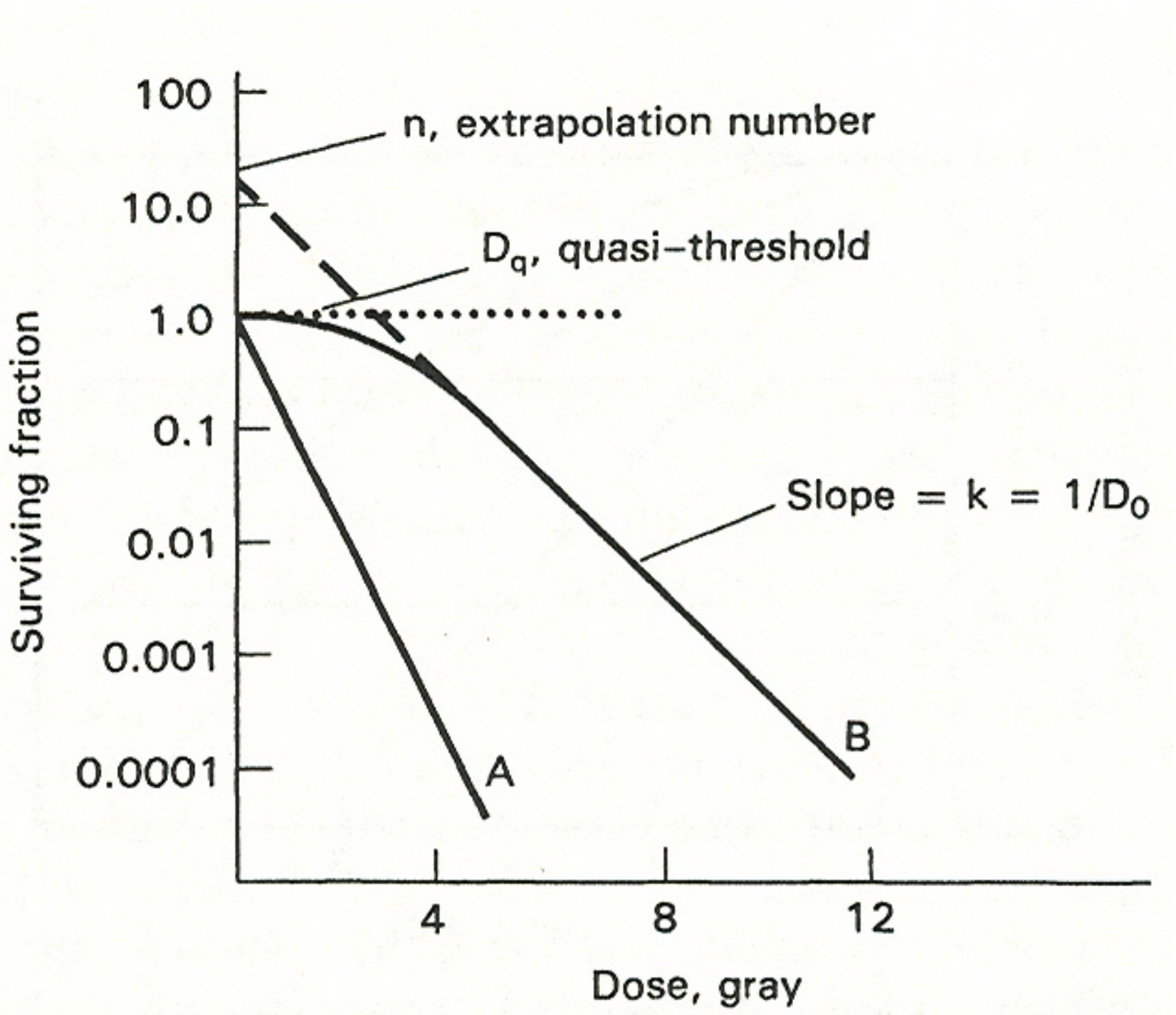
Module 6
Survival curves

- This lesson will focus on modifications to the radiation response of the cell, particularly the effects of oxygen and energy transfer on the response of the cell to radiation.
- The effects of sex, age, water, chemicals (radiosensitizers and radioprotectors) on irradiation will be discussed.
- Dose rate will be addressed, in context with radiotherapy.


100
10.0
1.0
0.1
0.01
0.001
0.0001
Surviving fraction
n, extrapolation number
D_q, quasi–threshold
Slope = k = $1/D_0$
A
B
4
8
12
Dose, gray

Dose-Response Curves

- Several clonogenic assays can determine dose-response relationships for cells of normal tissues. The assays for skin colony, testes, kidney tubule, and jejunum crypts can determine the reproductive integrity of individual cells. Bone marrow cells, thyroid cells, and mammary cells all involve transplantation into another site. Skin reactions in pigs, lung response in mice, and spinal cord damage can be used to determine functional endpoints for dose-response curves.

- In general, the response of a tissue or organ to radiation depends on two factors: (1) inherent sensitivity of the individual cells, and (2) kinetics of the population as a whole of which the cells are a part.

- In highly-differentiated tissues that have specialized functions, the cell survival curves are irrelevant due to the lack of mitotic future of the cells. The radiation needed to destroy the function of the cell is much greater than that to stop mitosis of the cell.

Survival Curves

- The survival curves of mammalian cells can be used to obtain direct information on their response to radiation.
- When the logarithm of a typical cell survival is plotted versus dose on a linear scale, several features become evident. At larger doses of radiation, the graph becomes a straight line on this scale. At low doses, a shoulder on the curve is found, which is quantified by the threshold dose. The width of shoulder of the survival curve indicates the degree of sublethal damage, which is more readily repaired in the S phase.

D_m, D_0, D_q

- The ***mean lethal dose***, D_m, is defined as that amount of radiation required to reduce the survival by 37%. It is equal to D_{37} in the linear portion of the curve. A large D_m indicates a radioresistant line of cells, whereas a small D_m is characteristic of cells with a high radiosensitivity.
- The D_0 is the ***slope of the exponential portion of the curve***, which is the nonlethal dose. The ***threshold dose***, D_q, is a measure of how much damage occurs before it is lethal. The D_q is a measure of the width of the shoulder. A large D_q indicates that a cell can readily recover.
- The D_0 value is the measure of intrinsic radiosensitivity of a cell type, whereas the clinical radiosensitivity depends upon the size of the shoulder and the cellular environment.

