

Cell Survival Curves

Cell death

A cell that is able to proliferate indefinitely and form a large colony from a single cell is said to be **clonogenic**.

Tumor cells can be grown indefinitely in cell culture.

Normal cells must be **transformed** to grow indefinitely in culture.

For cells growing in culture, the loss of the ability to continue growth is termed **reproductive death**.

Following irradiation, cells may still be intact and able to produce proteins, synthesize new DNA and even go through one or two cell divisions. But if it has **lost the capability to reproduce indefinitely**, it is considered dead.

Very high radiation doses (10,000 rads or 100 Gy) can cause the breakdown of all cellular functions.

In contrast, the **mean lethal dose** for loss of reproductive capability is usually less than 2 Gy.

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Fig. 3.1 in Hall, Eric J. *Radiobiology for the Radiologist*, 5th ed.
Philadelphia, PA: Lippincott Williams & Wilkins, 2000.

Clonogenic Survival Assay:

- Cells from an actively growing stock are harvested by gentle scraping or by the use of trypsin.
- The number of cells per unit volume is determined manually (hemocytometer) or electronically (Coulter Counter).
- Known numbers of cells can then be plated into fresh dishes. If allowed to incubate for 1-2 weeks, clonogenic cells will form macroscopically visible colonies that can be fixed, stained and counted.
- Not every cell seeded will form a colony, even in the absence of irradiation, due to factors such as errors in counting, stress of manipulation, suboptimal growth medium, etc. The **plating efficiency (PE)** is defined as the number of colonies observed/ the number of cells plated.

- $$PE = \frac{\textit{colonies observed}}{\textit{number of cells plated}}$$

- Parallel dishes are seeded with cells that have been exposed to increasing doses of radiation. The number of cells plated is increased so that a countable number of colonies results. **Surviving fraction (SF)** is the colonies counted divided by the number of colonies plated with a correction for the plating efficiency.

- $$SF = \frac{\textit{colonies counted}}{\textit{cells seeded} \times (PE / 100)}$$

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Fig. 3.2 in [Hall].

Cell survival curves

Cell survival data are generally plotted as logarithm of the surviving fraction versus dose.

For comparing curves, it is convenient to represent them mathematically, based on hypothetical models for the mechanisms behind lethality.

The interpretation of the shape of the cell survival curve is still debated, as is the best way to fit these types of data mathematically.

