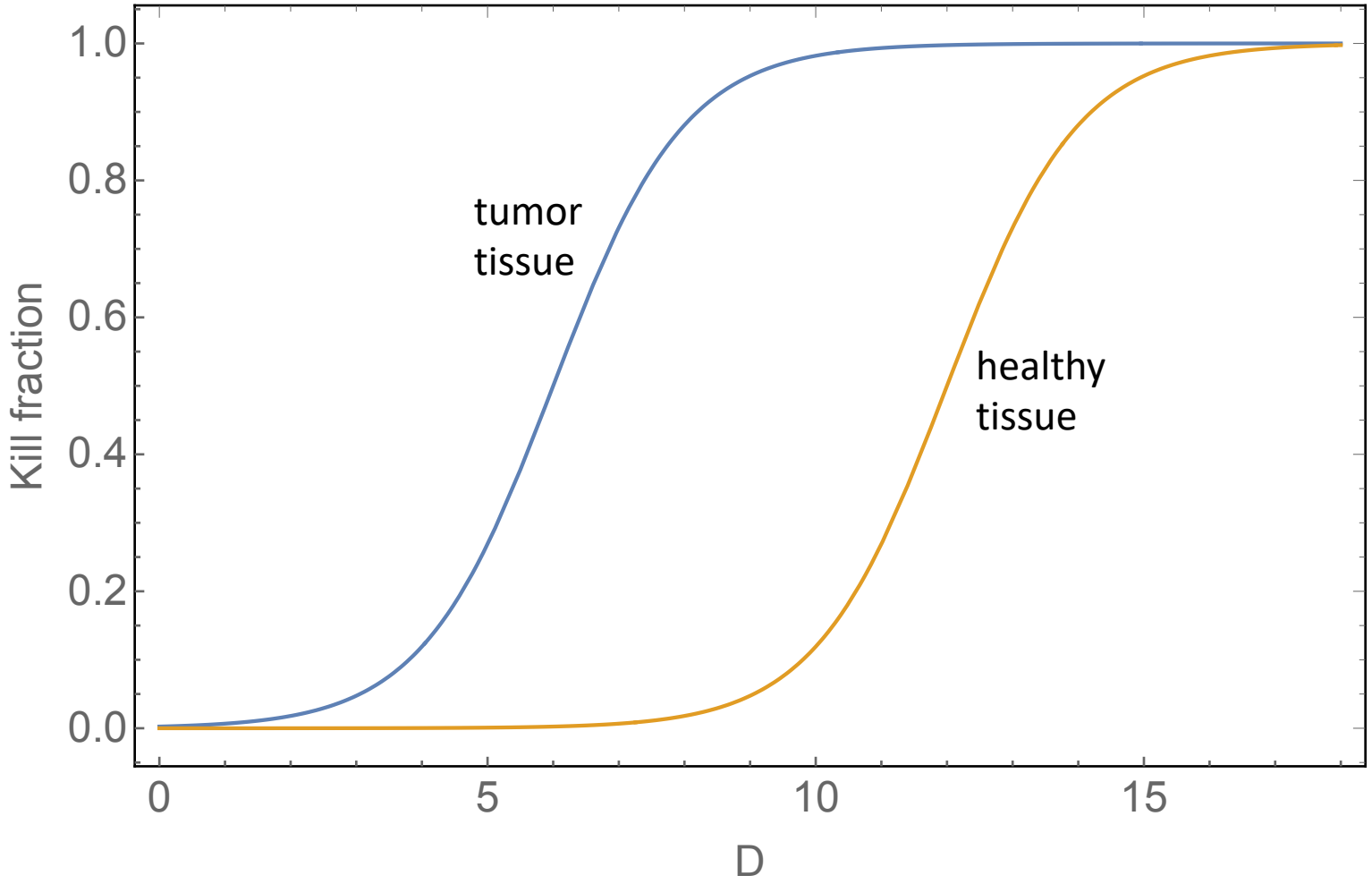
At the peak difference

Fraction of killed tumor cells: 88%
Fraction of killed healthy cells: 12%

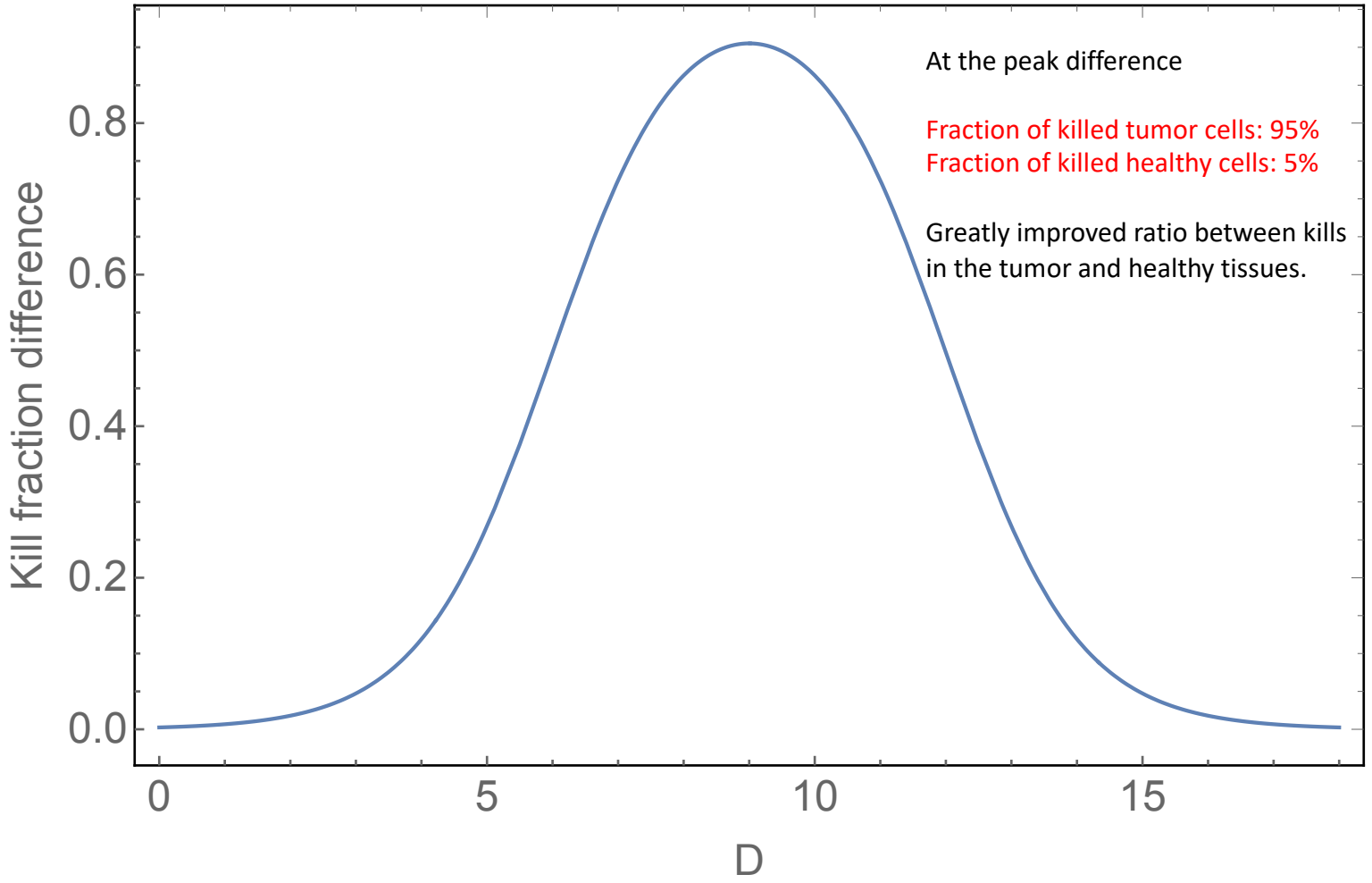
Too many kills in the healthy tissue.

Moving to lower doses does not help, there are too many surviving tumor cells. Moving to higher doses increases the fraction of kills in the tumor tissue but also in healthy tissue

Higher separation of response curves



Dose response for tumor tissue and healthy tissue



Cell death and cell survival curves

In radiotherapy we wish to kill tumor cells, and to properly grade the dose we must first study how irradiated cells die.

Experimental input: laboratory tests provide cell survival curves (surviving fraction against absorbed dose) that describe the **relationship between the surviving fraction of cells**, i.e., the fraction of irradiated cells that maintain their reproductive integrity (clonogenic cells) **and the absorbed dose**.

Cell survival against dose is graphically represented by plotting the surviving fraction *S(D)* on a logarithmic scale on the ordinate against dose *D* on a linear scale on the abscissa.

ACTION OF X-RAYS ON MAMMALIAN CELLS*, ‡

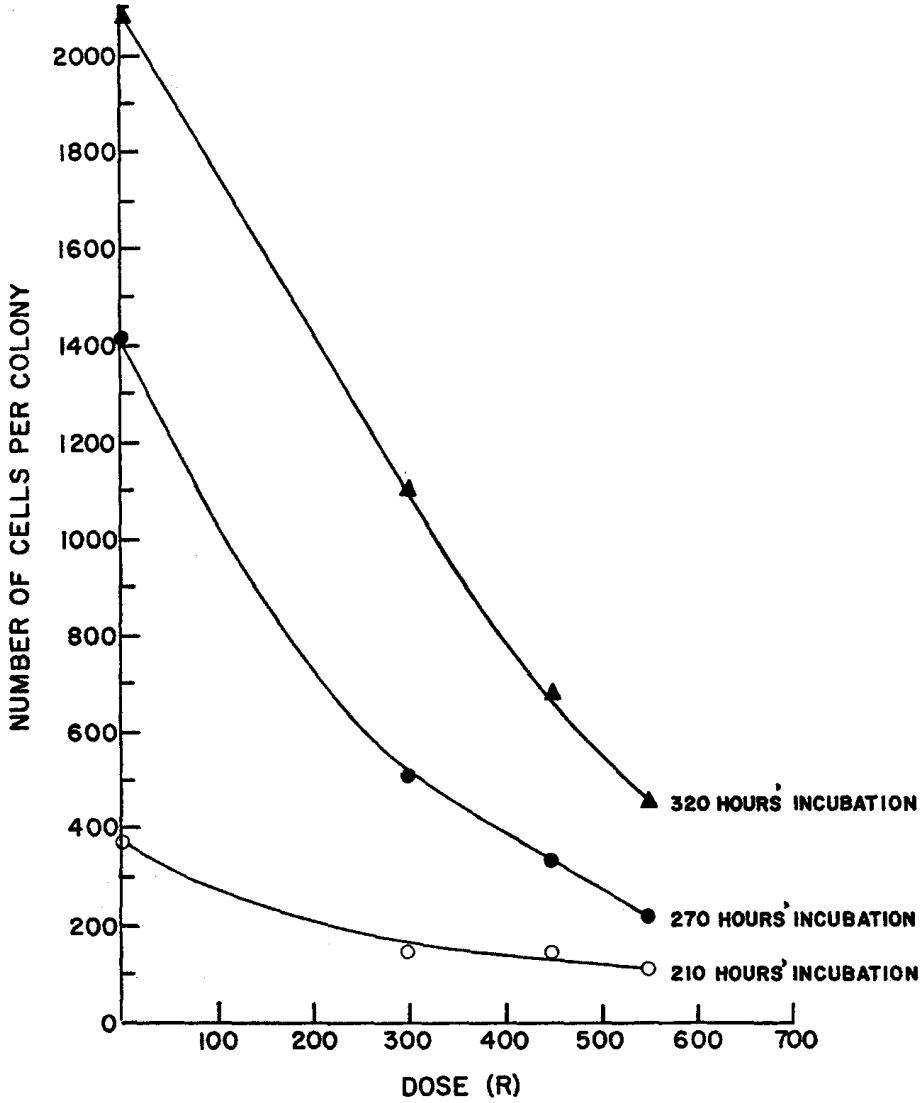
By THEODORE T. PUCK, PH.D., AND PHILIP I. MARCUS

(*From the Department of Biophysics, Florence R. Sabin Laboratories, University of Colorado Medical Center, Denver*)

(Received for publication, February 3, 1956)

Unirradiated cells exhibit 100 per cent colony-forming efficiency under the conditions employed. Hence, it is reasonably certain that the experimental procedure subjects these cells to no major stress other than that of the irradiation. Throughout this paper the words, "survival," "viable," and "killing" are used in the sense which has become standard in microbiology; *i.e.*, referring only to the ability of the individual cell to multiply into a macroscopic colony.