Table 7-1. The reported mean lethal dose (D_0) and threshold dose (D_q) for various experimental mammalian cell lines

Cell Type	D_0 (rad)	D_q (rad)
Mouse oocytes	91	62
Mouse skin	135	350
Human bone marrow	137	100
Human fibroblasts	150	160
Mouse spermatogonia	180	270
Chinese hamster ovary	200	210
Human lymphocytes	400	100

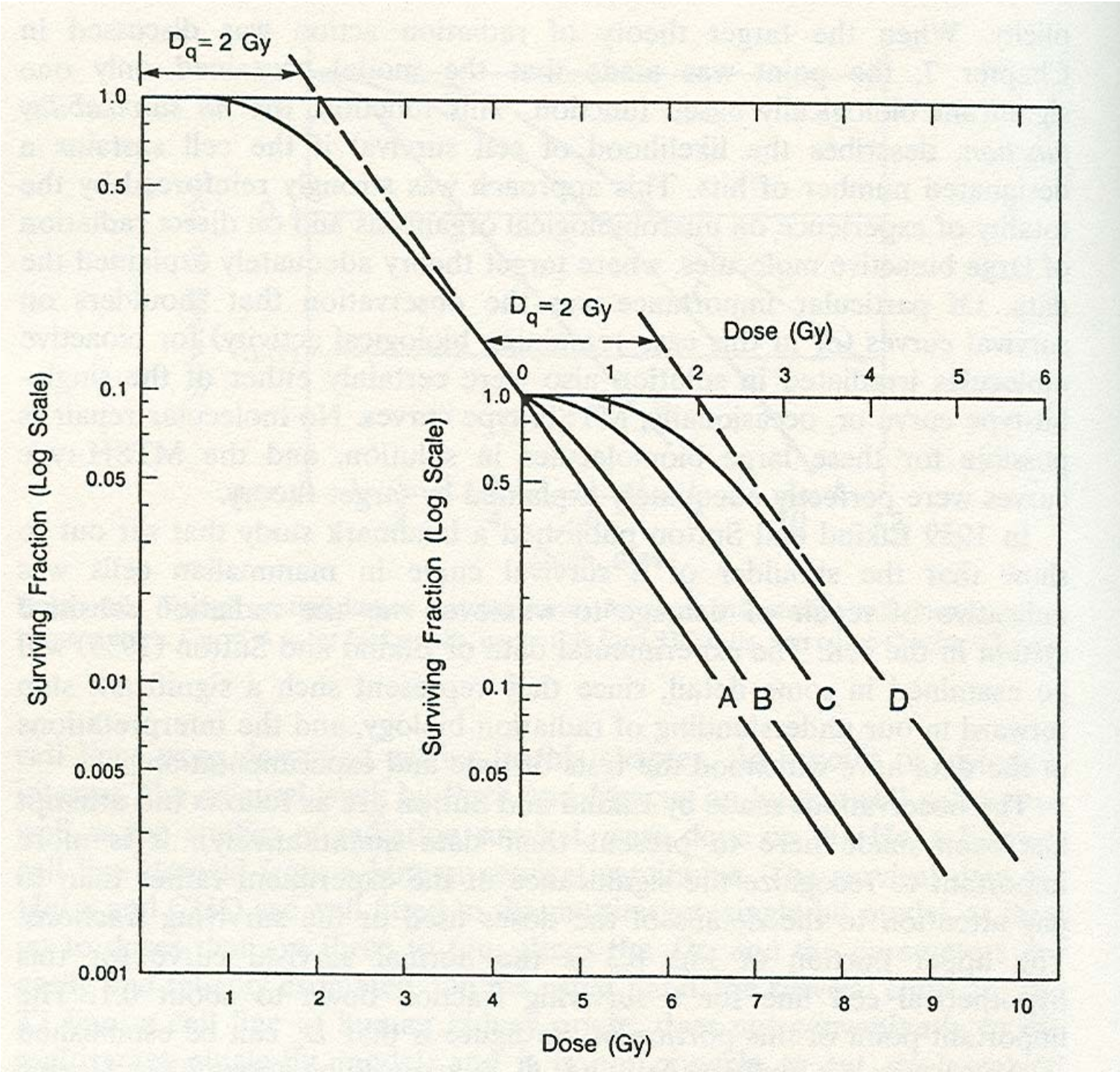
Adapted from: Stewart C. Bushong, *Radiologic Science for Technologists: Physics, Biology, and Protection,* (Elsevier Mosby, St. Louis, 8th ed. 2004), page 512.

Quasithreshold, D_q

- The shoulder of the survival curve is explained in various theories:
  - o According to the target theory, the shoulder is due to the interaction of sublethal lesions.
  - o The repair model assumes the shoulder is due to the repair of single lesions produced by single tracks of radiation in proportion to dose, a mechanism which may become saturated.

Application of Survival Curves

- The existence of a threshold in cell-survival curves implies that some damage must accumulate before it is fatal to the cell.
- The larger the value of D_q, the more damage that must accumulate before reproductive death. This damage to cells prior to cell death is called sublethal damage.
- In radiation therapy it is very important to note that when a dose is split into two parts separated by enough time, a threshold is observed for each part of the dose. Thus by properly spacing treatment, it is possible to reduce the damage to healthy cells during radiation treatment. This concept of dose fractionation will be further explored later in this lesson.



