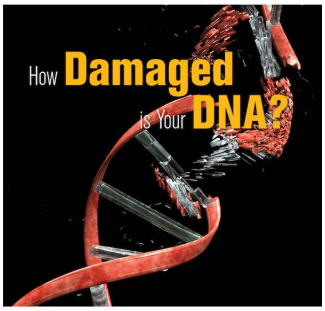
Cellular Radiobiology

1. Cellular Responses to DNA Damage: An Overview

Once a cell has been irradiated and registered DNA damage, it has several possible fates:

Repair of DNA Damage (or at least, *attempted* repair...) - cells naturally have a number of enzymes or enzyme complexes which can repair certain types of damage to DNA, assuming these are not too severe, not too frequent, and the repair systems are operating properly



☞For damaged or lost bases in DNA - cell uses the excision repair pathway

☞For single stranded breaks in DNA - cell uses the SSB repair pathway

☞For double stranded breaks in DNA - cell uses the DSB repair pathway

Recovery from "Cellular" Damage - the observable manifestation of the repair of DNA damage is that the survival of cells is higher than if the repair was somehow prevented; cellular recovery was actually observed well before radiobiologists knew anything about DNA repair!

☞*The main type of cellular recovery (often called, incorrectly, cellular "repair") is:*

Sublethal Damage Recovery (SLD/SLDR)

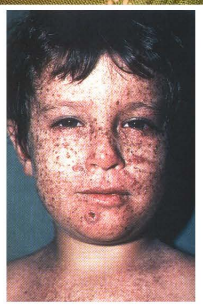
Patients with mutations in DNA repair-related genes usually have multiple health problems – although the severity can vary from mild to severe – the most common of which is cancer proneness

a) symptoms vary depending on whether the defect is in excision repair, SSB repair or (most important for radiotherapy) DSB repair, but can include:

- *Exquisite sensitivity to either UV, chemo drugs or ionizing radiation*
- *Immunodeficiency*
- *Premature aging*
- *Neurological problems*
- *Cancer proneness*

Xeroderma pigmentosum -

a disease associated with mild-to-severe sensitivity to UV light (which is also found in fluorescent lights) and some chemotherapy drugs, and skin scarring/cancer from a young age



the molecular defect is the reduced or inability to perform excision repair of DNA damage

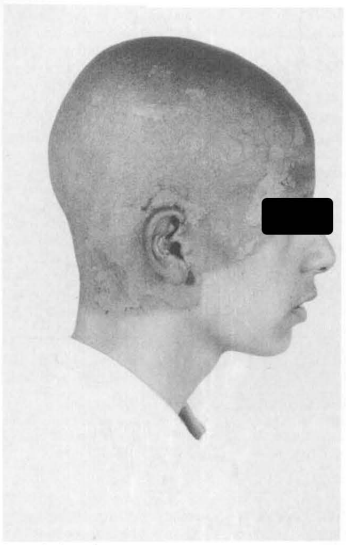
Ataxia Telangiectasia (AT) -

a disease associated with moderate-to-severe sensitivity to ionizing radiation and some chemotherapy drugs, partial or complete ataxia, immunodeficiency and assorted types of cancer, usually starting at a young age that is observed in the general population (e.g., breast cancer in a 30 year old who isn't a BRCA1/2 carrier)

the molecular defect is the inability of the cell to "sense" the presence of DNA double strand breaks, meaning that if the cell doesn't know they're there, they won't be repaired

Luckily, the full-blown disease is very rare (1 in 40,000 - 80,000 live births), because these patients are in BIG trouble when it comes to getting radiotherapy for their cancer...

Adverse Reactions to Radiotherapy in AT Patients



Lateral view of scalp erythema and skin desquamation immediately after radiotherapy.

This patient (with ALL) received ***1800 cGy in 10 daily fractions*** of prophylactic cranial irradiation following chemotherapy, resulting in brisk scalp and skin desquamation by the end of treatment. He also experienced an extreme case of somnolence syndrome that resolved only very slowly. By 3-4 months post radiotherapy, he had developed bilateral osteitis and a necrotic, right mastoid ulcer. After 7 months, an EEG revealed diffuse radiation-induced encephalopathy, that lead to his death soon thereafter.

Telangiectasia in the eye of a patient with AT

Int. J. Radiation Oncology Biol. Phys., Vol. 74, No. 5, pp. 1323–1331, 2009

Disorder	Age (y)/gender	RT (Gy)	Outcome
A-T	10.5/M	30	Died, 8 mo
A-T	9/M	27.5 (mediastinal); 27.5 (supraclavicular)	Died, 3 mo
A-T	3.9/M	3	Died, <1 mo
A-T	7/M	30	Died, 3 wk
A-T	3.8/F	30	Died, 9 mo
A-T	9/M	16	Severe mucosal ulceration
A-T	4.5/M	18	Leukoencephalopathy
A-T	7/M	24 (brain); 12 (spine)	Somnolescence syndrome
A-T	9/F	9	Died, 10 mo
A-T	15.2/M	15.5	Died, 1 mo
A-T	3.9/M	3	Died, 3 mo
A-T	1.5/M	18 (brain); 3 (chest)	No excessive toxicity
A-T	2.5/M	24 (brain); 6 (spine)	Leukoencephalopathy, 10 mo

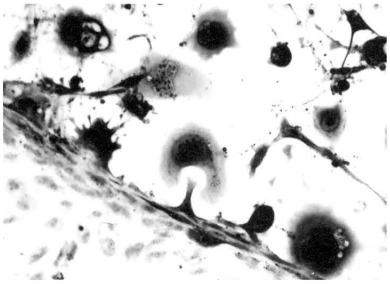
"Tolerance" of DNA Damage - when the damage occurs in parts of DNA that aren't critically important for the cell's structure, function and/or survival; then, the cell can return at a later time to repair the damage on a low-priority basis. And if the cell forgets? It becomes a permanent mutation.