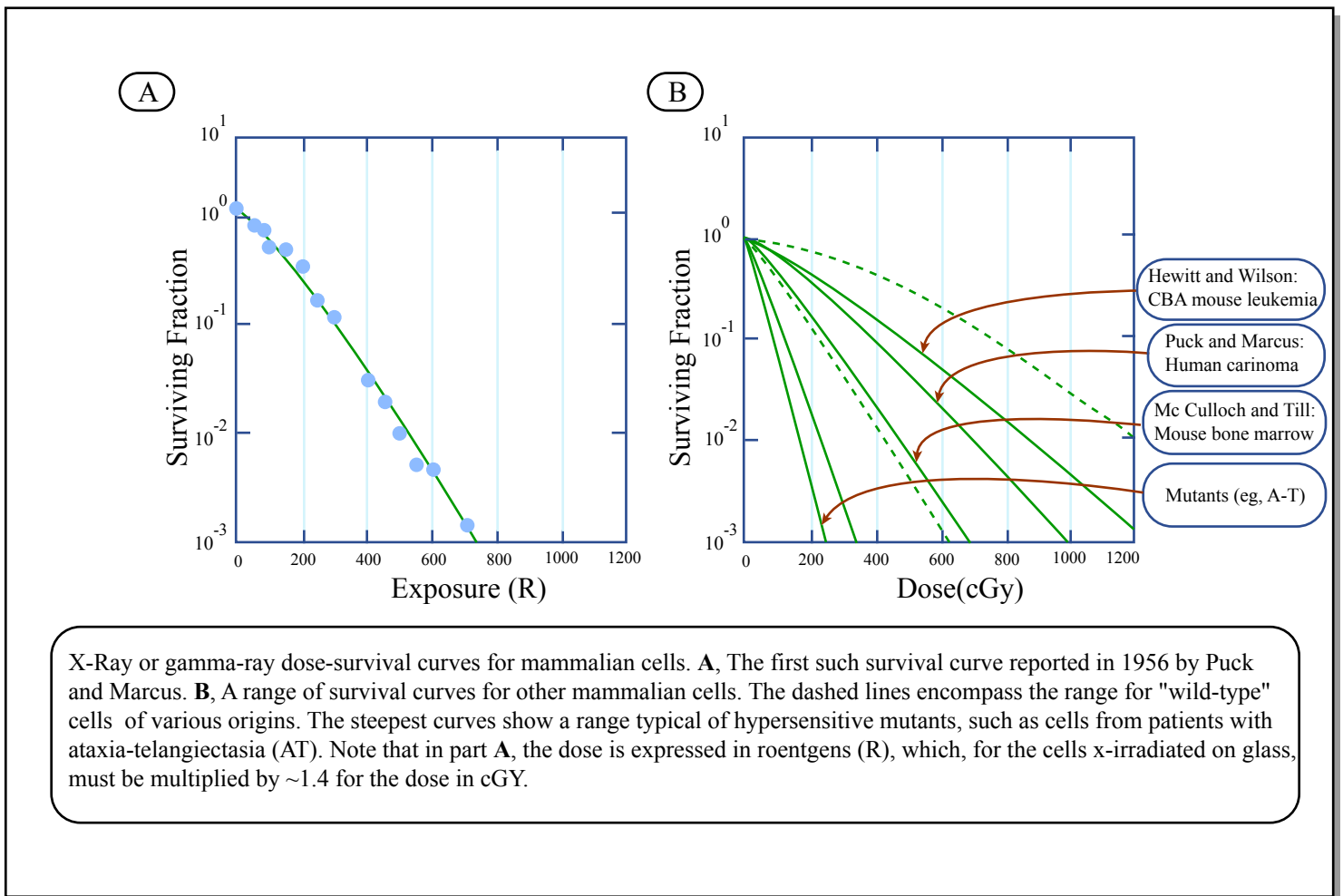


Figure by MIT OCW.

There are two basic types of cell survival curve:

Linear (exponential) or "curved"

Radiation sensitivity and the cell cycle

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Fig. 4.11 in [Hall].

Example of cell cycle times

Cell cycle phase	CHO hamster	HeLa human
T_C	11	24
T_M	1	1
T_S	6	8
T_{G1}	3	4
T_{G2}	1	11

Image removed due to copyright restrictions.
Fig. 1.5 in [Turner].

Target Theory

Target theory originated from work with *exponential dose response curves*.

It was assumed that each “hit” results in an inactivation, i.e., a “single-hit, single-target model”.

- Each cell has a single target.
- Inactivation of the target kills the cell.

Linear Survival Curves

Irradiation of cells with high-LET radiation produces linear survival curves. The relationship between the surviving fraction S and the dose D is then:

$$S = e^{-\alpha D} \quad \text{where:}$$

S is the number of surviving cells,
 $-\alpha$ is the slope, and
 D is the radiation dose delivered.

This relationship is more commonly represented as $S = e^{-D/D_0}$
by defining D_0 as $1/\alpha$.

When $D = D_0$, $S = e^{-1} = 0.37$

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Poisson Distribution

All calculations of hit probability are governed by Poisson statistics, where the probability of n events is given by

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

where x = the average number of events
and n = the specific number of events

If each “hit” is assumed to result in cell inactivation, then the probability of survival is the probability of not being hit, $P(0)$.

From the Poisson relationship, where $x = 1$, and $n = 0$,

$$P(0) = \frac{(e^{-1} \cdot 1^0)}{0!} = e^{-1} = 37\%$$

For this reason, D_0 is often called the *mean lethal dose*, or the dose that delivers, on average, *one lethal event per target*.

Exponential dose response relationships are found in certain situations

- Certain types of sensitive cells (e.g., haemopoietic stem cells)
- Synchronized populations in M and G₂
- Irradiation with high-LET radiation ***

Cell Survival Curves with Shoulders

Survival curves for most mammalian cells exposed to **low-LET radiation** show some **curvature**.