(From Puck TT, Markus PI: Action of x-rays on mammalian cells. *J Exp Med* 103:653-666, 1956)



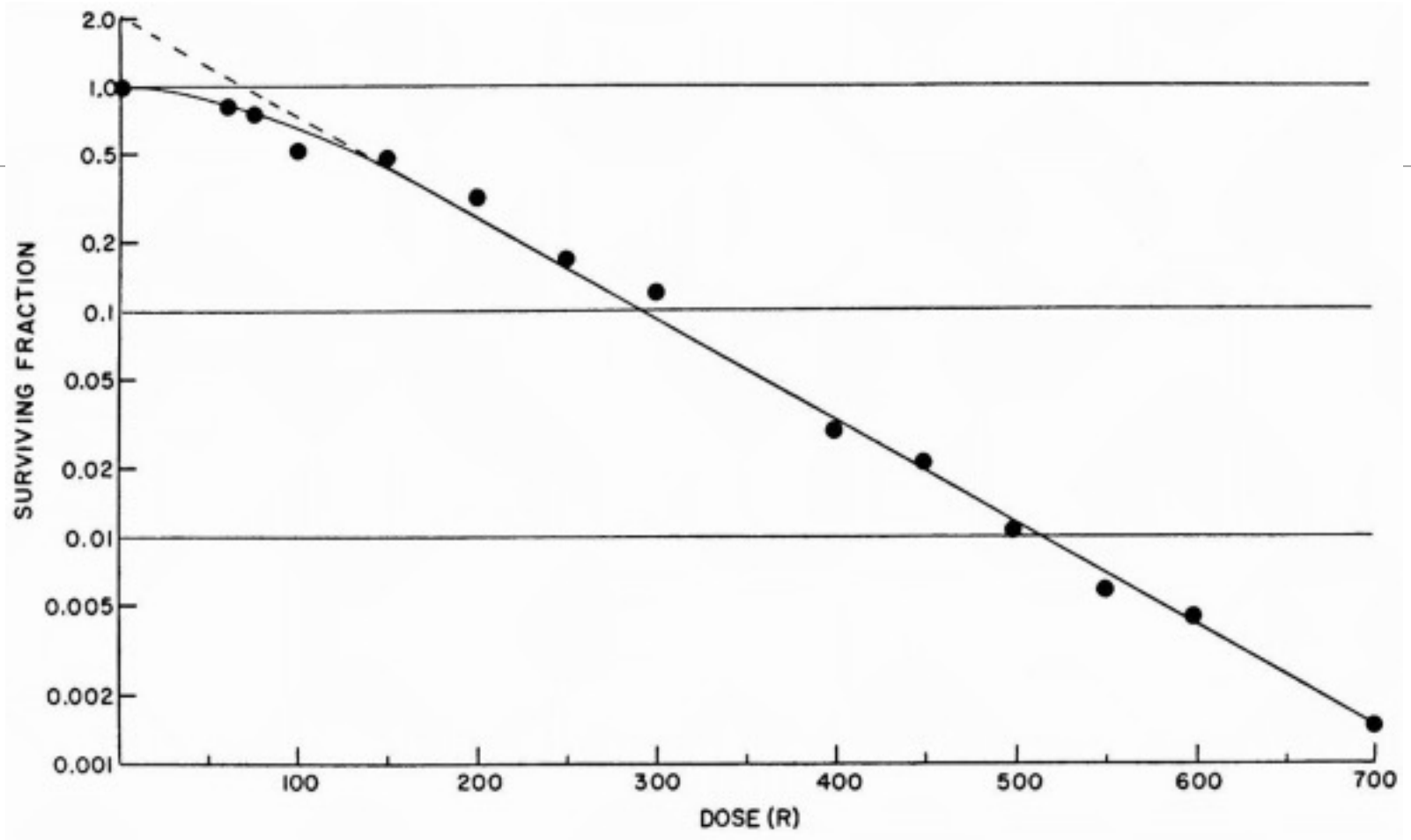
TEXT-FIG. 1. Demonstration of growth-slowing action of x-irradiation on single HeLa cells. Plates containing single cells deposited in the standard manner were x-irradiated, incubated for the times shown, then fixed and stained. The cells per colony were counted by microscopic examination, for all the colonies on each plate, or if these were too large in number, for a random sample of 10 to 20 colonies. In order to avoid weighting the data through the effect of abortive colonies, only colonies with more than 50 cells were counted.

1 R ≈ 1 cGy in soft tissue

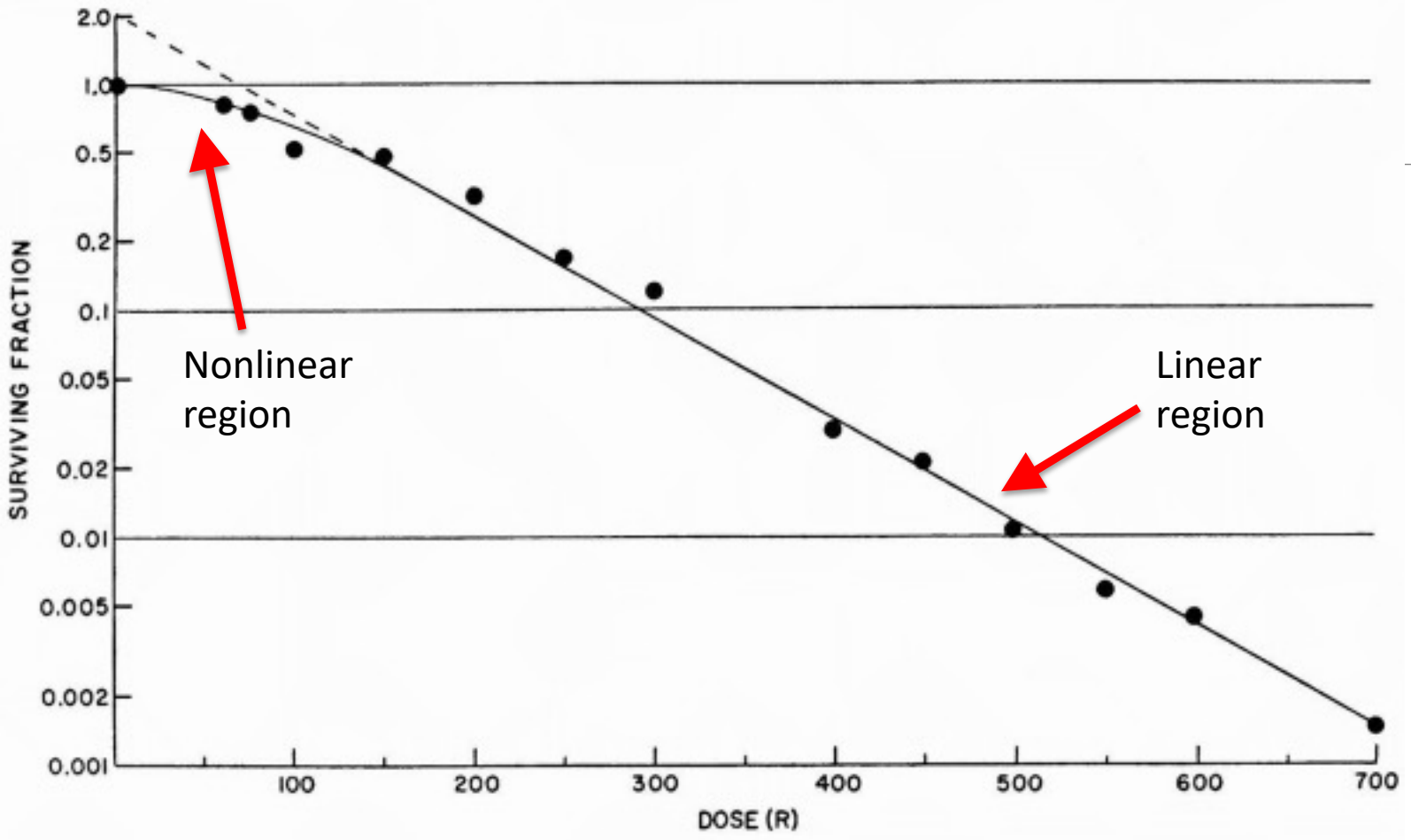
(From Puck TT, Markus PI: Action of x-rays on mammalian cells. *J Exp Med* 103:653-666, 1956)

A typical example will illustrate the results obtained: A series of plates, each seeded with 200 S3 cells, was irradiated with 300 r, and incubated for 9, 12, and 17 days, respectively. The plate stained after 9 days had a total of 117 aggregates visible to the unaided eye, ranging in diameter from 0.1 to 1.0 mm. Estimation of the actual number of these which would go on to further growth is difficult with the eye alone. This uncertainty was eliminated on further incubation, for the 12 day and 17 day plates unequivocally differentiated between cell aggregates which had remained less than 1 mm. in diameter, and those which now formed large colonies of 2 to 5 mm. The number of these latter was 31 and 35 on the plates incubated for 12 and 17 days respectively, values agreeing well within sampling uncertainty. However, by employing the criterion that each colony with 50 or more normal cells is to be counted as a survivor, the counts obtained on all 3 plates agreed, consisting in 35, 36, and 36, for the 9, 12, and 17 day incubations, respectively. It is impossible to avoid a certain degree of arbitrariness in the criterion which will indicate loss of reproductive function. We believe the operational definition employed in this study is biologically analogous to that which has proved useful in microorganisms, in addition to furnishing a readily reproduceable end-point.

(From Puck TT, Markus PI: Action of x-rays on mammalian cells. *J Exp Med* 103:653-666, 1956)



Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (From Puck TT, Markus Pl: Action of x-rays on mammalian cells. *J Exp Med* 103:653-666, 1956)



Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (From Puck TT, Markus Pl: Action of x-rays on mammalian cells. *J Exp Med* 103:653-666, 1956)

Linear-quadratic model

Actual data are fit reasonably well by the following mathematical expression for the surviving fraction

$$S(D) \approx e^{-\alpha D - \beta D^2}$$

The dose D_{eq} at which the linear and the quadratic term contribute equally is obtained from

$$\alpha D_{\mathrm{eq}} = \beta D_{\mathrm{eq}}^2$$

and therefore

$$D_{\mathrm{eq}} = \alpha/\beta$$

Target theory

Here we assume that a cell has one sensitive target, and that successive hits by ionizing particles are all statistically independent, so that we can use the Poisson statistics.

The probability of hitting *n* times a given target, when the average number of good hits is *a,* is

$$P(n) = \frac{a^n}{n!} e^{-a}$$

Then the probability missing the target is:

$$P(0) = e^{-a}$$

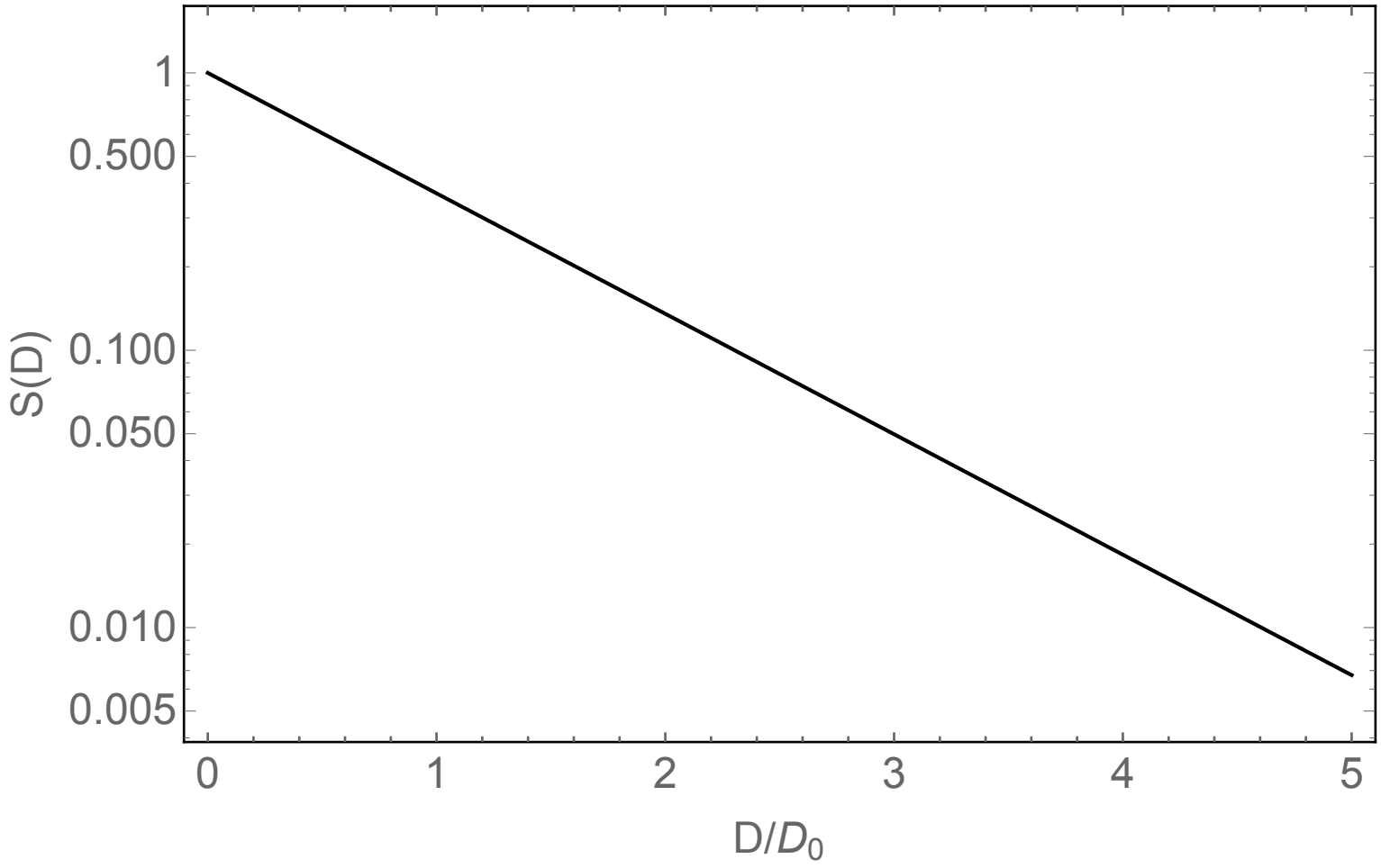
If the target is a vital function in a cell and the average number of hits depends on radiation dose *D*

$$a = D/D_0$$

then the survival probability is just the probability that the target is NEVER hit

$$S(D) = P(0, D) = e^{-D/D_0}$$

The surviving fraction in the simple Poisson approximation does not explain the LQ law



Multitarget model, asymptotic behavior and threshold effect.