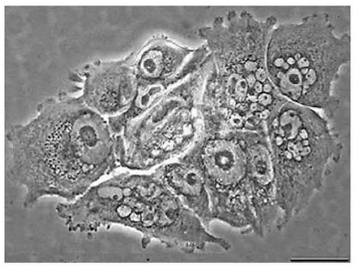
Cell Death - what happens when the DNA damage is irreparable, or else when the cell tries, but can't complete, the repair process; cells can die by several different processes *(see below for those most associated with cell killing by ionizing radiation)*

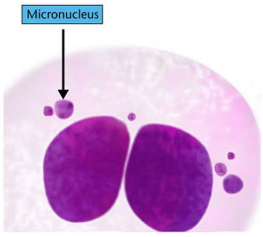
1. the three modes of cell death most relevant to the way radiation kills cells are:

MITOTIC CATASTROPHE (used to be called "clonogenic death") - ***by far, the most common mode of radiation-induced cell death***; it occurs when the cell tries to divide, but can't complete the process due to chromosome and/or mitotic spindle damage

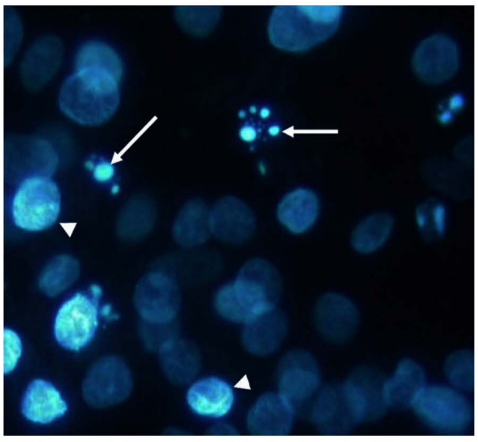
Cells that undergo mitotic catastrophe (left images) look a bit like fried eggs and often have multiple nuclei along with several "micronuclei".







APOPTOSIS or "Programmed Cell Death" - a mode of cell death that occurs in interphase of the cell cycle (usually in G_1/G_0 phase); it takes place when the cell senses that the damage is so severe that it can't be repaired, so instead of risking passing on the damage to progeny, the cell "deliberately" commits suicide



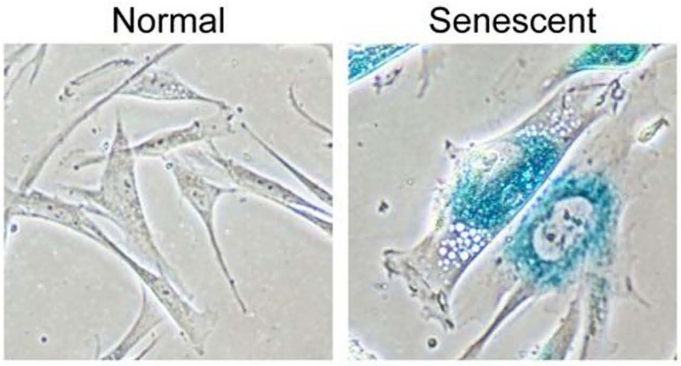
Cells that undergo apoptosis (right image) look more as if they've chewed up their own nuclei and impoded, i.e., show very little remaining cytoplasm.

The surface of apoptotic cells gets all "bubbly", followed by it shrinking down to just a handful of fragments that are consumed by nearby cells (left image)

SENESCENCE - a mode of cell death in which the cell doesn't physically die in the short term, but just shuts down, stops dividing, over-produces cell cycle inhibitory proteins (such as p16), stops trying to repair DNA damage, the chromatin condences into a "closed" form, etc.; this is more likely to occur for high radiation doses (think: hypofractionation) than low ones, and after exposure to certain chemotherapy drugs

a) ***senescence is a natural process – cells just petering out over time like this is why we all have limited lifespans – however it can also be induced by excessive amounts of cellular damage (to DNA, proteins, lipids and everything else)***

b) cells undergoing senescence over-produce certain proteins that can be detected by staining...in this example the blue stain indicated the presence of "senescence-associated β galactosidase"



Mutagenesis - occurs when the cell contains residual DNA damage that is not so severe as to kill the cell, but may be sufficient to cause other genetic defects; these defects would be inherited by all cells that subsequently arise from the original cell

Neoplastic Transformation and Carcinogenesis - technically, a subset of "mutagenesis", in which the genetic defect leads to the formation of a malignant cell (transformation), and later, a tumor (carcinogenesis)

Division Delay - an interference with movement through the cell cycle which happens to ALL cells exposed to ionizing radiation, regardless of whether they are destined to live, die, mutate, transform etc.

2. Cell Death and Cell Survival

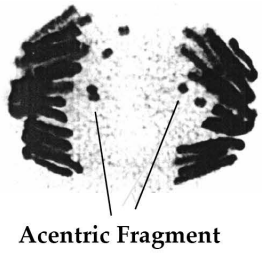
a) What is it about the DNA damage that can't be repaired that makes it lethal to the cell?