Delay in dose

A = Continuous

B = 30 minutes

C = 2 hours

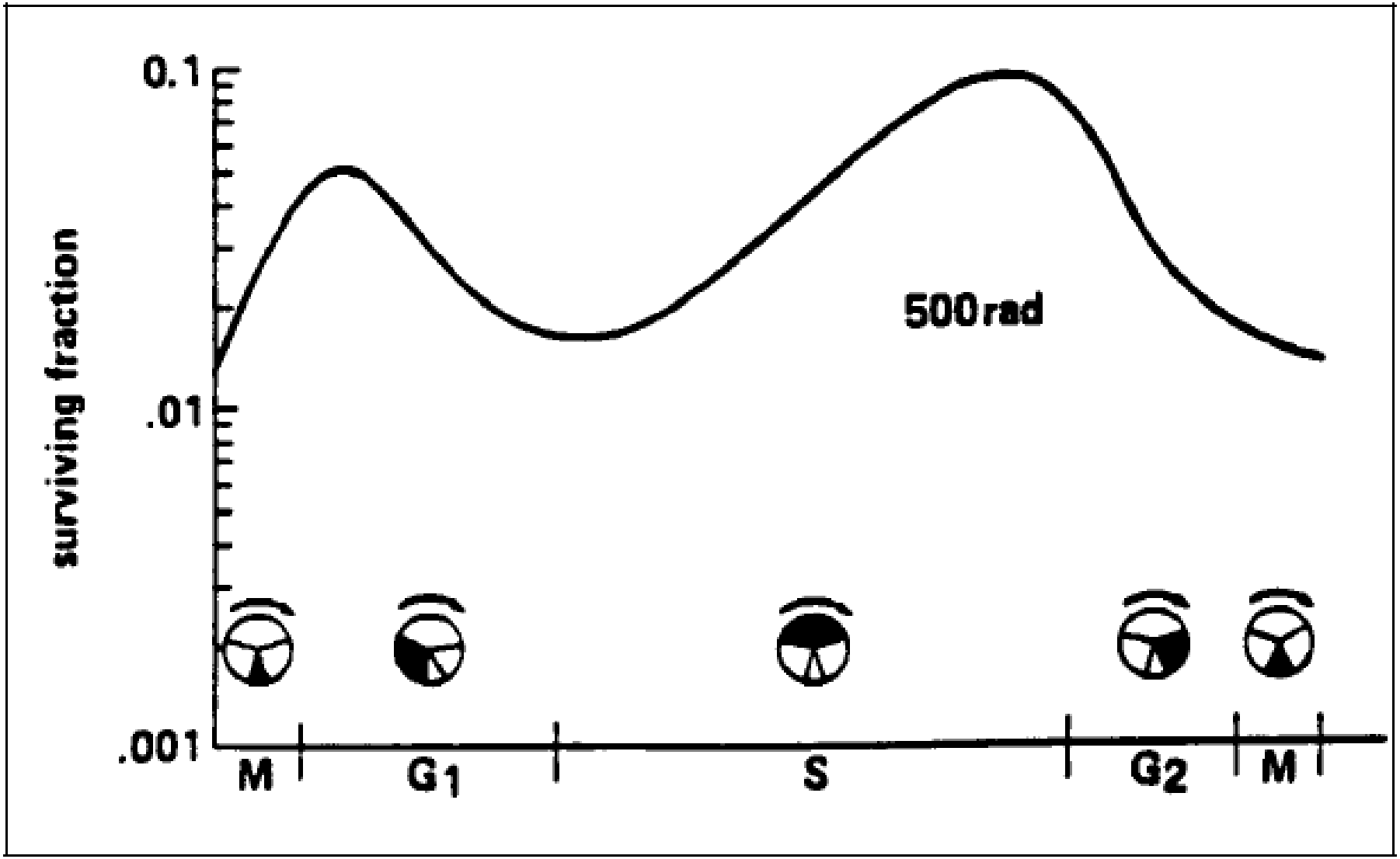
D = 6 hours

Types of Radiation Damage

- Non-lethal damage involves a lesion which does not prevent proliferation, but it affects the rate of such proliferation.
- Lethal damage is damage which is irreparable, irreversible, and leads irrevocably to cell death.
- Sublethal damage is damage which is repaired in hours unless additional sublethal damage is added, with which it interacts to form lethal damage. Basically, sublethal damage, which is chiefly evident at low dose levels, is shed after the passage of a relatively short period of time. It has been shown that the repair of sublethal damage reflects the repair and the rejoining interval of double strand breaks before they can interact to form lethal lesions.
- Potentially lethal damage is the damage that can be modified by postirradiation environmental conditions. This type of damage is manifest in those cell populations which are proliferating and are well nourished. This type of damage is only lethal if cells are stimulated to divide before repair occurs. Therefore, if mitosis can be delayed by suboptimal growth conditions, the DNA damage can be repaired. Potentially lethal damage limits the effectiveness of radiotherapy on tumors. There is no potentially lethal damage repair following exposure to high linear energy transfer radiations.

Cell Cycle Effects

- During the cell cycle the radiation sensitivity of the cells can change dramatically. Studies using cells which have their cell cycles synchronized have shown the results.
- The mitosis phase, where the cell divides, is always the most sensitive, while late in the S phase, where DNA synthesis occurs, is the least sensitive. This knowledge is useful in the planning of cancer treatments.