If there are multiple targets, say *n* targets, all of which must be hit to kill a cell, then the probability of missing at least one of them – i.e., the survival probability – is

$$S(D) = 1 - (1 - e^{-D/D_0})^n$$

then, for large dose

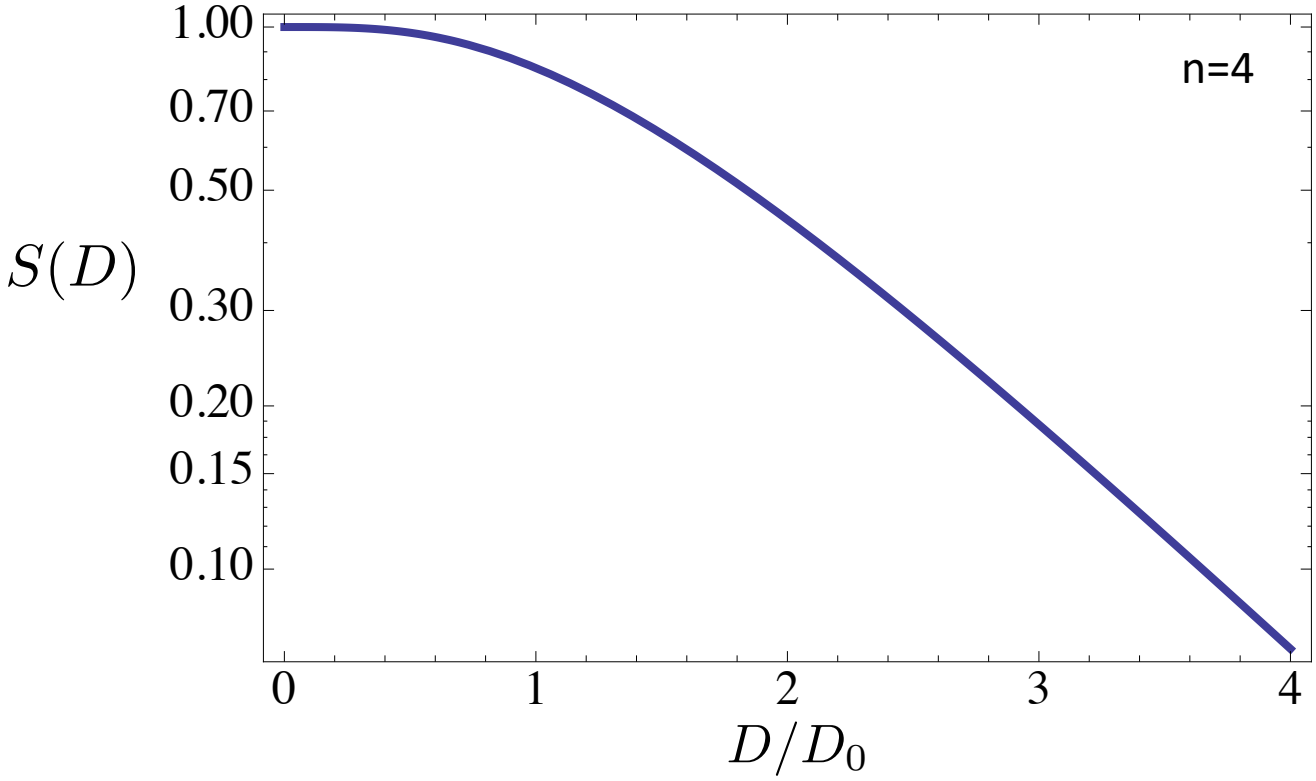
$$S(D) \approx n e^{-D/D_0}$$

i.e.,

$$\ln S(D) \approx \ln n - D/D_0$$

which is a linear relation with intercept ln *n*, and slope -1/D_0.

$$S(D) = 1 - (1 - e^{-D/D_0})^n$$



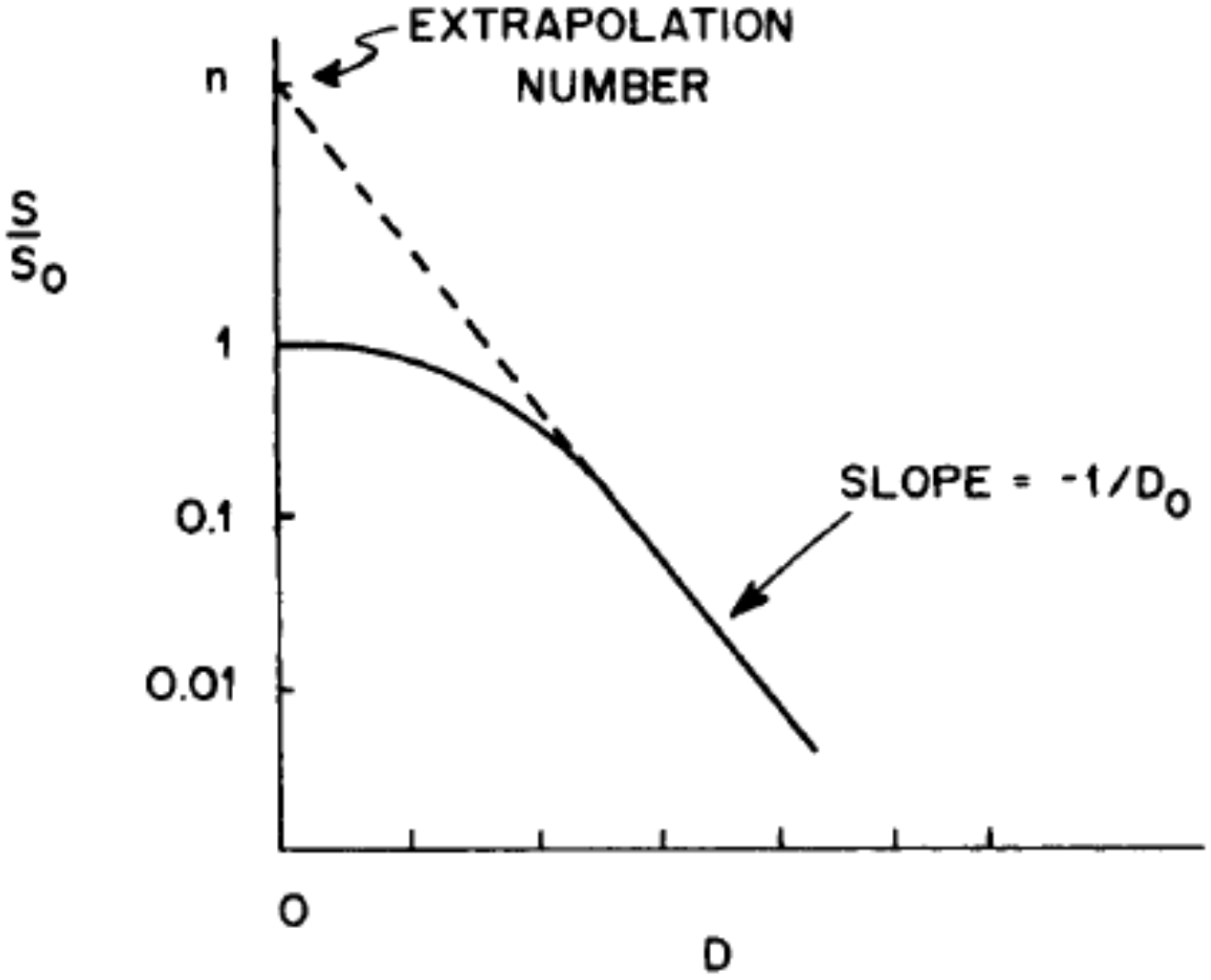


Fig. 13.14 Semilogarithmic plot of multitarget, single-hit survival.

Notice that

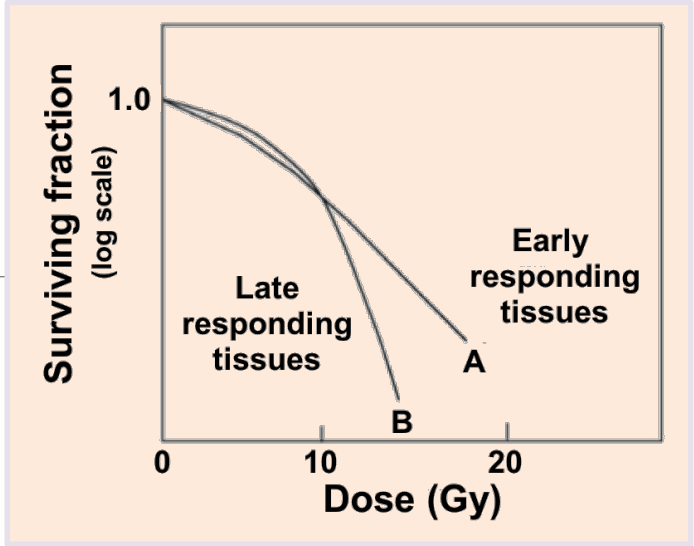
$$\left[\frac{d}{dD}e^{-\alpha D-\beta D^2}\right]_{D=0} = (-\alpha - 2\beta D)e^{-\alpha D-\beta D^2}\Big|_{D=0} = -\alpha$$

and that

$$\frac{d}{dD}\left[1-(1-e^{-D/D_0})^n\right]_{D=0} = -n\,\frac{e^{-D/D_0}}{D_0}(1-e^{-D/D_0})^{n-1}\Big|_{D=0} = 0$$

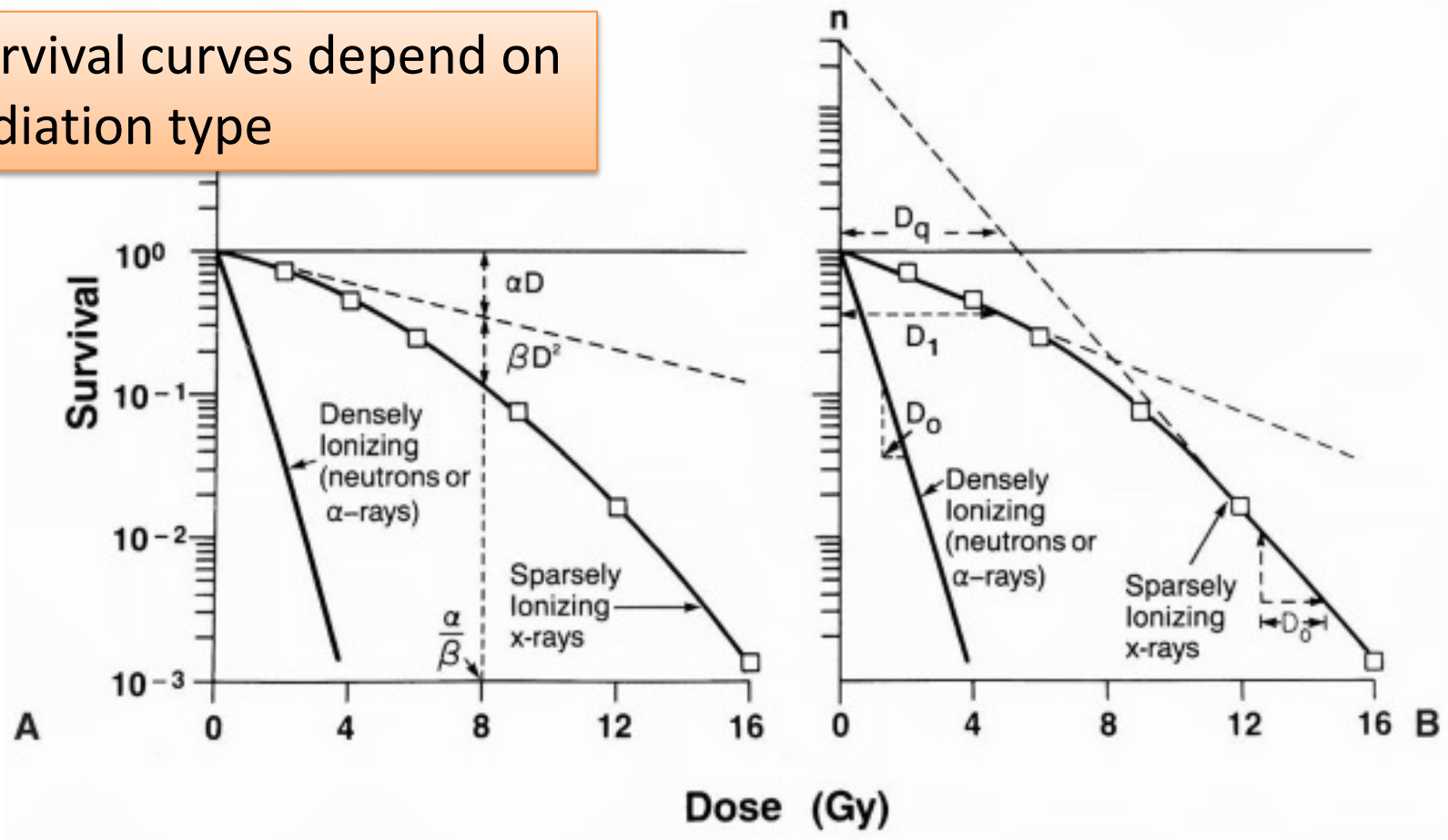
The derivatives differ in the origin, and the multitarget model does not exactly reproduce the observed linear-quadratic law.
Other explanation of the LQ law exist, but there is no general agreement on any of them.