1. Answer: the next time the cell tries to divide, this residual damage is expressed in the form of broken or rearranged chromosomes, called **chromosome aberrations**

(a) chromosome aberrations are *usually* fatal to the cell because:

they physically interfere with the division process, in which case, the cell would die right then and there while trying to divide

OR



broken parts of chromosomes float away during the division process (because the pieces have no centromeres), and then the cell later finds itself missing hundreds, if not thousands, of genes, so it dies a gradual, lingering death over several days..and it might even divide a few more times before finally dying

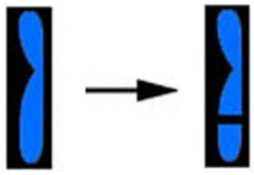
2. how to classify different chromosome aberrations

(a) the main way chromosome aberrations are classified is according to the way they were produced:

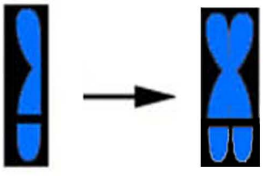
*whether they resulted from a single radiation "hit" (**single-hit type**) or from an interaction between damages from two different "hits" (**two-hit type**, most common)*

(b) examples of different types of chromosome aberrations:

Terminal Deletion (one-hit type)

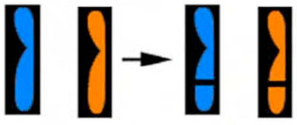


Irradiate in G1 causing 1 break in 1 chromosome

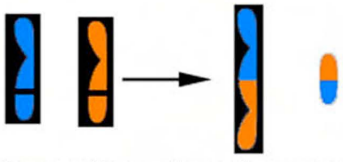


By G2 phase, the chromosomal material has been duplicated, including the acentric, terminal fragments

Dicentric and Acentric Fragment (two-hit type)

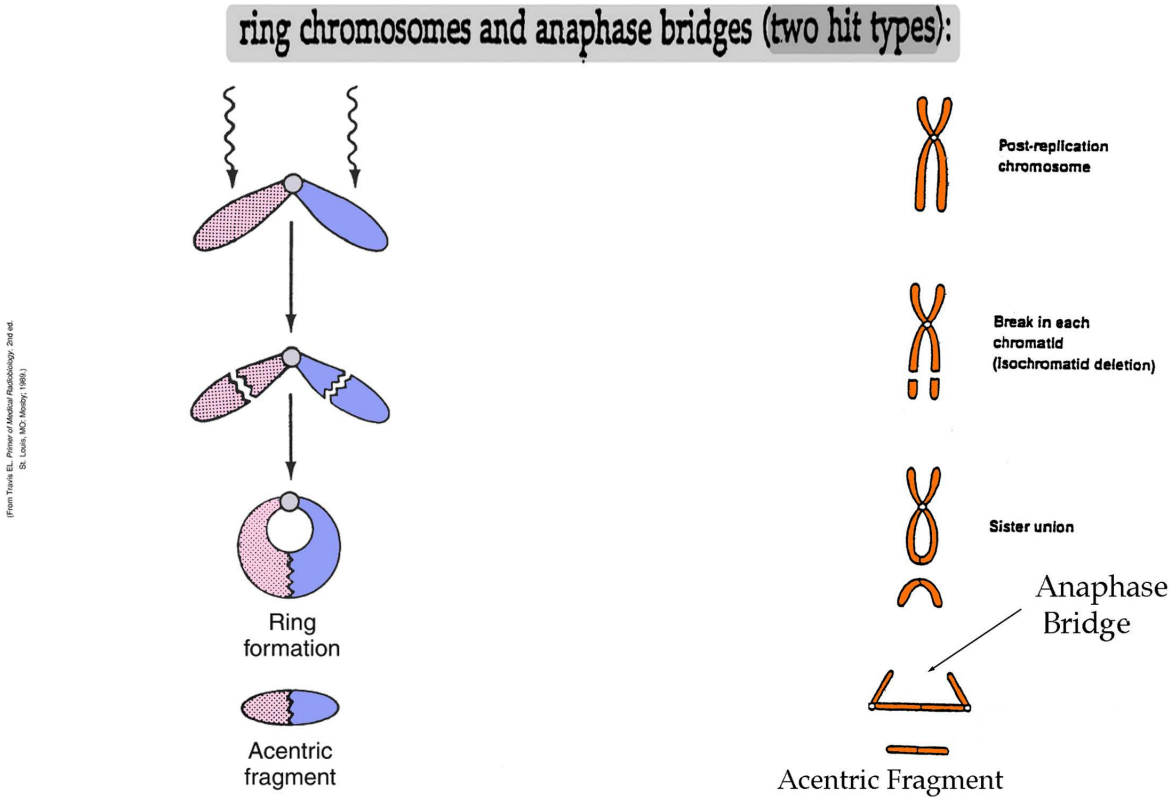


Irradiate in G1 causing 2 breaks in 2 different chromosomes



Breaks rejoin incorrectly, causing a chromosomal exchange (i.e., a dicentric and acentric fragement)

By G2 phase, the chromosomal material has been duplicated, including the misrejoined pieces