The initial low dose region in which there is less cell inactivation per unit dose than at high doses is called the **shoulder**.

Often the higher-dose region tends towards a straight line.

The parameter D_0 can then be used to characterize the radiosensitivity in this region of the curve.

Extrapolation of the terminal straight line portion of the curve back to the abscissa defines a value, n , the **extrapolation number**.

In the shoulder region of the curve the proportion of the cells killed by a given dose increases with the dose already given. Two interpretations are possible:

- Cell death results from the **accumulation of events** that are individually incapable of killing the cell, but which become lethal when added together (target models).
- Lesions are individually repairable but become irreparable and kill the cell if the **efficiency of the enzymatic repair** mechanisms diminishes with number of lesions and therefore the dose (repair models).

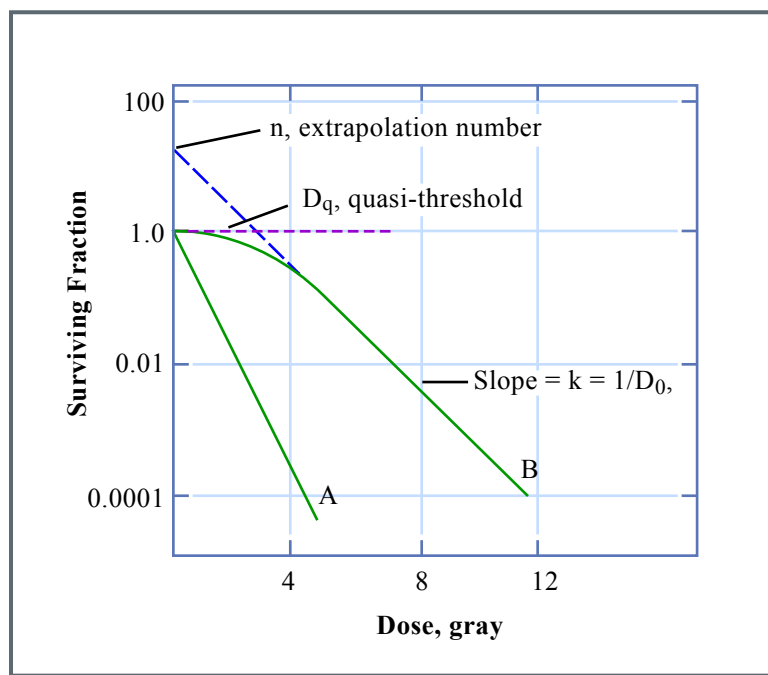


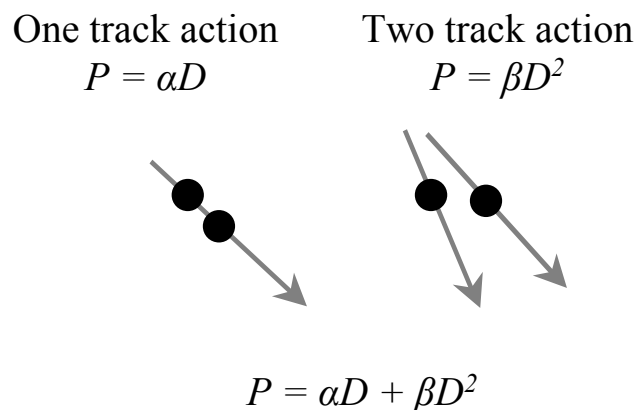
Figure by MIT OCW.

Linear-Quadratic Model (two component model):

The linear quadratic model has evolved from two similar formulations, each with roots in target theory.

Theory of Dual Radiation Action

- Lesions responsible for cell inactivation result from the interaction of sublesions.
- At least two sublesions are required for cell inactivation.
- Sublesions can be produced by the passage of one or two radiation tracks.



Molecular Theory of Cell Inactivation

(Chadwick and Leenhouts, 1981)

- Cell inactivation results from unrepaired DNA double-strand breaks.
- At low-LET, a dsb can result from either a single event (linear component) or two separate events (quadratic component).
- Alternatively, cell inactivation results from chromosome aberrations.
- Some aberrations are produced by a single event.
- Some aberrations are produced by two separate breaks.