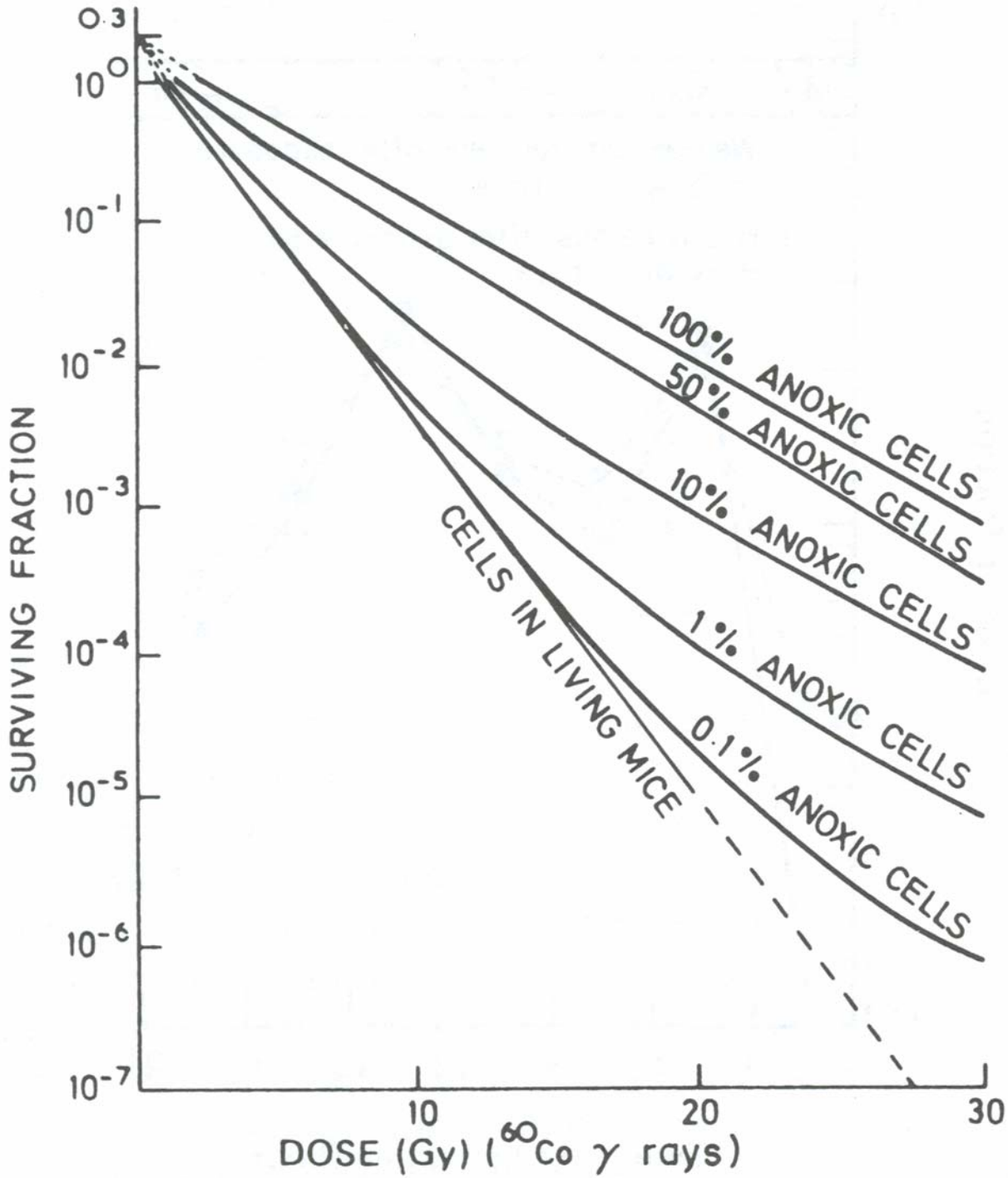

0.1
.01
.001
surviving fraction
500 rad
M
G1
S
G2
M

The Effect of Oxygen and LET on Cell Survival

- The most biologically variable factor that modifies the radiation response is the amount of oxygen in the system.
- A measure of the effect of oxygen is OER (oxygen enhancement ratio) which is defined by the equation:

OER = D_0(anoxic)/ D_0 (oxygenated)

where D_0(anoxic) is the dose required to produce the same effect as D_0 (oxygenated), the dose in the oxygenated system.


0·3
10^0
10^-1
10^-2
10^-3
10^-4
10^-5
10^-6
10^-7
SURVIVING FRACTION
100% ANOXIC CELLS
50% ANOXIC CELLS
10% ANOXIC CELLS
1% ANOXIC CELLS
0·1% ANOXIC CELLS
CELLS IN LIVING MICE
10
20
30
DOSE (Gy) (60Co γ rays)

Chemical Effects on Dammage

$$R-H \rightarrow RH^{\bullet +} + e^{-} \rightarrow R^{\bullet} + H^{+} + e$$

$$RH + OH^{\bullet} \rightarrow R^{\bullet} + H_2O$$

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$$

$$R^{\bullet} + X(\text{sensitizer}) + H_2O \rightarrow ROH + X^{\bullet} + H+$$

$$R^{\bullet} + R'SH \rightarrow RH + R'S^{\bullet}$$

Oxygen Effect in Tumors

- This section will illustrate the practical importance of the oxygen effect in radiotherapy. The presence of a relatively small proportion of hypoxic cells in tumors can limit the success of radiotherapy. Tumors consist of two populations of cells—one well-oxygenated fraction and the hypoxic fraction, which may account for 10-15% of a human tumor. As the tumor is irradiated, the radiation will kill the more radiosensitive outer cells but not the more radioresistant, oxygen-deprived inner cells. As a result, there will be an increase in the supply of nutrients to these inner cells, which will again start proliferating if they have retained their reproductive potential. Therefore, though these cells are only a small fraction of the tumor, they play an important role in the radioresponsiveness of a tumor. Administration of hyperbaric oxygen is used to ensure the radiosensitivity of the tumor. Hyperbaric oxygen administration increases the success rates of radiotherapy for head and neck tumors.
- Survival curves are biphasic for tumors, with a steep initial slope for the well-oxygenated proportion of the tumor cell population and a shallower final curve over the higher doses where the radioresistant hypoxic cells predominate. Overall, the most sensitive tumors have much less than 1% hypoxic cells, whereas resistant tumors have more than 10% hypoxic cells.