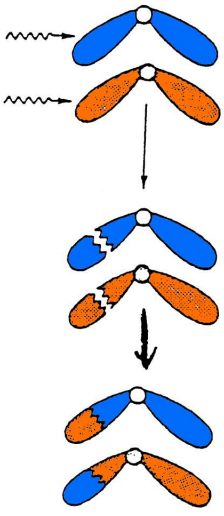


(c) all of the above examples of chromosome aberrations leave "acentric fragments" behind and most of the time, the loss of all the genes on these fragments will kill the cell

(d) however, there are other types of chromosome aberrations that *don't* leave fragments behind, so genes aren't lost – although they do get "rearranged" – and the cell is more likely to survive

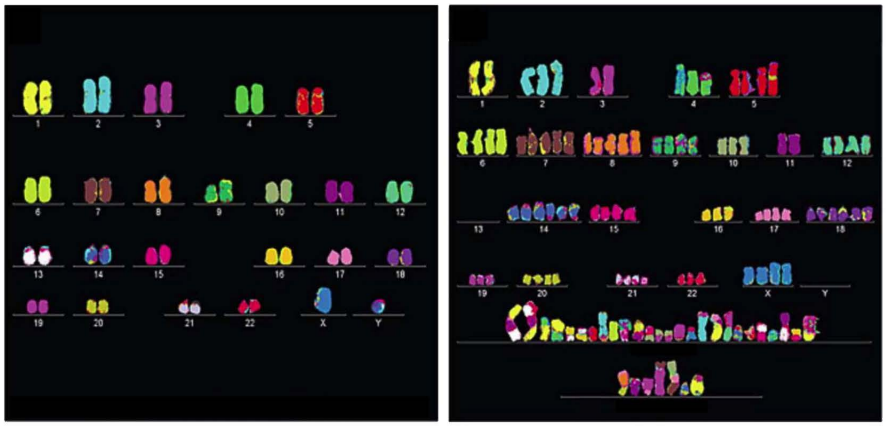
1. ***these are particularly dangerous because the rearrangement of genes may cause some to be turned on or off that normally shouldn't be that way, and this is a major way that cancers start***

Examples: Translocations and Inversions



Translocation

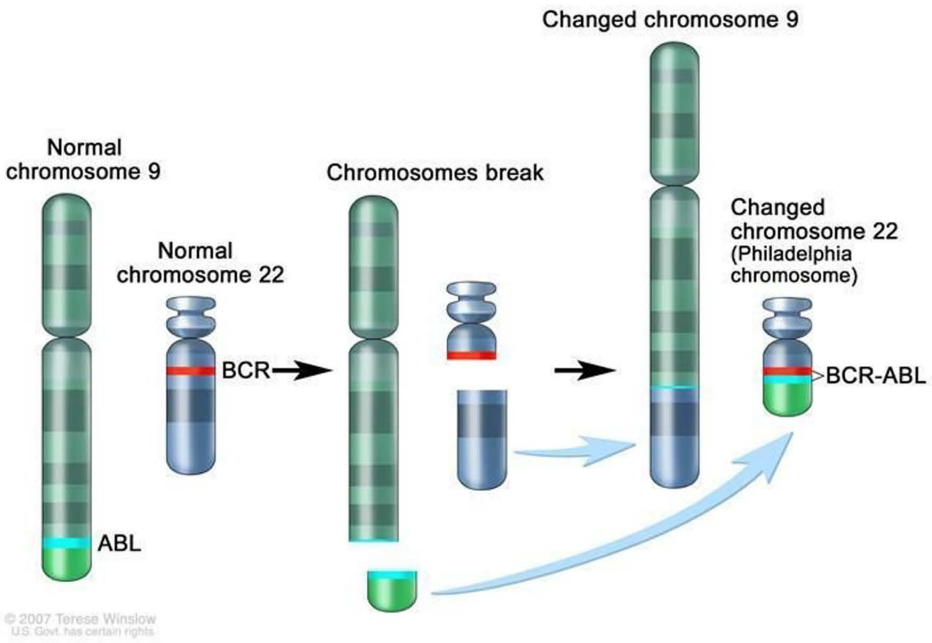
(exchage between two different chromsomes)



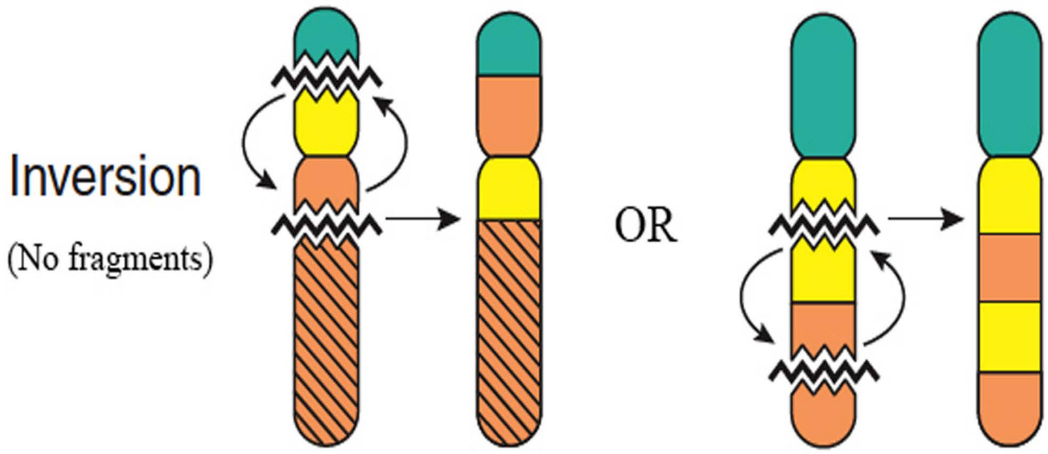
Staining chromosomes with different dyes highlights the orderly nature of the normal human karyotype (left), that is, humans have precisely two copies of each chromosome with no leftovers