Properties of cell survival curves

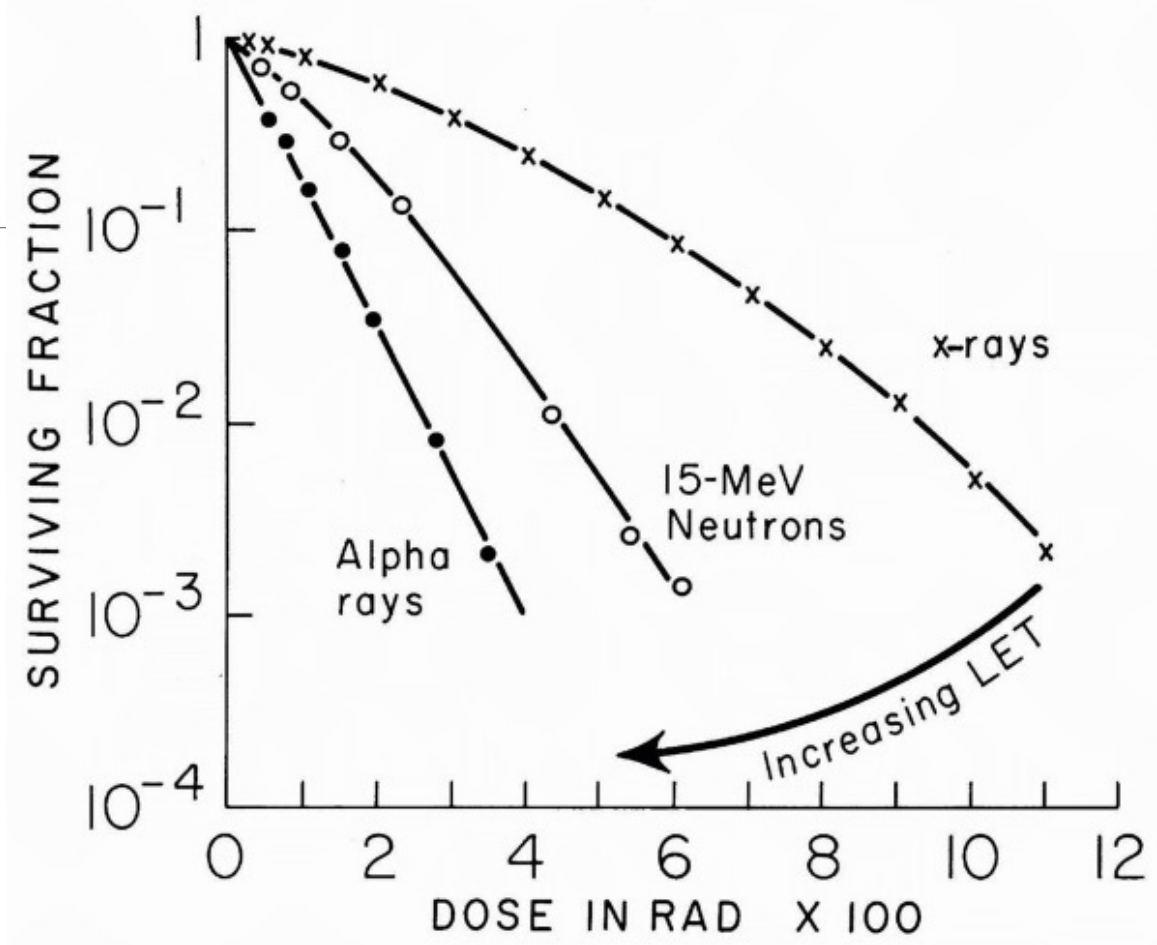


- For late responding tissues the survival curves are more curved than those for early responding tissues.
- For early effects the alpha/beta ratio is large; for late effects it is small.
- For early effects alpha dominates at low doses.
- For late effects beta has an influence at doses lower than for early responding tissues.
- The alpha and beta components of mammalian cell killing are equal at the following doses:
  - alpha/beta ≈ 10 Gy for early responding tissues.
  - alpha/beta ≈ 3 Gy for late responding tissues.

Survival curves depend on radiation type



For α-particles or low-energy neutrons (said to be densely ionizing), the dose-response curve is a straight line from the origin (i.e., survival is an exponential function of dose). The survival curve can be described by just one parameter, the slope. For x- or γ-rays (said to be sparsely ionizing), the dose-response curve has an initial linear slope, followed by a shoulder; at higher doses, the curve tends to become straight again. **A:** The linear quadratic model. The experimental data are fitted to a linear-quadratic function. There are two components of cell killing: One is proportional to dose (αD); the other is proportional to the square of the dose (βD^2). The dose at which the linear and quadratic components are equal is the ratio α/β. The linear-quadratic curve bends continuously but is a good fit to experimental data for the first few decades of survival. **B:** The multitarget model. The curve is described by the initial slope (D_1), the final slope (D_0), and a parameter that represents the width of the shoulder, either n or D_q. (taken from Eric J. Hall, Amato J. Giaccia: "Radiobiology for the Radiologist" Lippincott Williams & Wilkins (2005))



Survival curves for cultured cells of human origin exposed to 250-kVp x-rays, 15-MeV neutrons, and 4-MeV α-particles. As the linear energy transfer of the radiation increases, the slope of the survival curves gets steeper and the size of the initial shoulder gets smaller. (Adapted from Broerse JJ, Barendsen GW, van Kersen GR: Survival of cultured human cells after irradiation with fast neutrons of different energies in hypoxic and oxygenated conditions. *Int J Radiat Biol Relat Stud Phys Chem Med* 13:559-572, 1968; and Barendsen GW: Responses of cultured cells, tumors, and normal tissues to radiation of different linear energy transfer. *Curr Top Radiat Res Q* 4:293-356, 1968.)

RBE (Relative Biological Effectiveness)

If a dose D of a given type of radiation produces a specific biological endpoint, then RBE is defined as the ratio

$$\mathrm{RBE} = \frac{D_X}{D}$$

where D_X is the X-ray dose needed under the same conditions to produce the same endpoint.

RBE is not a constant

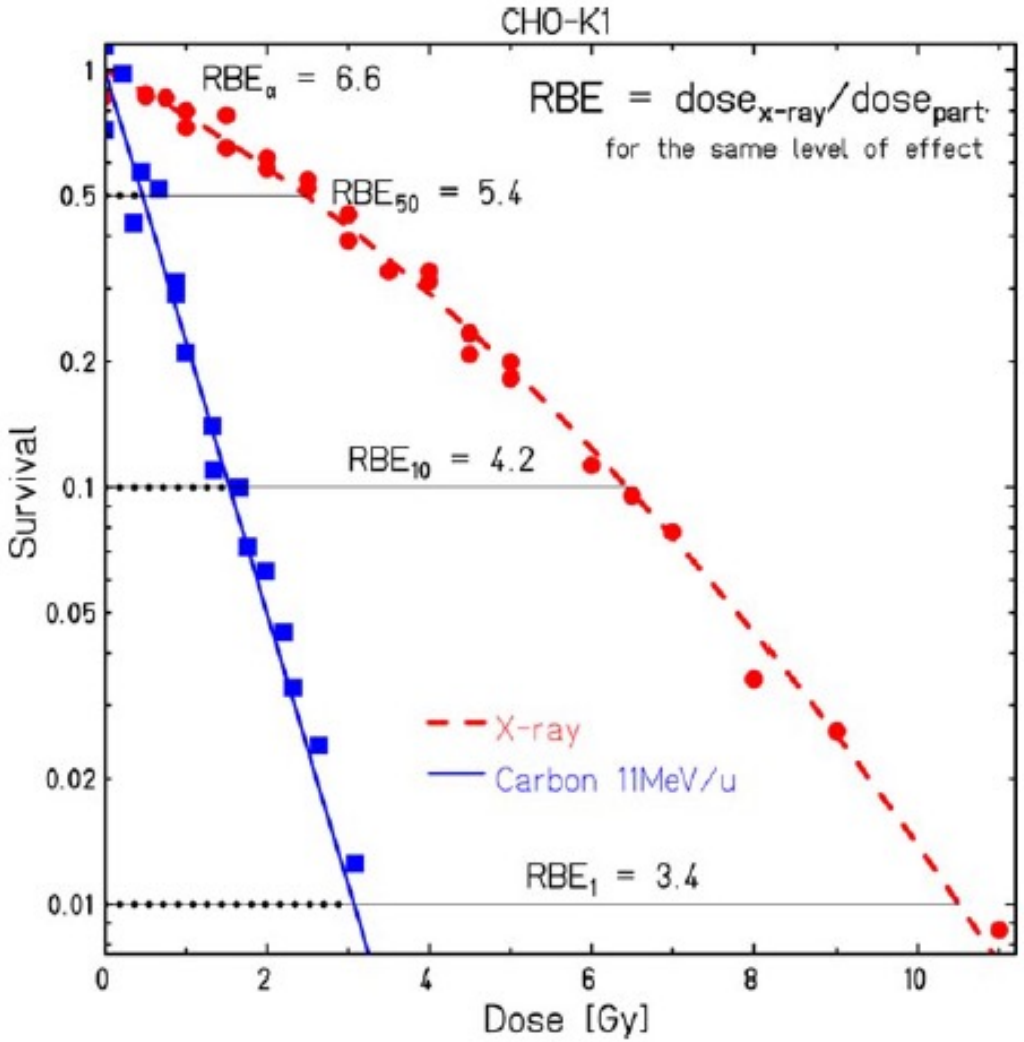
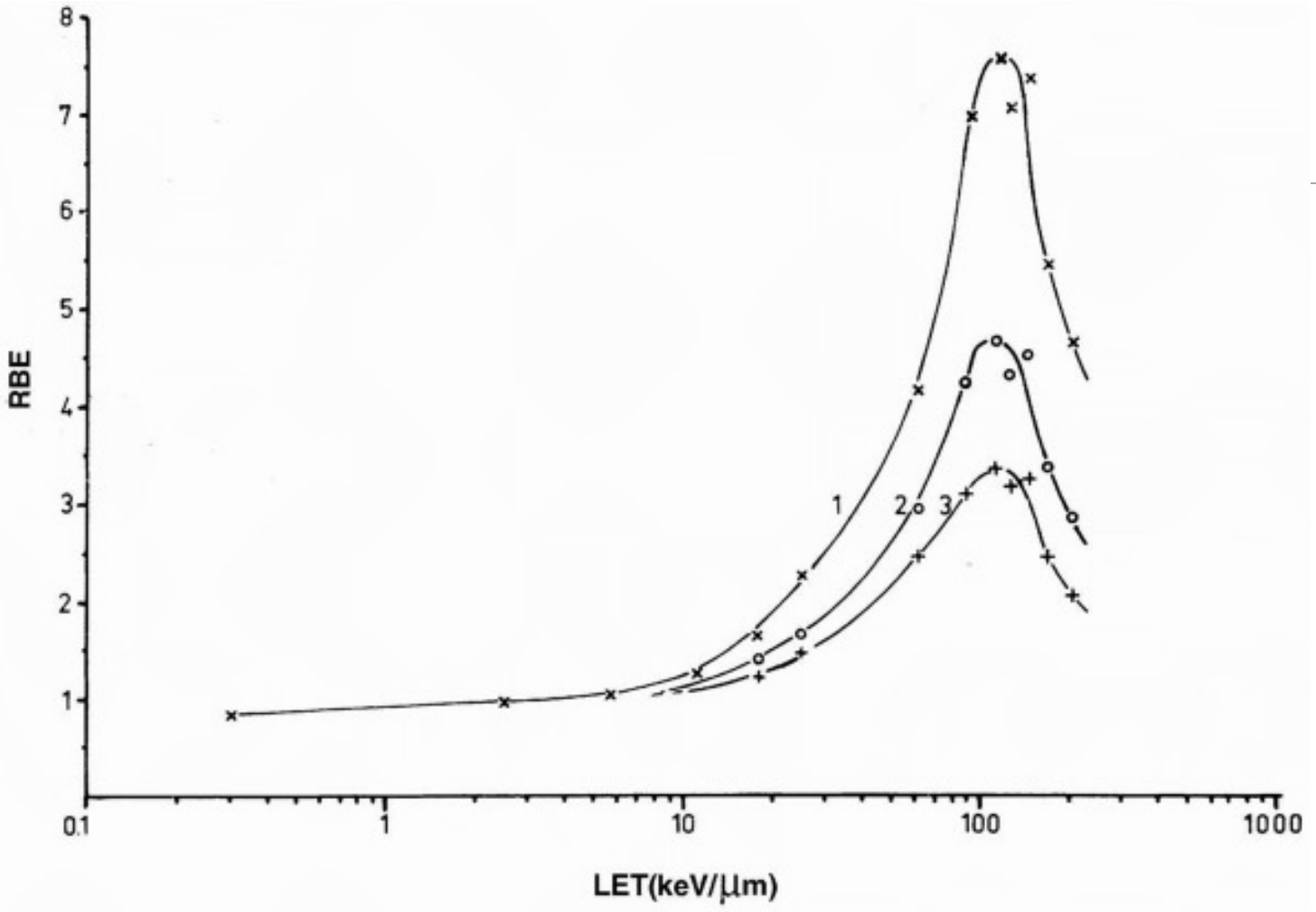
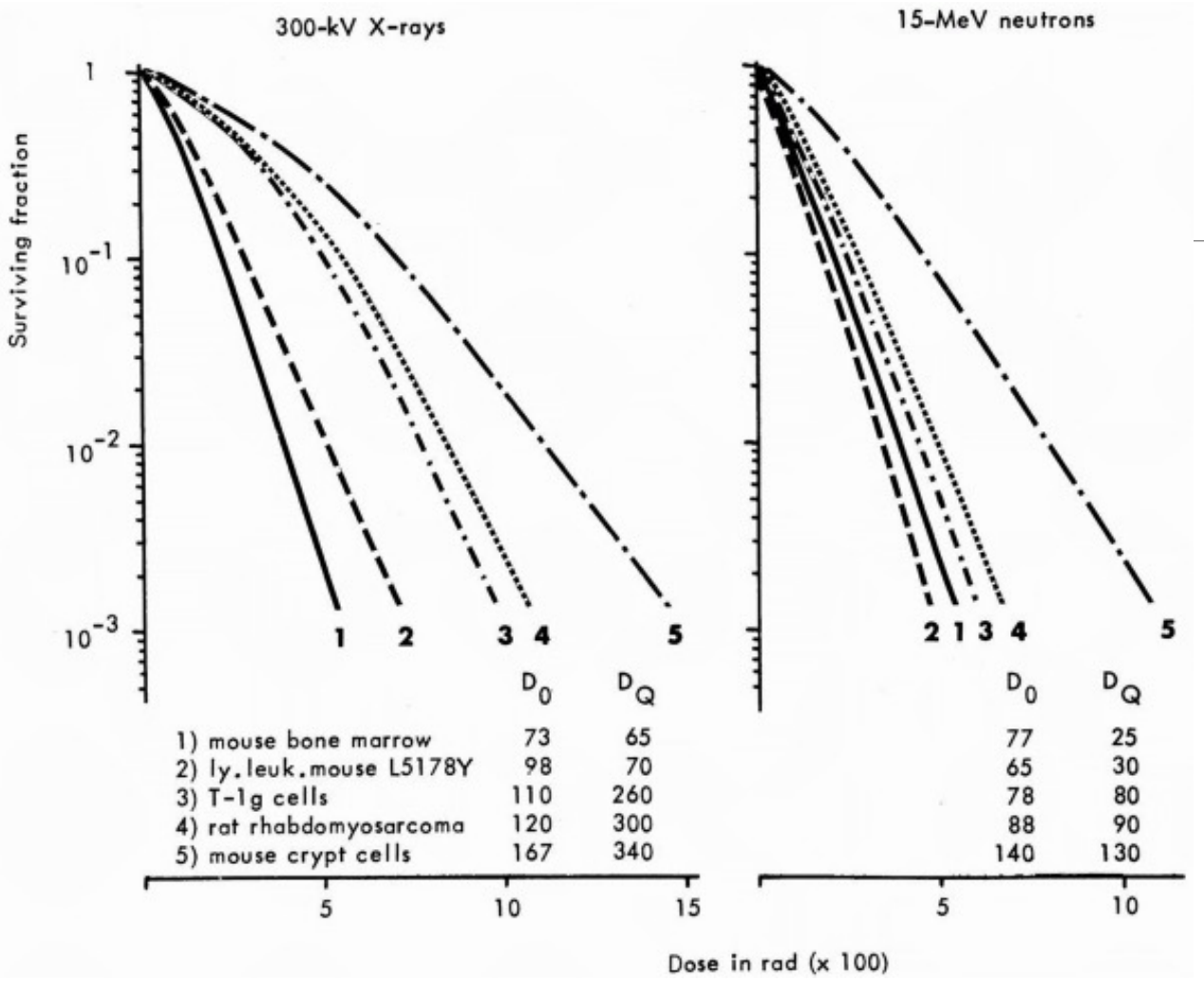