Image removed due to copyright restrictions.

Observations of chromosome damage led to the assumption that since DNA has 2 strands, it must take two events to break a strand.

Linear-quadratic model

The linear quadratic model assumes that a cell can be killed in two ways.

- Single lethal event
- Accumulation of sublethal events

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If these modes of cell death are assumed to be independent,

$$S = S_1 S_n$$

Where S_1 is the **single event killing** or $e^{-\alpha D}$

And S_n is the **two event killing** which can be represented as $e^{-\beta D^2}$

The most common expression is

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

S is the fraction of cells surviving a dose D and α and β are constants.

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Fig. 3.6 in [Hall].

Linear-quadratic model

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Fig. 3.3 in [Hall].

Useful parameters from linear quadratic cell survival curves:

- D_1 , the **initial slope**, due to single event killing, the dose to reduce survival to 37%
- D_0 , the **final slope**, interpreted as multiple-event killing, the dose to reduce survival by 67% from any point on the linear portion of the curve.
- some quantity to describe **the width of the shoulder**. The extrapolation of the final slope D_0 , back to the y axis yields n , the **extrapolation number**. The larger the value of n , the larger the shoulder on the survival curve.

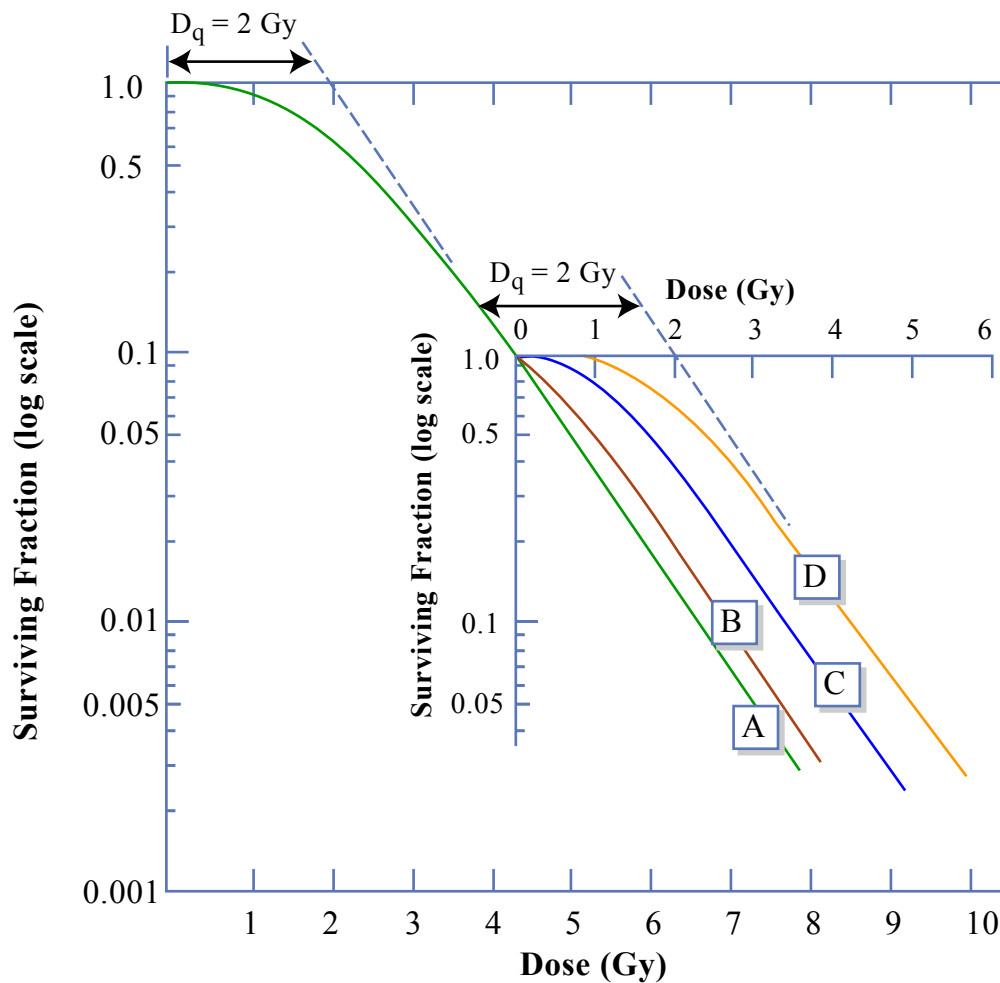
Linear survival curves are easy to understand

The curvature in the shoulder continues to be “interpreted”

Repair is definitely involved...