A bladder cancer cell (right) has extra copies of some chromosomes, a few missing normal chromosomes, and a lot of hybrid or marker chromosomes, which characterize cancer cells

Real world example of a single chromsomal translocation causing cancer:



The translocation between chromosomes 9 and 22 responsible for chronic myelogenous leukemia. The normal structures of chromosomes 9 and 22 are shown at the left. When a translocation occurs between them at the indicated site, the result is the abnormal pair at the right. The smaller of the two resulting abnormal chromosomes ($22q^-$) is called the Philadelphia chromosome, after the city where the abnormality was first recorded.



(two breaks in the same chromosome, but that rejoin "backwards")

Another real world example:

Gene "banding patterns" for human chromosome #5 before (left) and after (right) x-irradiation

Cell Survival Curves

The toxic (cell killing) effects of ionizing radiation exposure present additional risks to irradiated individual in the form of:

- Normal tissue complications that develop following radiation therapy
- Acute, whole-body radiation syndromes
- Embryonic and fetal effects (teratogenesis)
- Cataract formation

(...and, of course, let's not forget the "good killing", namely the cure of tumors!)

In order to determine the likelihood that a certain effect will occur following irradiation, it is necessary to have some measure—either direct or indirect—of the radiosensitivity of the cells whose deaths precipitate the effect. *This is accomplished by generating either a cell survival curve, and/or a tissue dose response curve.*

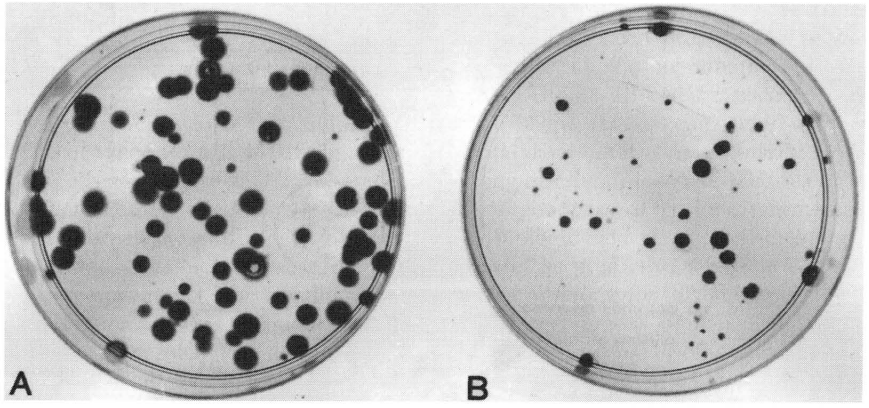
What is meant by cell death? [not what you might think...]

1) to the radiobiologist, **cell death means "loss of reproductive integrity" or "clonogenic death"**, not necessarily "real" death (as in cessation of metabolic activity and energy production, with eventual cell lysis)

2) **the cells that are reproductively dead may not die in the literal sense for days, weeks, months or EVER...and some of them can even divide a few more times before stopping, but the bottom line is, they CANNOT DIVIDE INDEFINITELY (and most of the time, are "really" dead within a few days)**

A. How do you measure the survival of individual cells?

1) Answer: by determining whether, after irradiation, they can form "colonies" in a petri dish, with each colony arising from a single cell that has reproduced hundreds if not thousands of times (meaning that such cells are NOT "clonogenically dead"; the ones that are cannot form a macroscopic colony)

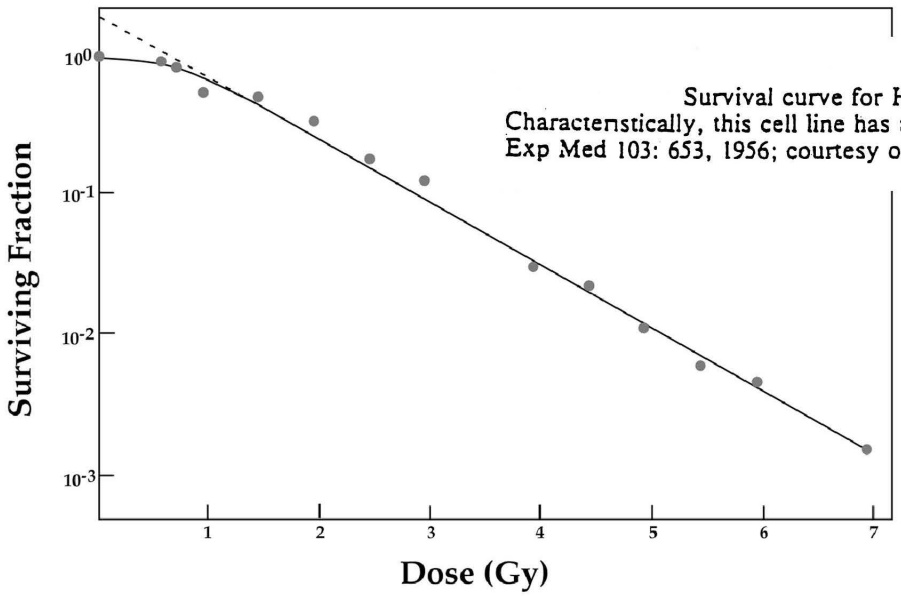


From Hall, Radiobiology for the Radiologist, 5th Edition

Colonies obtained with Chinese hamster cells cultured in vitro. **A:** In this unirradiated control dish 100 cells were seeded and allowed to grow for 7 days before being stained. There are 70 colonies; therefore the plating efficiency is 70/100, or 70%. **B:** Two thousand cells were seeded and then exposed to 800 rad (8 Gy) of x-rays. There are 32 colonies on the dish.