Fig. 1. Clonogenic survival curves illustrating the higher efficiency of the carbon ions compared with X-rays [10] (courtesy of the author, dr. Wilma K. Weyrather).



Variation of relative biologic effectiveness (RBE) with linear energy transfer (LET) for survival of mammalian cells of human origin. The RBE rises to a maximum at an LET of about 100 keV/μm and subsequently falls for higher values of LET. Curves 1, 2, and 3 refer to cell survival levels of 0.8, 0.1, and 0.01, respectively, illustrating that the absolute value of the RBE is not unique but depends on the level of biologic damage and, therefore, on the dose level. (From Barendsen GW: Responses of cultured cells, tumors, and normal tissues to radiation of different linear energy transfer. *Curr Top Radiat Res Q* 4:293-356, 1968.)

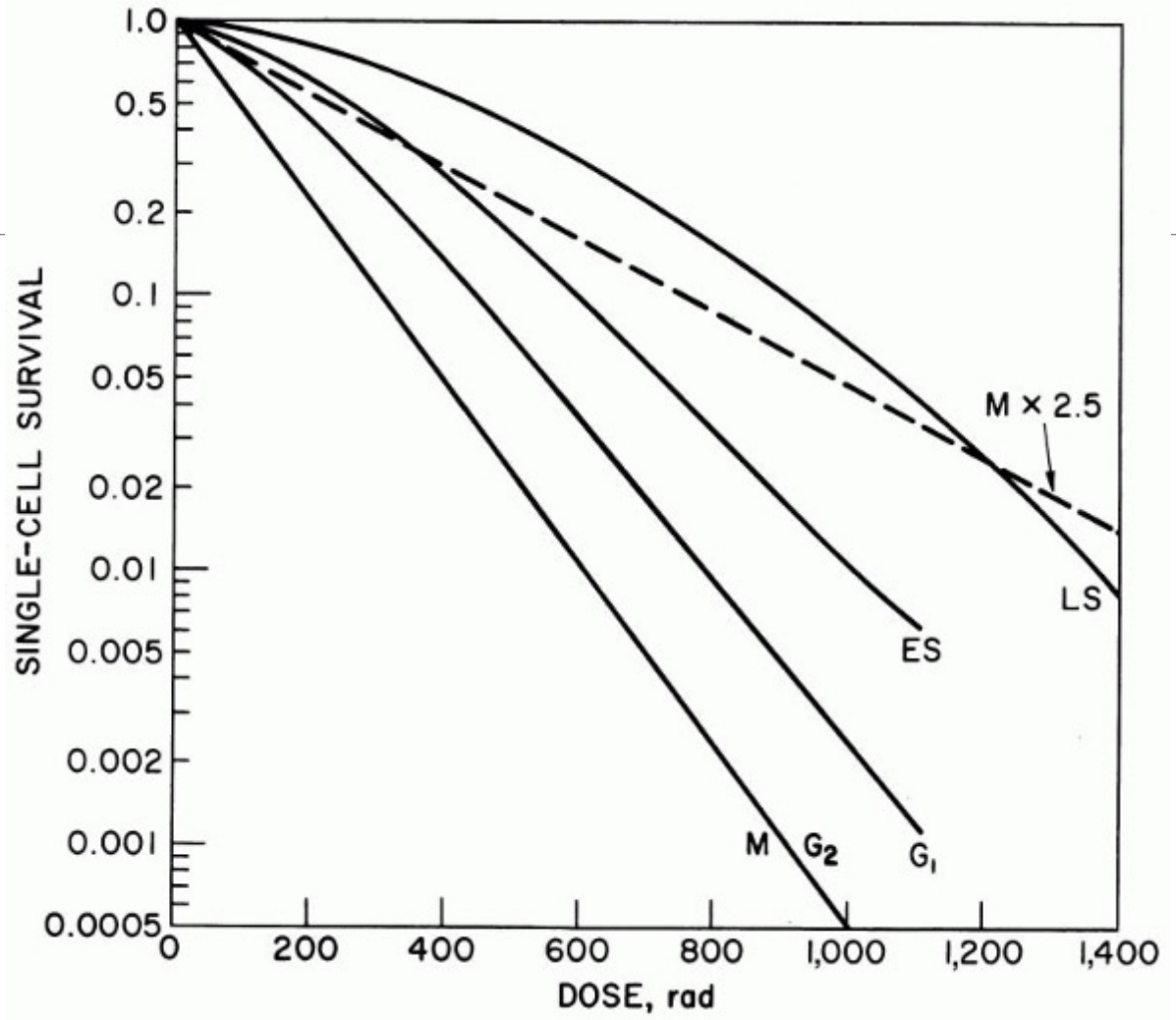
Survival curves depend on cell line



Survival curves for various types of clonogenic mammalian cells irradiated with 300-kV x-rays or 15-MeV $d^+ \rightarrow T$ neutrons: curve 1, mouse hematopoietic stem cells; curve 2, mouse lymphocytic leukemia cells L5178Y; curve 3, T1 cultured cells of human kidney origin; curve 4, rat rhabdomyosarcoma cells; curve 5, mouse intestinal crypt stem cells. Note that the variation in radiosensitivity among different cell lines is markedly less for neutrons than for x-rays.

(From Broerse JJ, Barendsen GW: Relative biological effectiveness of fast neutrons for effects on normal tissues. *Curr Top Radiat Res Q* 8:305-350, 1973)

Survival curves depend on cell phase



Cell survival curves for ovarian Chinese hamster cells at various stages of the cell cycle. The survival curve for cells in mitosis is steep and has no shoulder. The curve for cells late in S phase is shallower and has a large initial shoulder. G_1 and early S phases are intermediate in sensitivity. The *broken line* is a calculated curve expected to apply to mitotic cells under hypoxia. (From Sinclair WK: Cyclic x-ray responses in mammalian cells in vitro. *Radiat Res* 33:620-643, 1968.)

Several factors can make cells less radio-sensitive

- Removal of oxygen to create a hypoxic state.
- Addition of chemical radical scavengers.
- Use of low dose rates or multi-fractionated irradiation.
- Synchronization of cells in the late S phase of the cell cycle.

Dissolved oxygen in tissue acts as a radio-sensitizing agent, this is the so-called **oxygen effect.**

The curves show the survival of cells irradiated under identical conditions, except that one culture contains dissolved O_2 (e.g., from the air) and the other is purged with N_2.