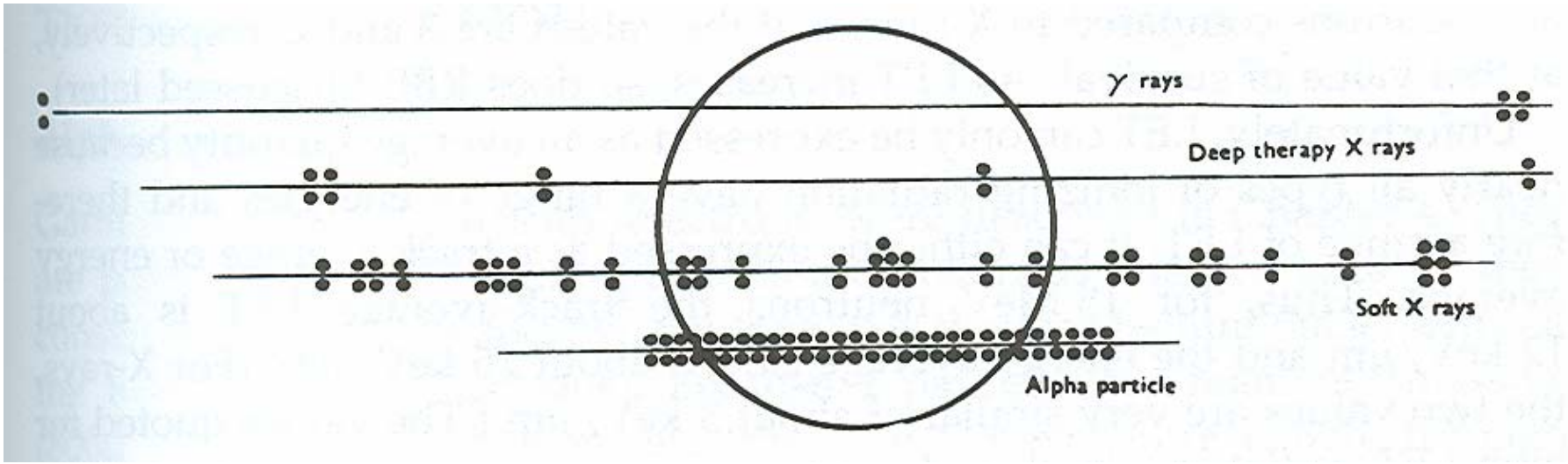
Calculation of Oxygen Enhancement Ratio

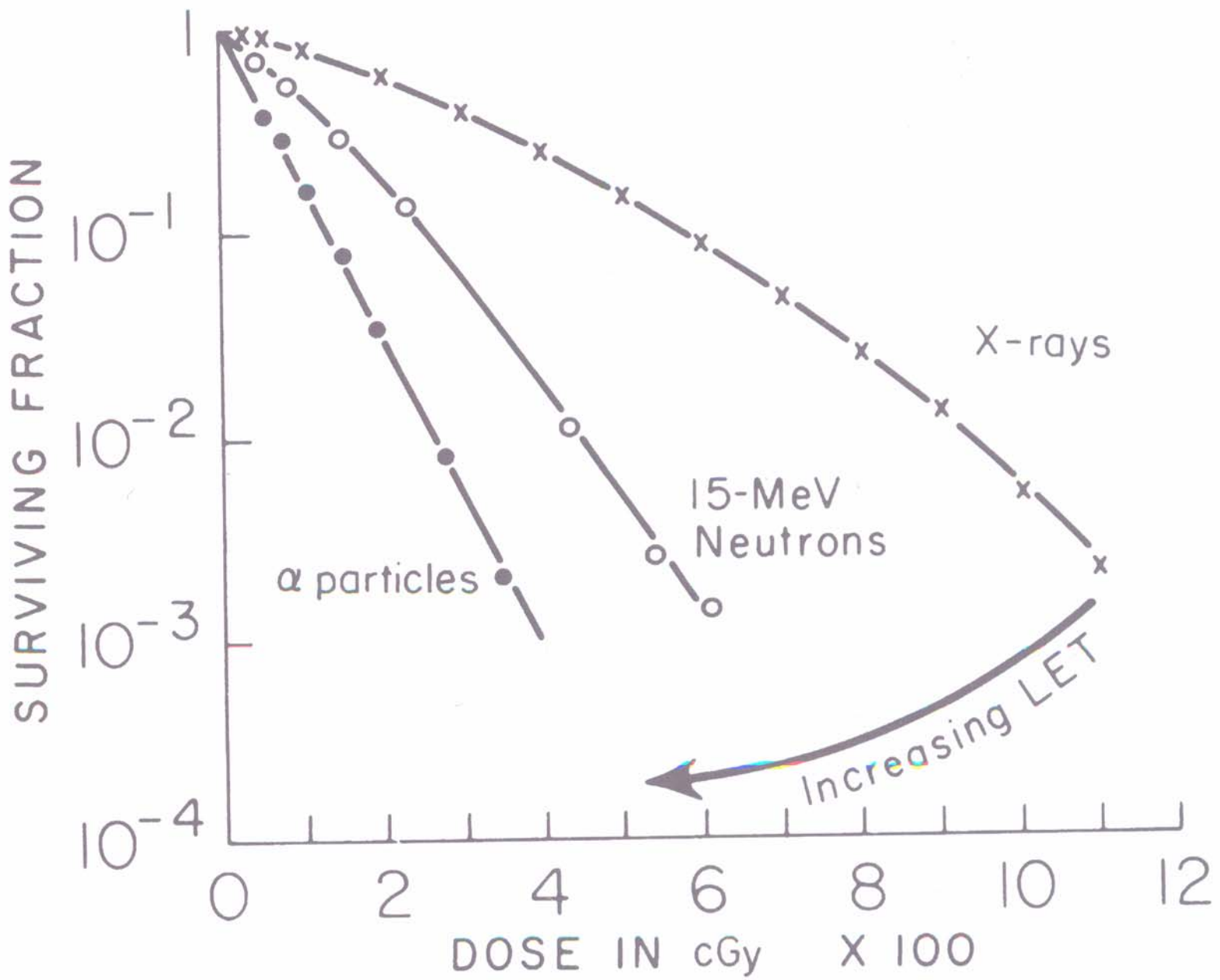
$OER = D_0(anoxic)/D_0(oxegenated)$

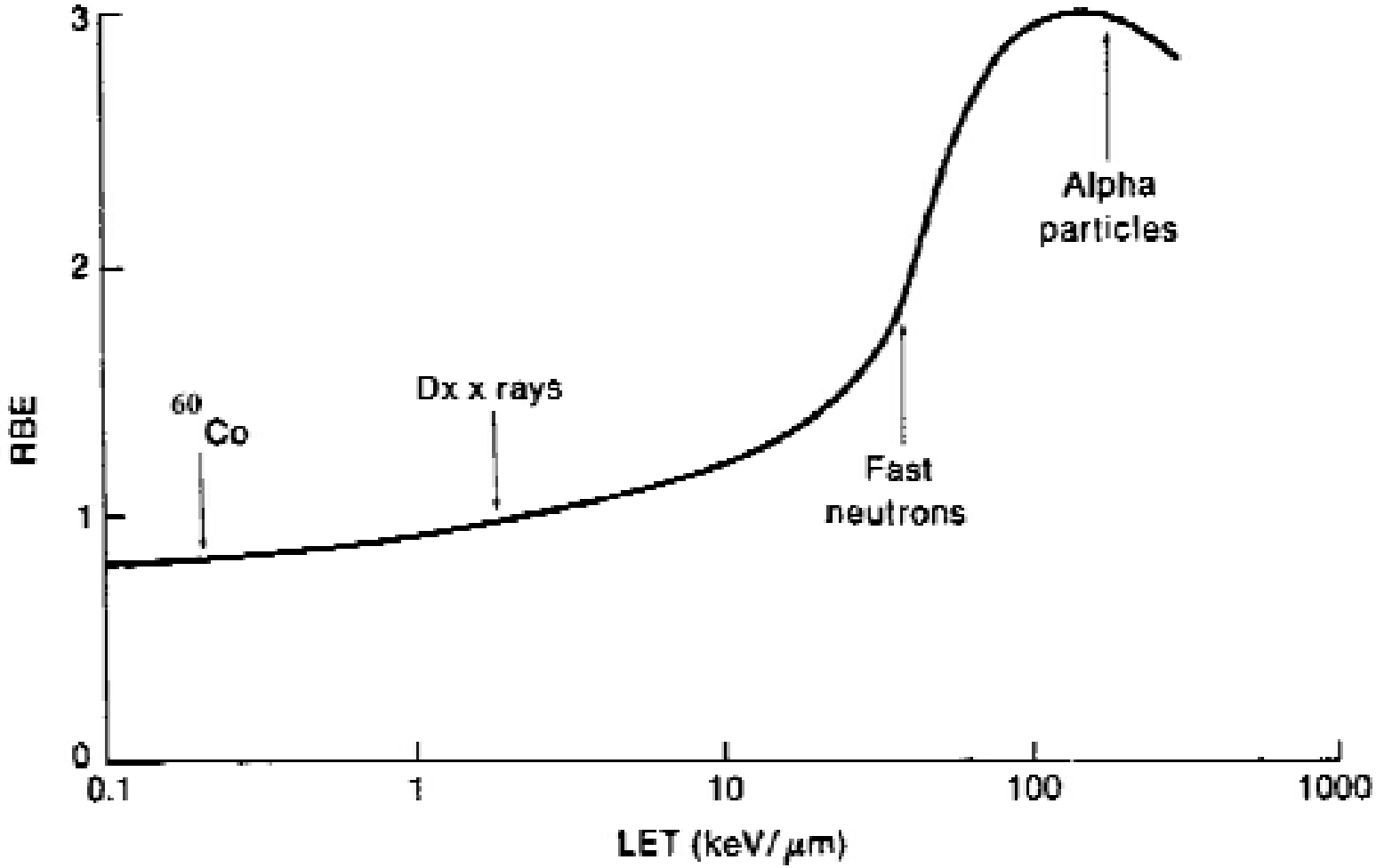
Linear Energy Transfer

- The amount of energy deposited per unit distance is known as linear energy transfer or LET. LET is only an average quantity.
- As the LET increases, the relative biological effectiveness (RBE) increases slowly at first, and then more rapidly as the LET increases beyond 10 KeV/µm.
- This value probably reflects the "target size" and is related to DNA content (the diameter of the DNA double helix). Radiation of this density is most likely to cause double strand breaks.
- High LET produces damaging effects through double strand breaks. Low LET radiation produces most of its damage by interaction of two sublethal events. There is little or no shoulder on the survival curve for high LET radiation.

Difference of Tract Properties




SURVIVING FRACTION
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
X-rays
15-MeV
Neutrons
α particles
Increasing LET
0
2
4
6
8
10
12
DOSE IN cGy X 100


3
2
1
0
RBE
^{60}Co
Dx x rays
Fast
neutrons
Alpha
particles
0.1
1
10
100
1000
LET (keV/μm)