2) by analyzing colony counts for lots of different cells irradiated with different doses, a survival curve can be generated

a. *the very first survival curve for mammalian cells was generated by Puck and Marcus in 1956, and this technological achievement ushered in the so-called "modern era of radiobiology"*



Survival curve for HeLa cells in culture exposed to x-rays. Characteristically, this cell line has a small initial shoulder. (Puck TT, Marcus PI: J Exp Med 103: 653, 1956; courtesy of the author and Rockefeller University Press)

The Immortal (-ized) Ms. Henrietta Lacks

b. the type of cell used for the survival curve was called "HeLa", and was derived from a human cervical carcinoma from a patient named **He**nrietta **La**cks; not only was this the first kind of cell used to generate a radiation survival curve, but it was also the very first mammalian cell type that could be grown at all in petri dishes (*"in vitro"*)

So, suffice it to say that HeLa cells are quite famous!

4) Survival Curve Terminology, Plotting Conventions and Mathematical Models

a. *survival curves are always plotted as the surviving fraction of cells on a log scale (Y-axis), as a function of the radiation dose on a linear scale (X-axis)...in other words, a SEMI-LOGARITHMIC PLOT*

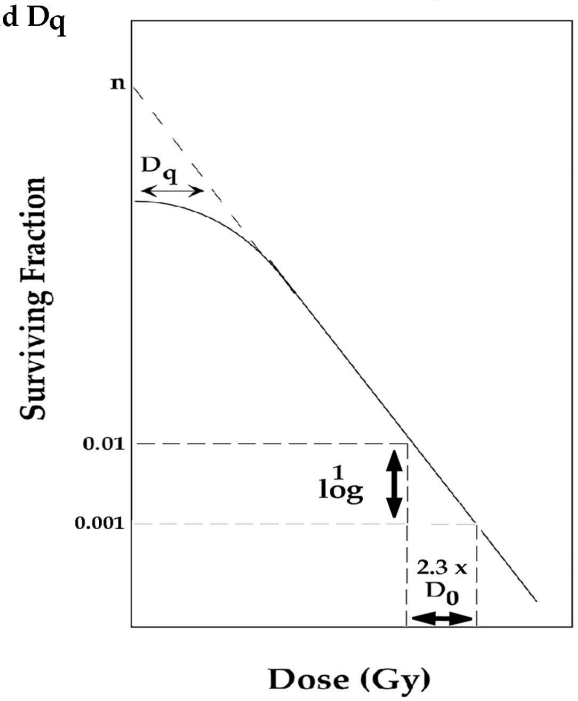
b. *for most cell types (both normal and cancerous), the shape of the cell survival curve is roughly a straight line at high doses ("straight line" on a log scale is called "exponential"), and a more curved, bent, "shoulder" at low doses*

NOTE: a few cell types show little or no "shoulder"

c. how would you describe a survival curve to somebody who wasn't actually looking at it?

ANSWER: use descriptive terminology!

1] **three descriptive, mathematical terms are commonly used to describe the shapes of radiation survival curves: D_0, n and D_q**



D_0 is defined as:

the reciprocal of the slope of the straight portion of the curve, i.e., a large value for D_0 means a shallow slope and a small value for D_0 means a steep slope

n, the extrapolation number, is defined as:

"a unit-less number corresponding to the back extrapolate of the exponential portion of the survival curve to the point where it intersects the y-axis"

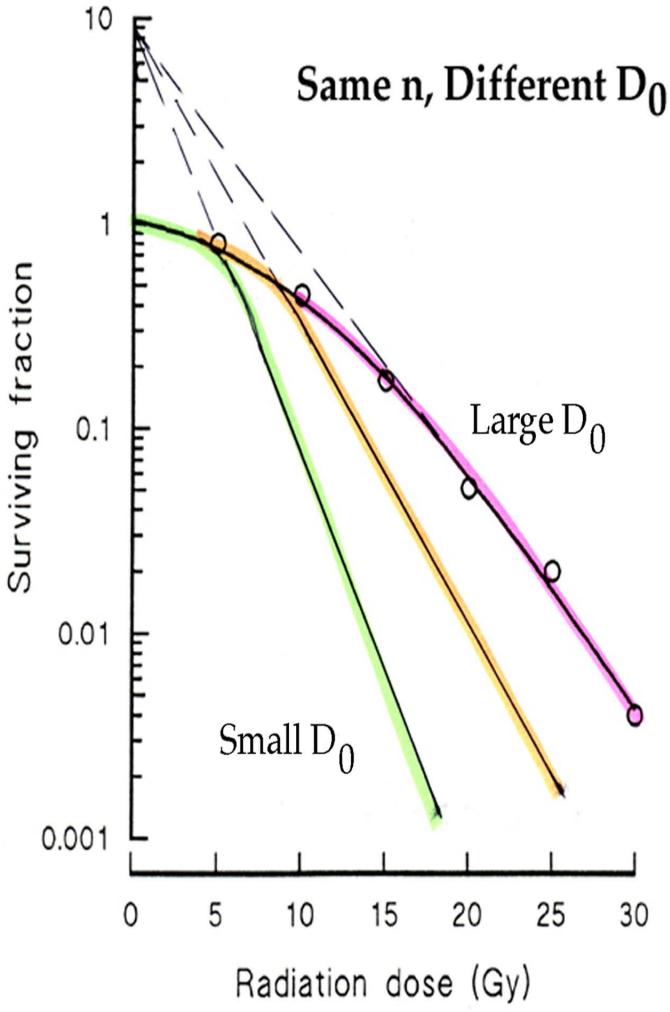
n provides partial information about the "steepness" of the shoulder region of the cell survival curve, i.e., the larger the extrapolation number, the steeper the shoulder (also note: a survival curve for which n = 1.0 has NO shoulder)