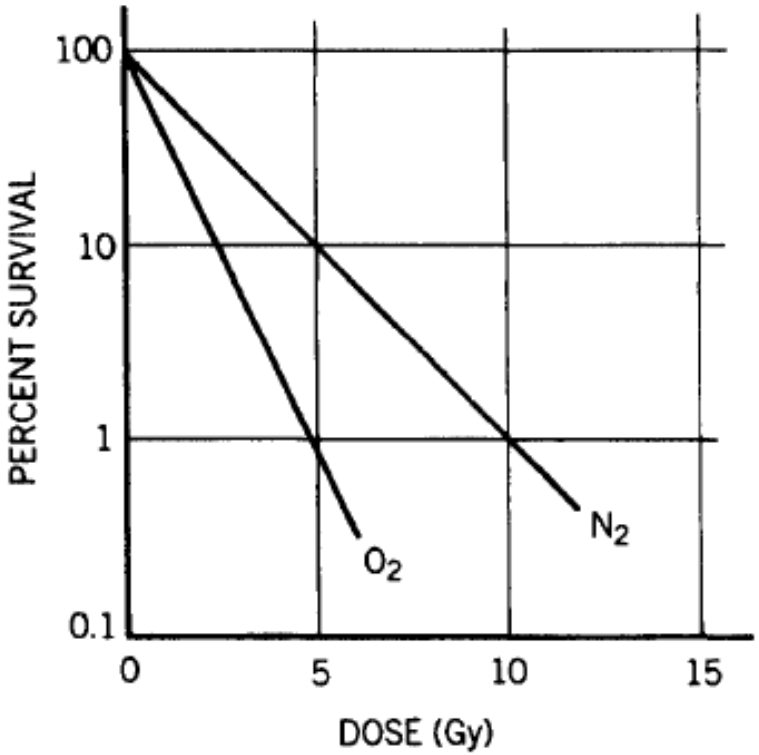


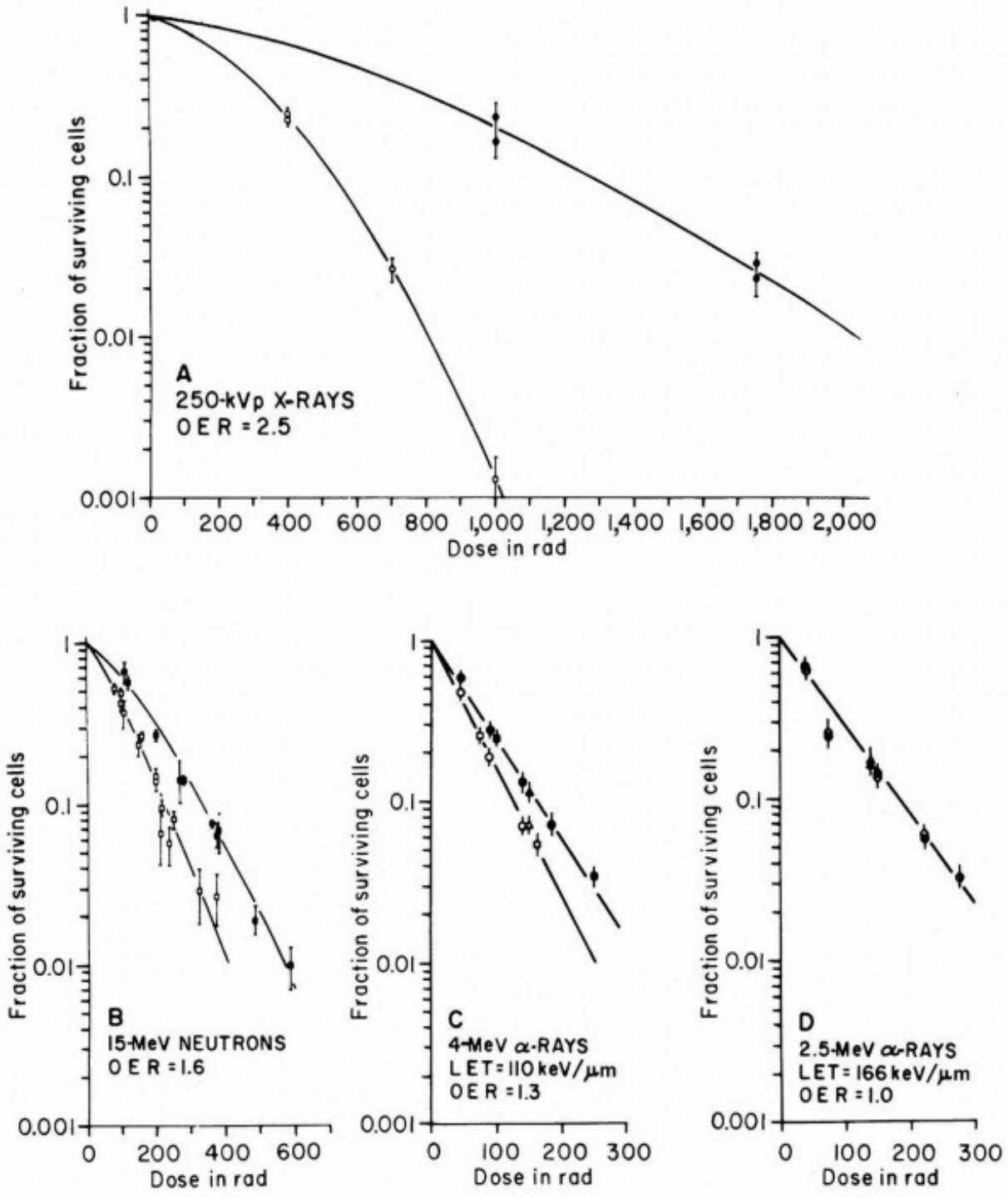
Fig. 13.18 Cell survival in the presence of dissolved oxygen (O_2) and after purging with nitrogen (N_2).

The effect of oxygen can be expressed quantitatively by means of the **oxygen enhancement ratio** (OER), defined as the ratio of the dose required under conditions of hypoxia and that under conditions in air to produce the same level of effect.

OER values are typically 2–3 for X rays, gamma rays, and fast electrons; around 1.7 for fast neutrons; and close to unity for alpha particles.

The existence of the oxygen effect provides strong evidence of the importance of indirect action in producing biological lesions.

Dissolved oxygen is most effective with low- rather than high-LET radiation, because intratrack reactions compete to a lesser extent for the initial reaction product.



Survival curves for cultured cells of human origin determined for four different types of radiation. Open circles refer to aerated conditions and closed circles to hypoxic conditions.

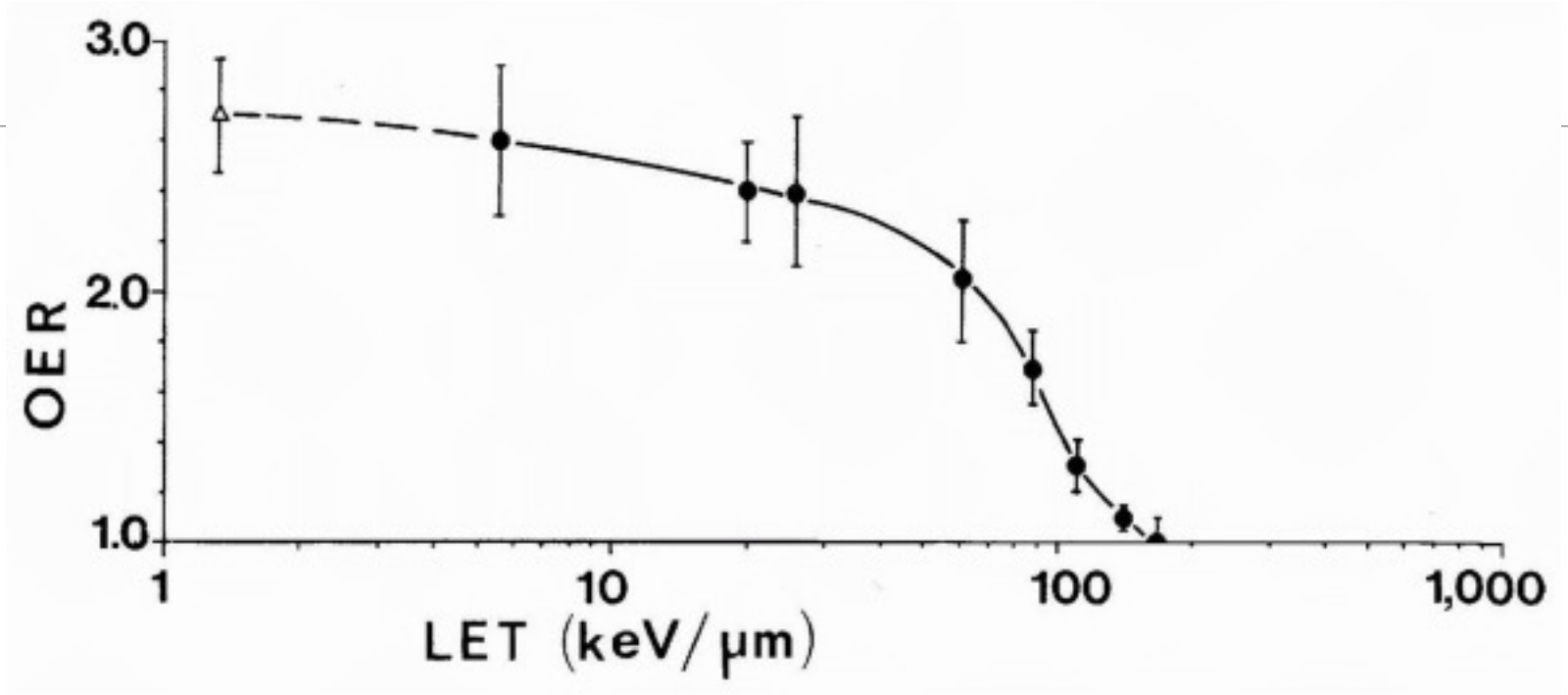
A: For 250-kVp x-rays, oxygen enhancement ratio (OER) = 2.5.

B: For 15-MeV $d^+ \rightarrow$ T neutrons, OER = 1.6.

C: For 4-MeV α-particles, linear energy transfer (LET) = 110 keV/μm, 0ER = 1.3.

D: For 2.5-MeV α-particles, LET = 166 keV/μm, OER = 1.

(Adapted from Broerse JJ, Barendsen GW, van Kersen GR: Survival of cultured human cells after irradiation with fast neutrons of different energies in hypoxic and oxygenated conditions. *Int J Radiat Biol Relat Stud Phys Chem Med* 13:559-572, 1968; and Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SB, Parnell CJ: The effect of oxygen on impairment of the proliferative capacity of human cells in culture by ionizing radiations of different LET. *Int J Radiat Biol Relat Stud Phys Chem Med* 10:317-327, 1966.)



Oxygen enhancement ratio as a function of linear energy transfer. Measurements were made with cultured cells of human origin. Closed circles refer to monoenergetic charged particles, the open triangle to 250-kVp x-rays with an assumed track average LET of 1.3 keV/μm.

(From Barendsen GW, Koot CJ, van Kersen GR, Bewley DK, Field SB, Parnell CJ: The effect of oxygen on impairment of the proliferative capacity of human cells in culture by ionizing radiations of different LET. *Int J Radiat Biol Relat Stud Phys Chem Med* 10:317-327, 1966.)

Comments on LQ

- *Most radiation oncologists use the LQ model:*
  - it is simple and has a microdosimetric basis
  - α/β is large (> 6 Gy) when survival curve is almost exponential and small (1-4 Gy) when shoulder is wide
  - the α/β value is useful to quantify the sensitivity of a tissue/tumor to fractionated therapy.
- *But:*
  - Both α and β vary with the cell cycle phase. At high doses, S phase and hypoxic cells become more important.
  - The α/β ratio varies depending upon whether a cell is quiescent or proliferative
  - **The LQ model best describes data in the range of 1 - 6Gy and should not be used outside this range**

Survival curves may deviate from the LQ model at low and high doses

- Certain cell lines and tissues, are hypersensitive at low doses of 0.05-0.2Gy.
- The survival curve plateaus over 0.05-1Gy
- Not seen for all cell lines or tissues, but has been reported in skin, kidney and lung
- At high doses, the model does not fit data well because D^2 dominates the equation

HT29 cells

(from Lambin et al., Int. J. Rad. Biol. 63 (1993) 639

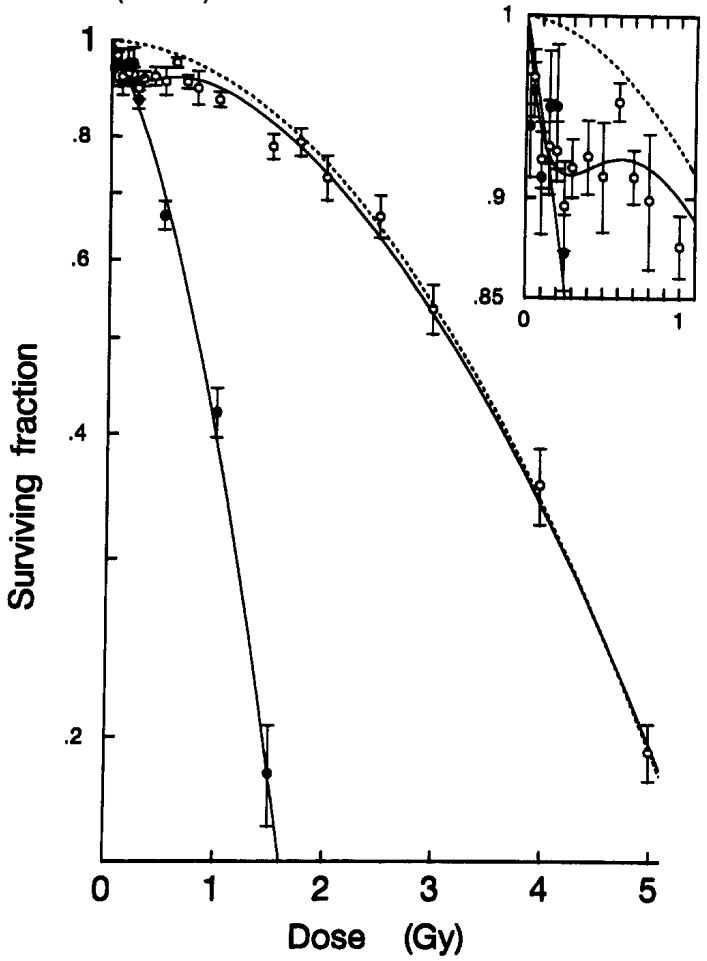


Figure 2. Survival of cells irradiated with 240 kVp X-rays (open symbols) and d(4)-Be neutrons (closed symbols). Each point shows the mean value ±SEM of the data from all five experiments. The dotted line shows the fit of the LQ model to the X-ray data ⩾2 Gy. The solid lines show the fit of the IR model to the X-ray data and the fit of the LQ model to the neutron data.