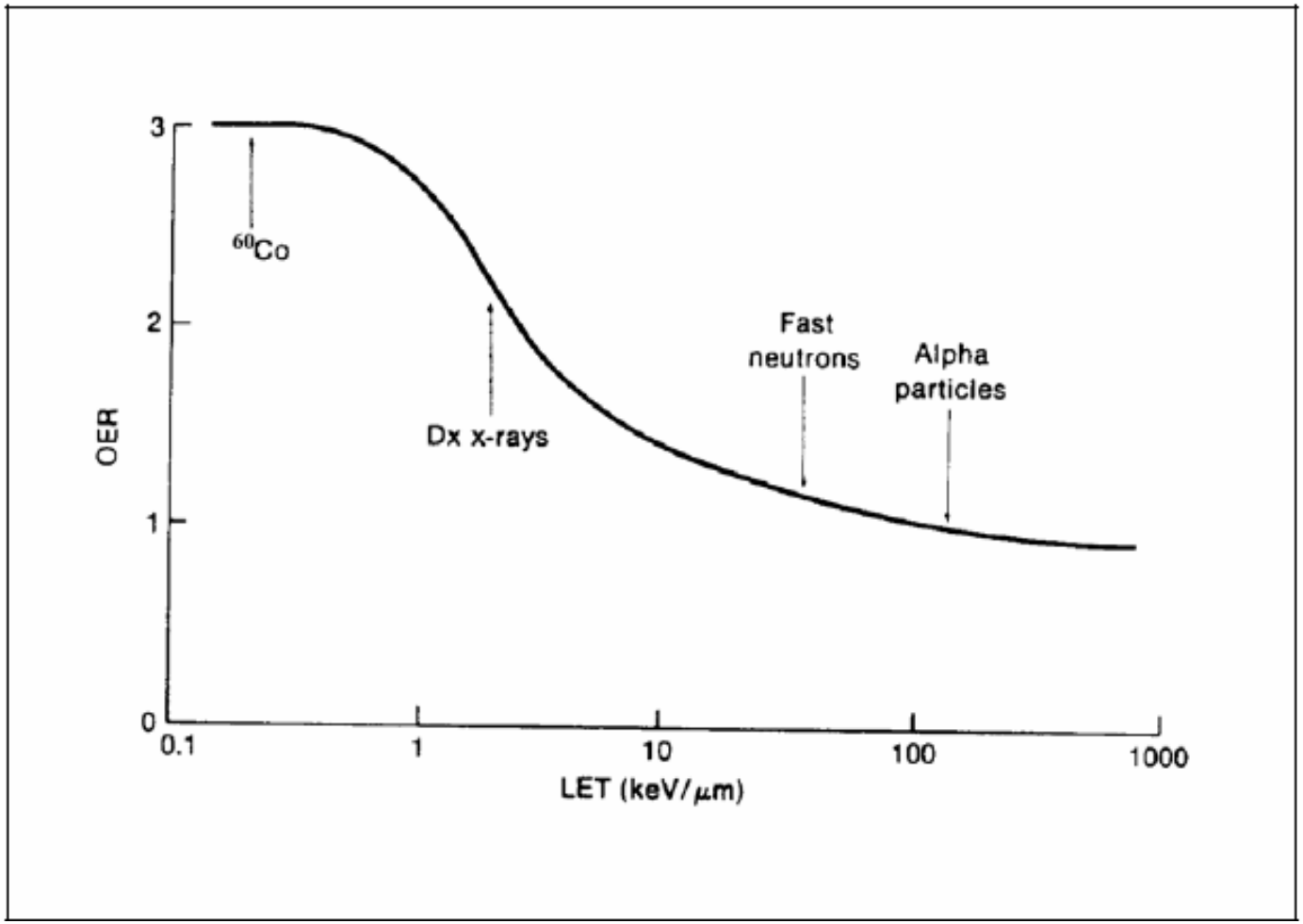
Relative Biological Effectiveness

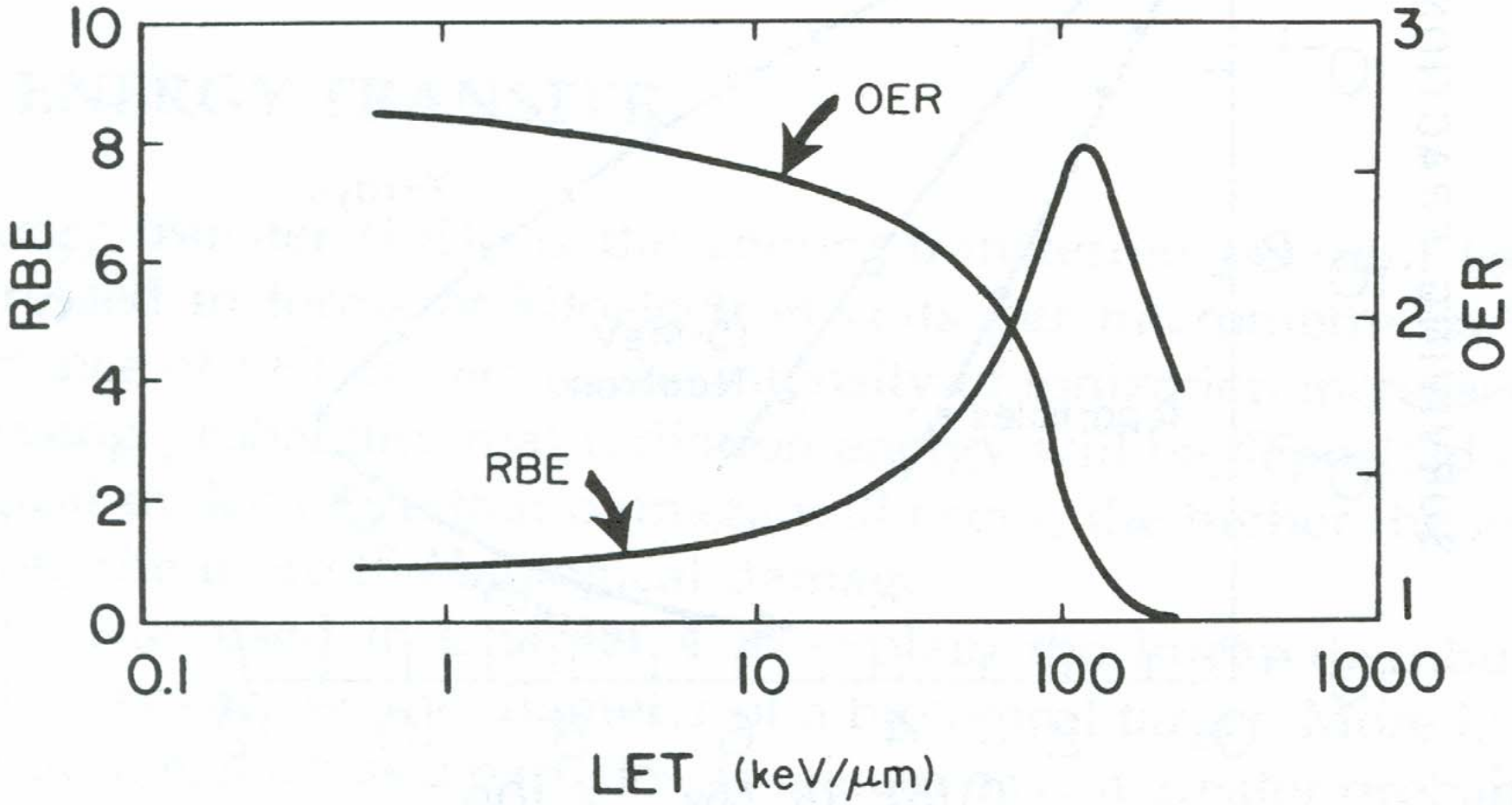
- **RBE = D_0 (standard radiation)/ D_0 (test radiation)**

where D_0 (standard radiation) is the dose of standard radiation required to produce the same effect as the dose of a test radiation, D_0(test radiation). The standard radiation is due to x-rays and gamma rays. As has been described previously, RBE is dependent on both the radiation and the LET.