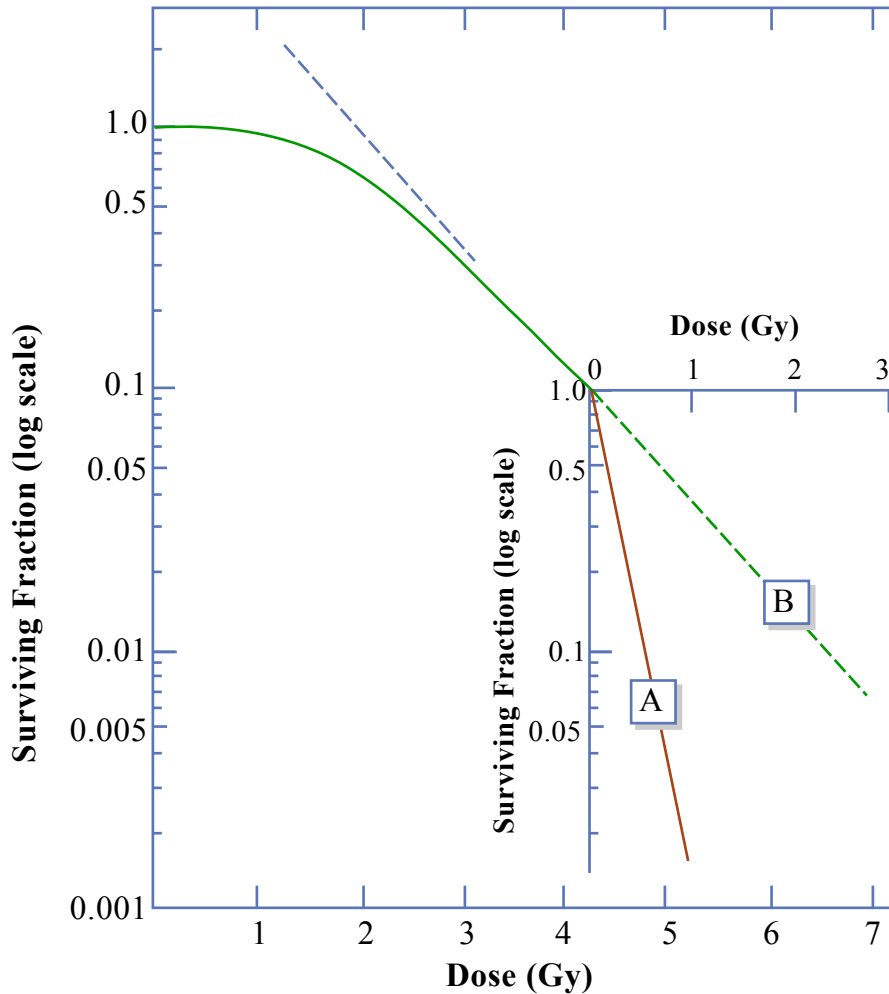
Classic split-dose experiments, Elkind and Sutton, 1959

Two doses of low-LET radiation



The Elkind-Sutton experiment. The cells are first irradiated to a surviving fraction of about 0.1. These preirradiated cells are then used as stock for further irradiations after holding the cells for various times. Curve A is for cells in which the irradiation is continuous with no interruption. Curves B, C, and D are for cells irradiated after 15 min, 2 h, and 6 h, respectively.

Low-LET followed by high-LET



Survival curves for an Elkind-Sutton type of experiment when the conditioning done is undertaken with low LET radiations, and the test exposure is done with high LET exposure. Curve B is the survival that would have occurred if the second irradiation were done with low LET radiation and no repair time had been allowed before the administration of the test irradiation. Curve A is the expected result for the second irradiation being administered with high LET radiation. The survival curve, A, is the same whether a time interval has been allowed between irradiations or not, and the survival curve after the high LET test dose is the same as if it were given to previously unirradiated cells.