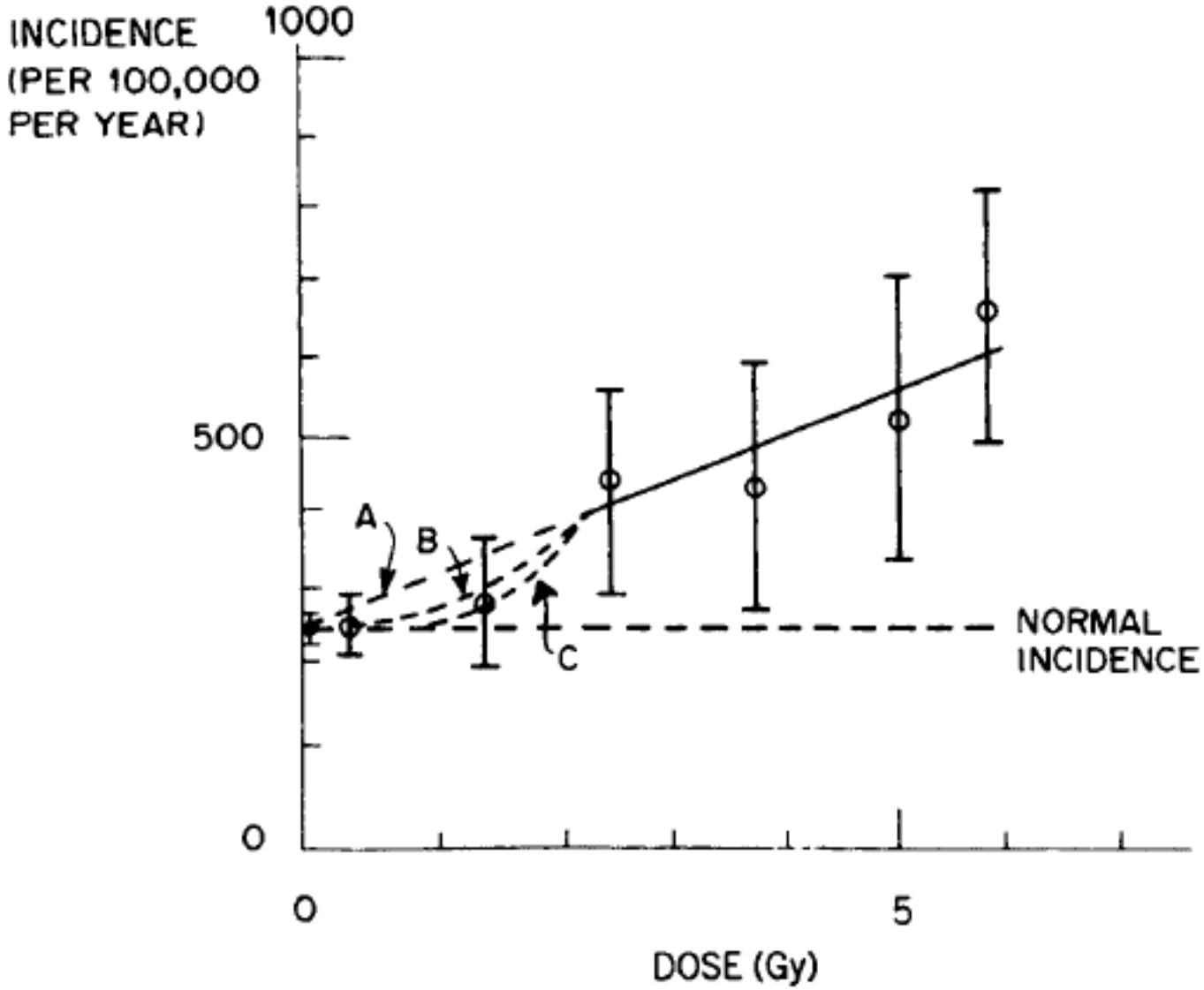


Fig. 13.11 Example of a dose–response curve, showing the incidence of an effect (e.g., certain cancers per 100,000 population per year) as a function of dose. Circles show measured values with associated error bars. Solid line at high doses is drawn to extrapolate linearly (dashed curve A) to the level of normal incidence at zero dose. Dashed curve B shows a nonlinear extrapolation to zero dose. Dashed curve C corresponds to having a threshold of about 0.75 Gy.

Low-level radiation

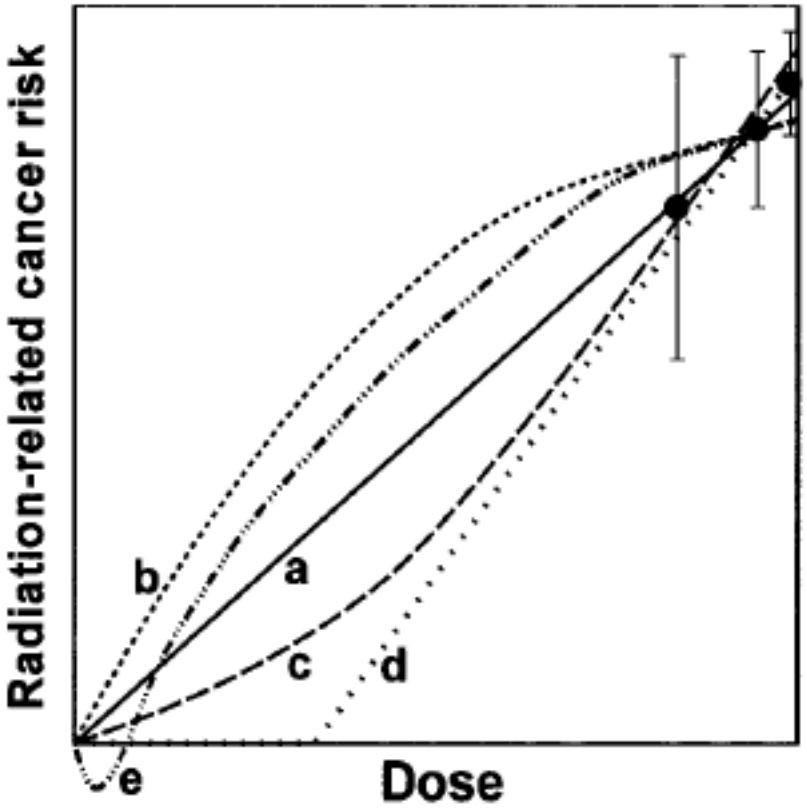


Fig. 3. Schematic representation of different possible extrapolations of measured radiation risks down to very low doses, all of which could, in principle, be consistent with higher-dose epidemiological data. Curve a, linear extrapolation; curve b, downwardly curving (decreasing slope); curve c, upwardly curving (increasing slope); curve d, threshold; curve e, hormetic.

from D. J. Brenner et al.: "Cancer risks attributable to low doses of ionizing radiation: Assessing what we really know", PNAS **100** (2003) 13761

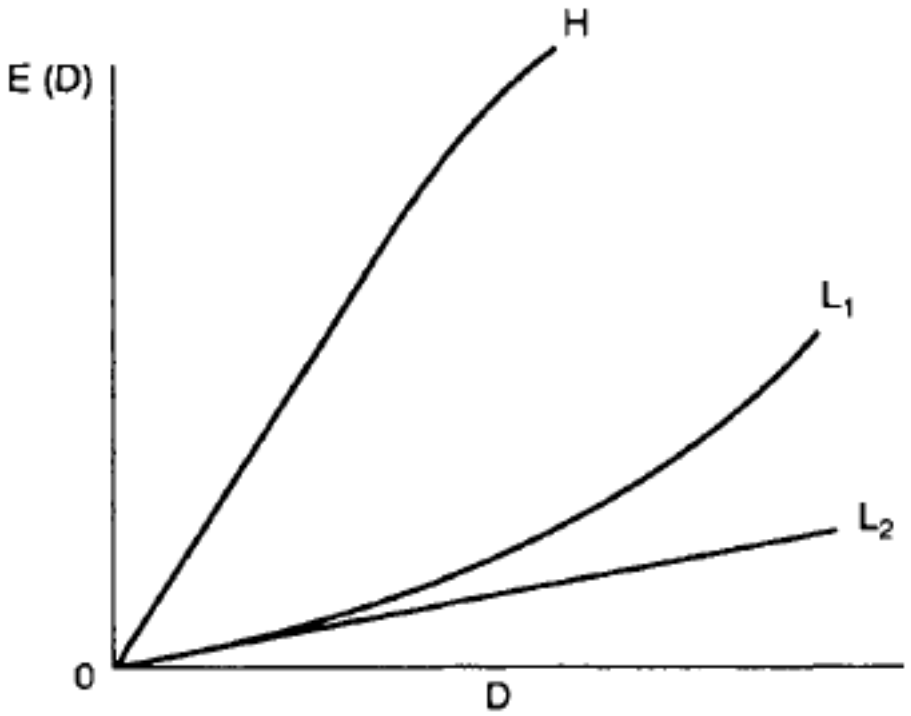


Fig. 13.12 Schematic representation of dose–response function $E(D)$ at low doses D for high-LET (curve H) and low-LET (curve L_1) radiations. L_2 is the extension of the linear beginning of L_1.

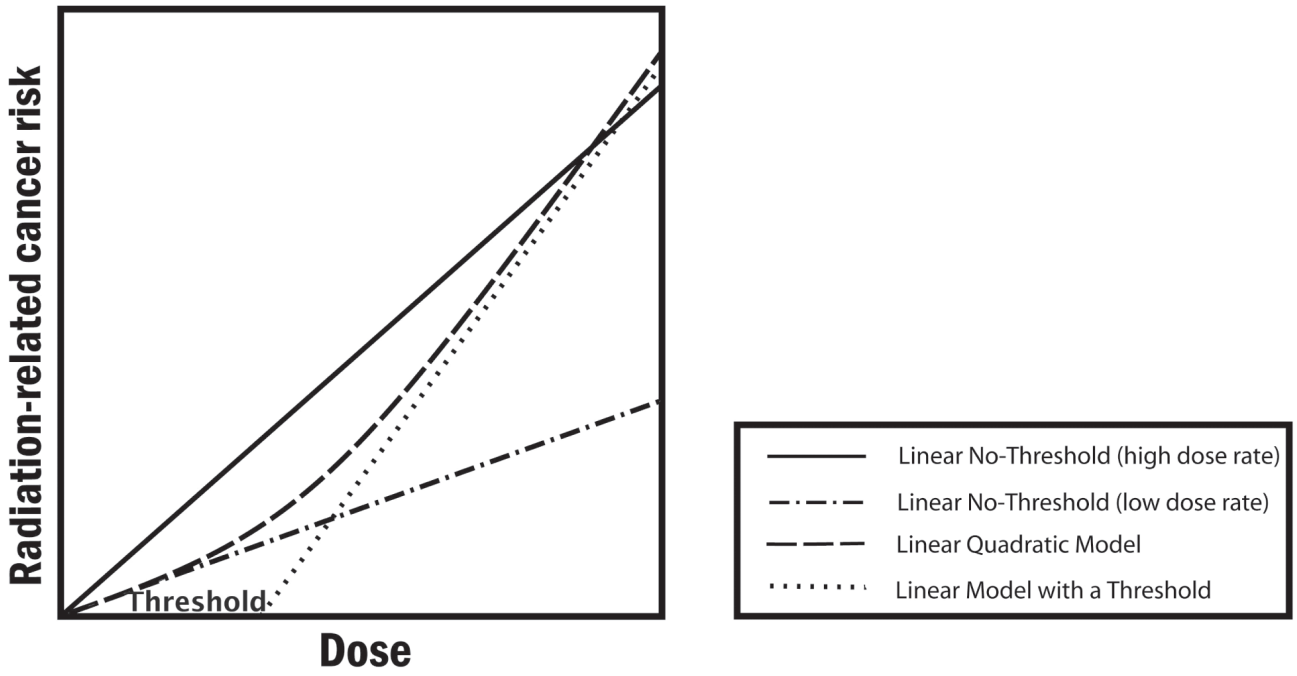


FIGURE PS-3 The committee finds the linear no-threshold (LNT) model to be a computationally convenient starting point. Actual risk estimates improve upon this simplified model by using a dose and dose-rate effectiveness factor (DDREF), which is a multiplicative adjustment that results in downward estimation of risk and is roughly equivalent to using the line labeled "Linear No-Threshold" (low dose rate). The latter is the zero-dose tangent of the linear-quadratic model. While it would be possible to use the linear-quadratic model directly, the DDREF adjustment to the linear model is used to conform with historical precedent dictated in part by simplicity of calculations. In the low-dose range of interest, there is essentially no difference between the two. Source: Modified from Brenner and colleagues.[17]

(source Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII – Phase 2)

Response of tissues: early- and late-responding tissues

The skin, intestinal epithelium, and bone-marrow cells, for example, are **rapidly dividing self-renewal tissues and respond early to the effects of radiation**.

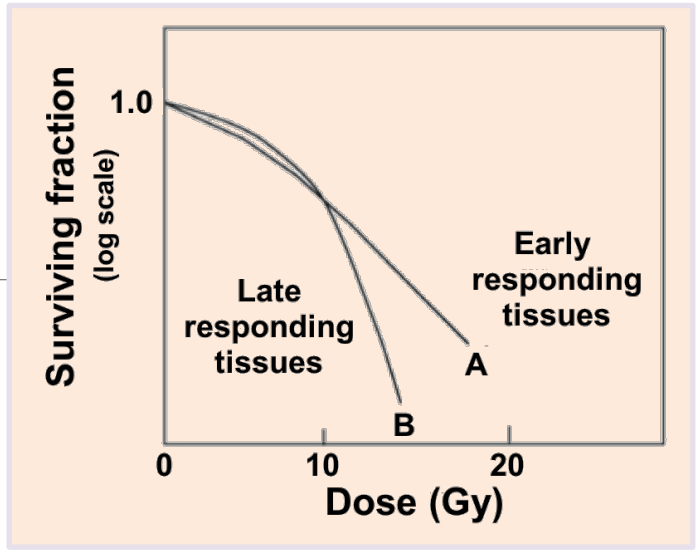
The spinal cord, lung, and kidney, by contrast, are **late-responding tissues**.

This reflects the current philosophy that the radiation response of *all* tissues results from the depletion of the critical parenchymal cells and that **the difference in time at which early- and late-responding tissues express radiation damage is a function simply of different cell turnover rates**.

Many older papers in the literature ascribe the response of late-responding tissues to vascular damage rather than to depletion of parenchymal cells, but this thesis is becoming increasingly difficult to accept.

(from Hall and Giaccia)

Properties of survival curves in tissues



- For late responding tissues, the survival curves are more curved than those for early responding tissues.
- For early effects, the alpha/beta ratio is large; for late effects it is small.
- For early effects alpha dominates at low doses.
- For late effects beta has an influence at doses lower than for early responding tissues.
- The alpha and beta components of mammalian cell killing are equal at the following doses:
  - alpha/beta ≈ 10 Gy for early responding tissues.
  - alpha/beta ≈ 3 Gy for late responding tissues.