Relative Biological Effectiveness Dependencies

- radiation dose
- number of dose fractions
- dose rate
- biological system or endpoint


3
2
1
0
OER
^{60}Co
Dx x-rays
Fast
neutrons
Alpha
particles
0.1
1
10
100
1000
LET (keV/μm)


10
8
6
4
2
0
RBE
3
2
1
OER
OER
RBE
0.1
1
10
100
1000
LET (keV/μm)

Factors in Radiation Sensitiviy

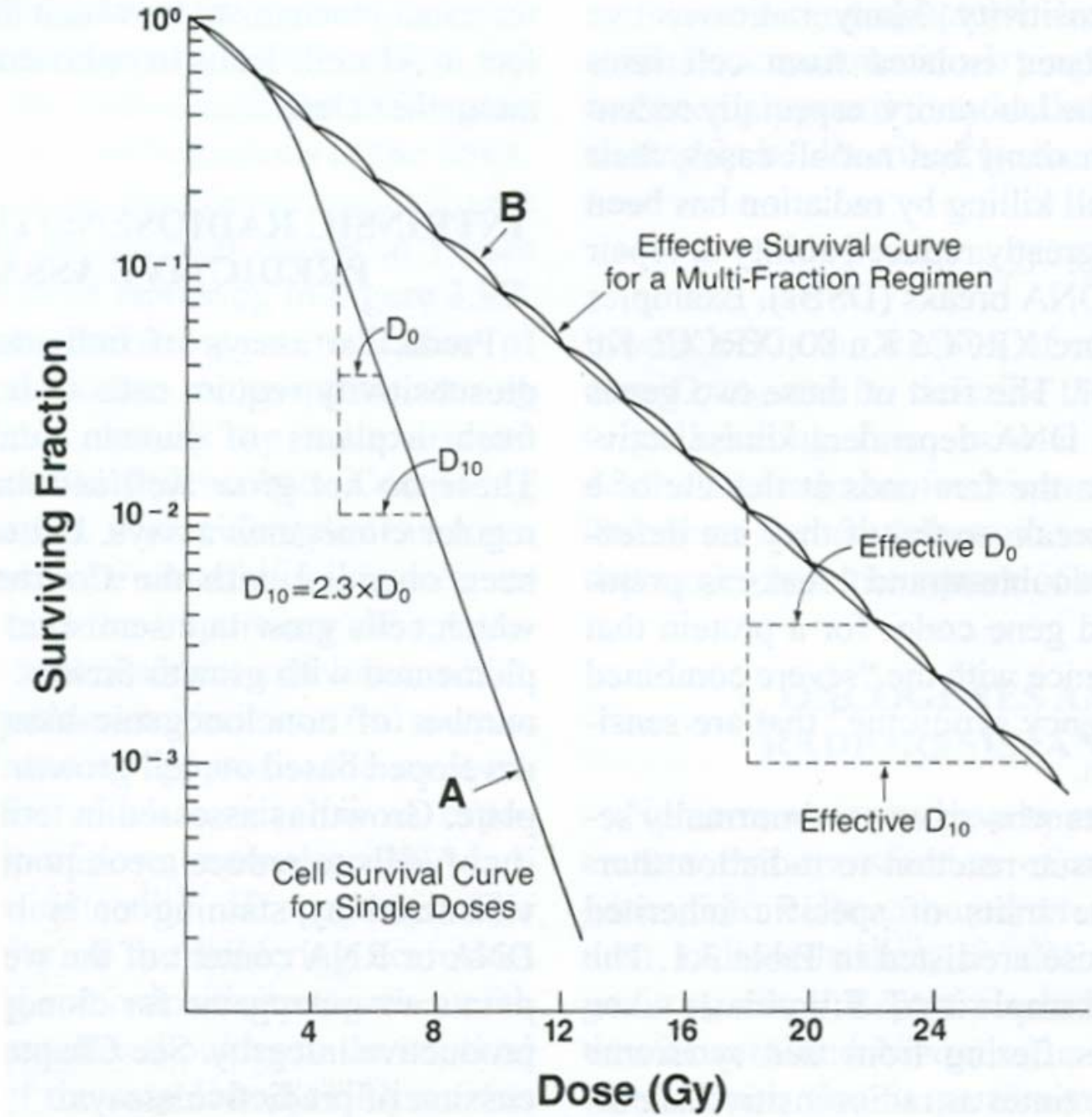
- **Water**
- **Age**
- **Sex**
- **Chemicals (Radiosensitizers and Radioprotectors)**
- **Temperature**
- **Dose Rate**

Dose Rate—Protraction

- Cellular sensitivity of the stem cells involved.
- Duration of the cell cycle (i.e., more damage to cells with a long cell cycle).
- Ability of some tissues to adapt to the new trauma of continual radiation.

Dose Rate—Fractionation

- The goal of fractionation in radiotherapy is to kill all tumor cells without producing serious damage to the normal surrounding tissues.
- This is best achieved by giving the total dose of radiation in a specific number of fractions over a time period, generally five treatments a week for six weeks.
- Repair, reassortment, repopulation, and reoxygenation, the four Rs of radiotherapy, determine the effectiveness of the fractionation.
- Fractions also increase damage to a tumor because of reoxygenation and reassortment of cells into radiosensitive phases of the cycle


Surviving Fraction
10^0
10^{-1}
10^{-2}
10^{-3}
B
Effective Survival Curve
for a Multi-Fraction Regimen
D_0
D_{10}
$D_{10}=2.3\times D_0$
Effective D_0
A
Effective D_{10}
Cell Survival Curve
for Single Doses
4
8
12
16
20
24
Dose (Gy)

Four R’s of Radiobiology

- In radiotherapy, these types of cellular damage are regulated by the four R’s of radiotherapy. The four Rs of radiobiology—repair, reassortment, repopulation, and reoxygenation—are affected by the type of radiation dose.
- There is a prompt repair of sublethal repair damage.
- There is a progression of cells through the cell cycle during the interval between the split doses, called reassortment.
- There is an increase of surviving fraction due to cell division, or repopulation. When the time interval between two dose fractions exceeds the cell cycle, there will be an increase in the number of cells surviving due to cell proliferation.
- The fractionation of the dose allows greater affect through reoxygenation of the tumour, which increases the radiosensitivity.

Summary

- The survival curves, which illustrate the survival of a population of cells, can show the effects of factors such as sex, age, and dose rate.
- Graphing a survival curve gives a great deal of pertinent information such as threshold dose and mean lethal dose.
- Oxygen has an important influence on the effects of radiation due to destruction of the cell by a free radical.
- The effects of radiation are influenced by cell cycle position, water, linear energy transfer, age, sex, chemicals, and temperature.
- The effects of dose rate on radiation is important for therapeutic radiation use.
- Radiosensitizers and radioprotectors are limited in their use due to toxicity.