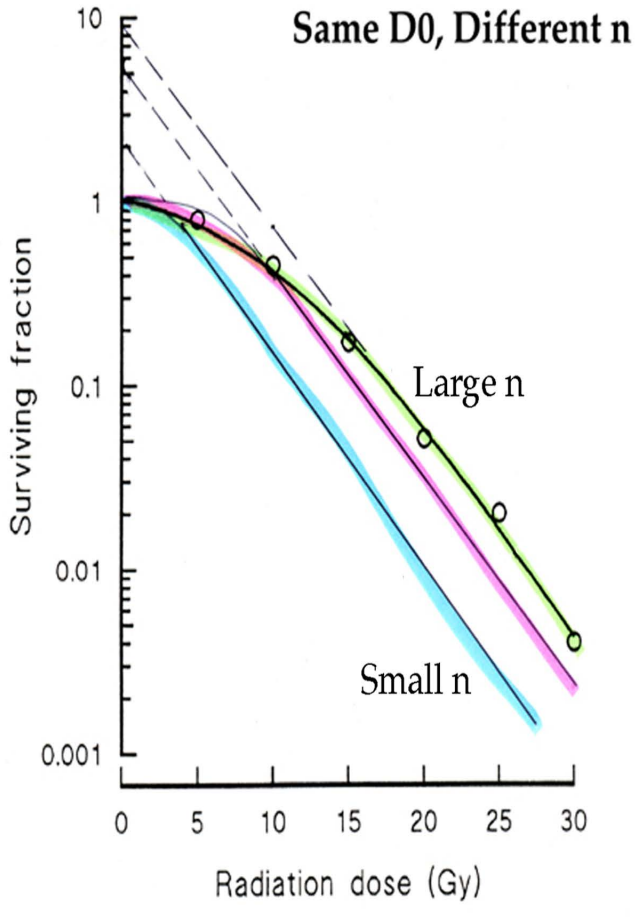
D_q, the quasi-threshold dose, is defined as:

the "width" of the shoulder region of the cell survival curve, i.e., the larger the value for D_q, the broader the shoulder; measured in units of dose (Gy)

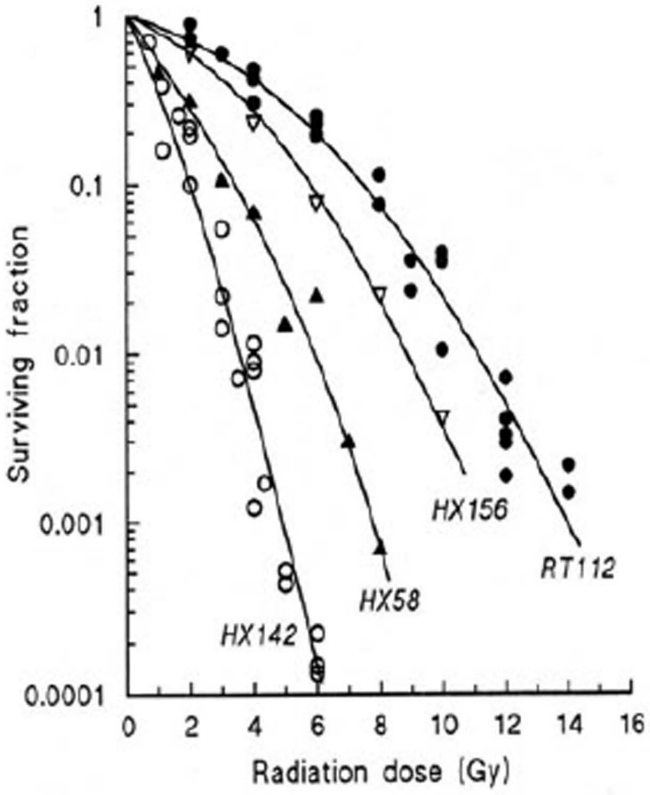
For most mammalian cells studied to date (under well-aerated conditions, and lacking genetic defects associated with exquisite radiosensitivity) , the extrapolation number varies between approximately 1.5 – 15, and D0 varies between about 0.9 – 2.5 Gy

Some Examples:





Real World Example - radiation survival curves for human tumor cells

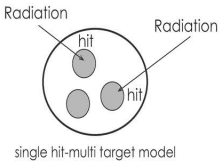


...note the range of radiosensitivities for the different types of cells; some are relatively radioresistant and others are more radiosensitive

d. Survival Curve Models and Theories AKA "Why do survival curves have the general shape that they do?"