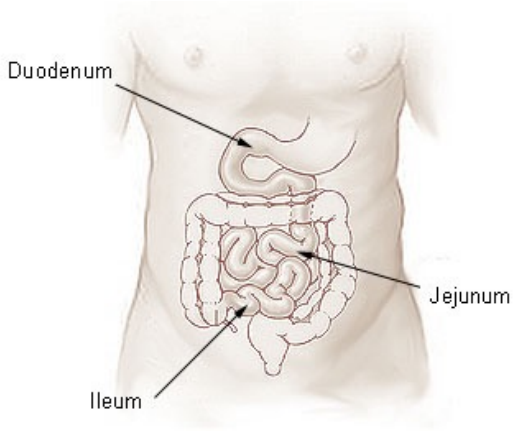
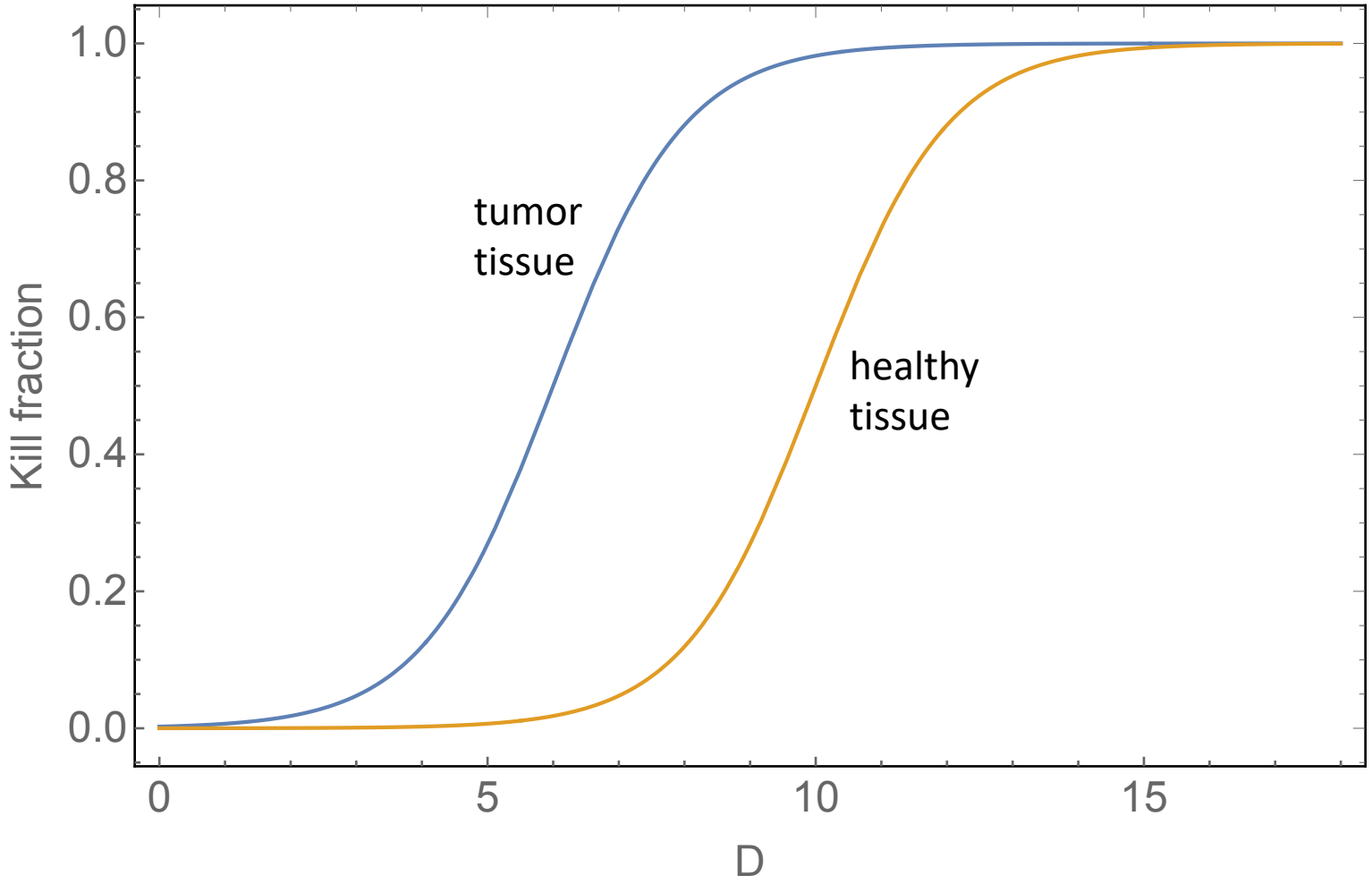


Table 8.1 Values for the α/β ratio for a variety of early- and late-responding normal tissues in experimental animals

Early reactions	α/β	References	Late reactions	α/β
Skin			**Spinal cord**	
Desquamation	9.1–12.5	Douglas and Fowler (1976)	Cervical	1.8–2.7
	8.6–10.6	Joiner *et al.* (1983)	Cervical	1.6–1.9
	9–12	Moulder and Fischer (1976)	Cervical	1.5–2.0
			Cervical	2.2–3.0
Jejunum			Lumbar	3.7–4.5
Clones	6.0–8.3	Withers *et al.* (1976)	Lumbar	4.1–4.9
	6.6–10.7	Thames *et al.* (1981)		3.8–4.1
				2.3–2.9
Colon				
Weight loss	9–13	Terry and Denekamp (1984)	**Colon**	
Clones	8–9	Tucker *et al.* (1983)	Weight loss	3.1–5.0
Testis			**Kidney**	
Clones	12–13	Thames and Withers (1980)	Rabbit	1.7–2.0
			Pig	1.7–2.0
Mouse lethality			Rats	0.5–3.8
30 days	7–10	Kaplan and Brown (1952)	Mouse	1.0–3.5
30 days	13–17	Mole (1957)	Mouse	0.9–1.8
30 days	11–26	Paterson *et al.* (1952)	Mouse	1.4–4.3
Tumour bed			**Lung**	
45 days	5.6–6.8	Begg and Terry (1984)	LD_{50}	4.4–6.3
			LD_{50}	2.8–4.8
			LD_{50}	2.0–4.2
			Breathing rate	1.9–3.1
			Bladder	
			Frequency, capacity	5–10

α/β values are in grays. LD_{50}, dose lethal to 50 per cent.

How do we translate this illustrative figure into a real optimization procedure? How do real curves behave?



Tumor Control Probability

The fraction of cells that survives a dose *D* is by definition *S(D)*, therefore when N cells are irradiated with dose *D*, on average there are *N S(D)* surviving cells.

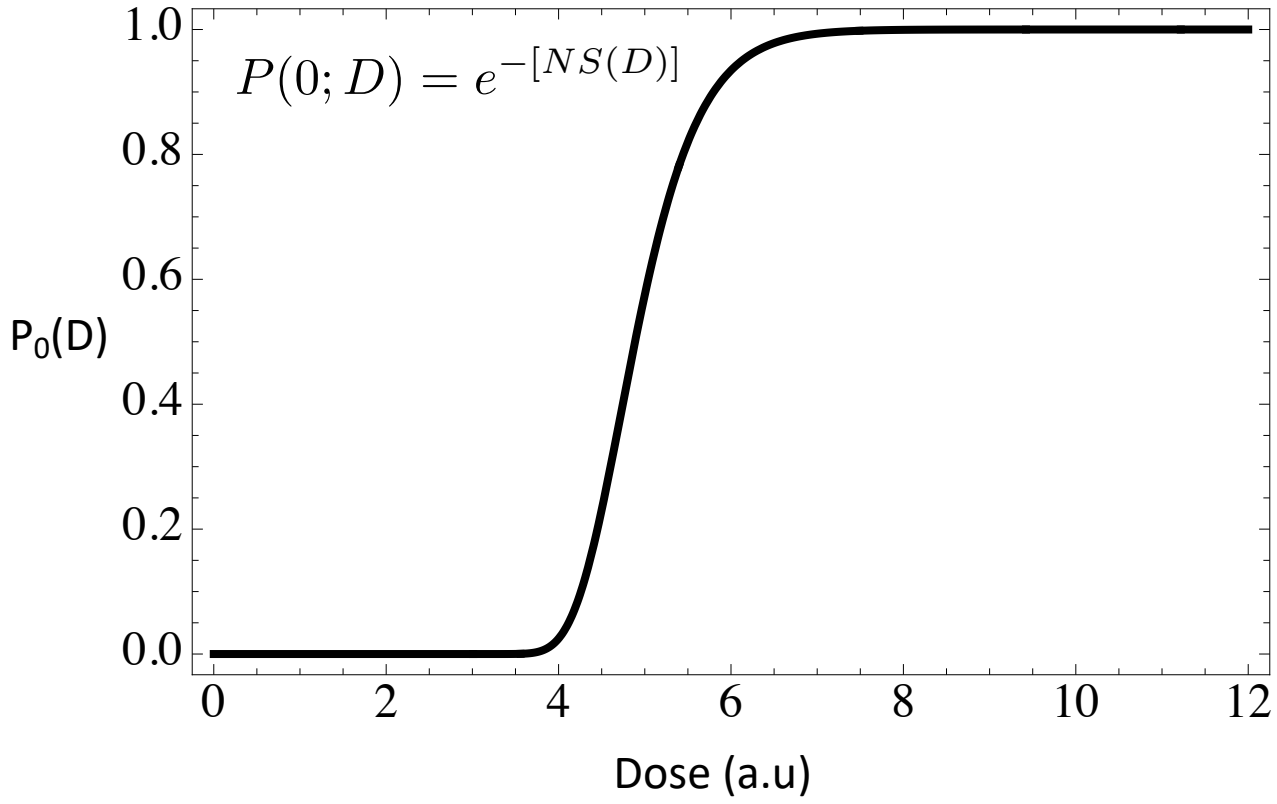
This means that when we use a Poisson probability model, the probability of finding *n* surviving cells is

$$P(n;D) = \frac{[NS(D)]^n}{n!} e^{-[NS(D)]}$$

and the probability of finding 0 surviving cells (a total kill !) is

$$P(0;D) = e^{-[NS(D)]}$$

using a linear-quadratic model for S(D), one finds a sigmoid curve



The quantity

tumor volume

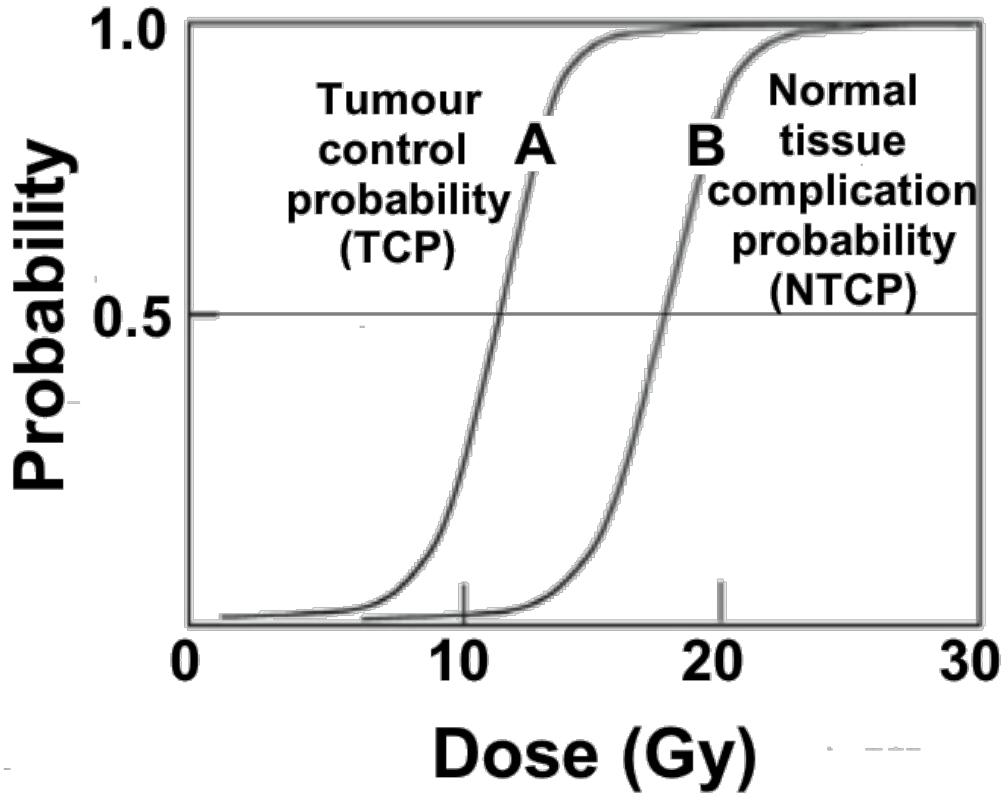
density of tumor clonogens

$$\mathrm{TCP} = P(0; D) = e^{-[NS(D)]} = e^{-\delta_C V S(D)}$$

is called the Tumor Control Probability (TCP).

Normal Tissue Complication Probability (NTCP)

Radiation is harmful for normal tissues as well as for tumors, and radiotherapy must avoid damage to normal tissues. The NTCP reproduces phenomenologically the shape of the TCP.



Lyman's mathematical description of the NTCP
(A naive sigmoid parameterization)

$$\mathrm{NTCP} = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{u} e^{-t^2/2} dt$$

with

$$u = \frac{D - TD_{50}}{m \cdot TD_{50}}$$

where m is a dimensionless parameter that tunes the slope about the midpoint of the sigmoid curve, and TD_{50} is the whole-organ dose for which NTCP = 50%