High-LET followed by low-LET

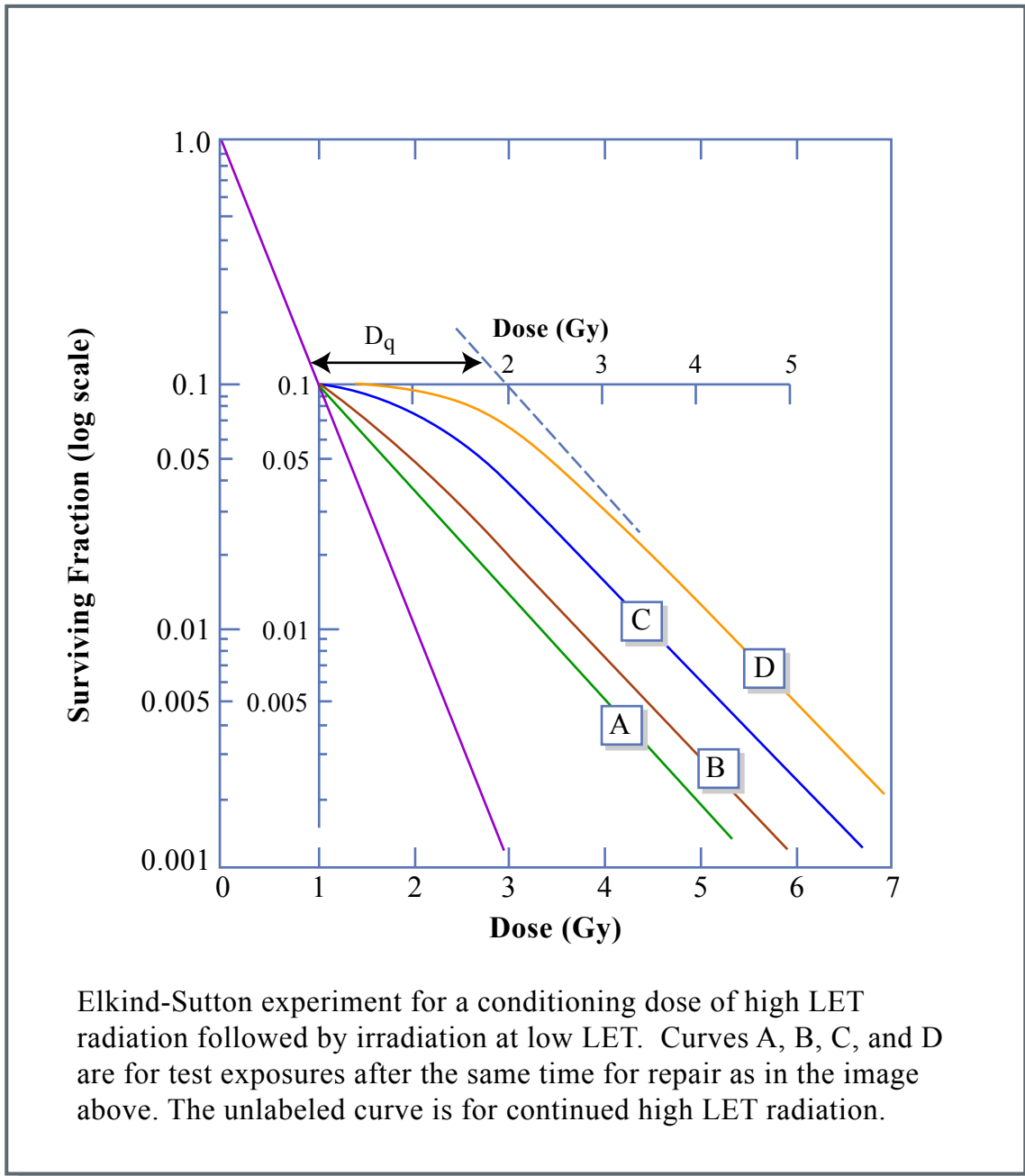


Figure by MIT OCW.