1. in an attempt to understand why radiation survival curves were "shouldered, and then exponential", radiation physicists and biologists developed mathematical models that "fit" the sets of data points

2. one of the earliest models developed (in the 1940's) was called "**Target Theory**" - the idea that cells contained one or more "targets" that needed to be "hit" by the radiation in order to kill the cell



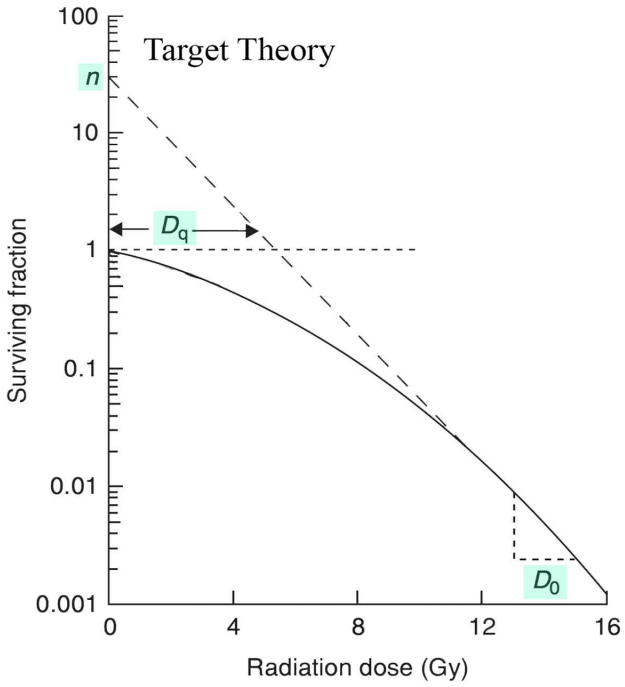
a) the terms "D_0", "n" and "D_q" come from this model

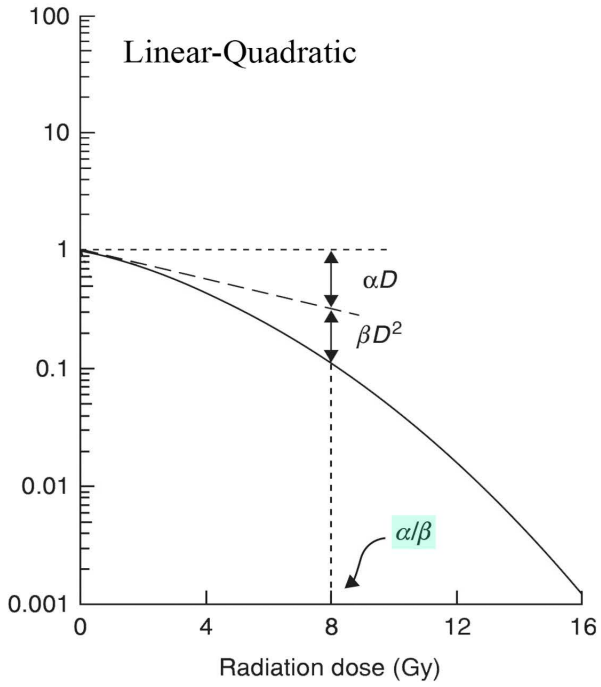
b) *ironically, even though the terminology persists to this day for descriptive purposes, the target theory model is no longer in favor, and has been replaced by a different one!*

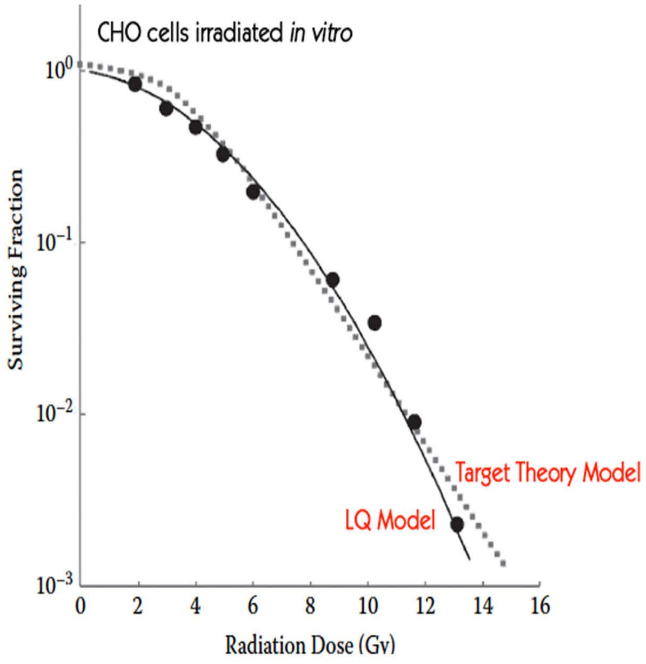
3. the current, model (first used in the 1970's) is called the "**Linear-Quadratic**" or "**Alpha-Beta (α/β)**" Model - it proposes that survival curves get their general shape as a result of two different types of radiation damage to the cell, the reparable kind and the irreparable kind, and how much of each determines the shape of the survival curve

a) in this model, instead of n's and D_0's, the descriptive terms are "α" and "β", or more commonly, the α/β ratio

The Two Survival Curve Models Compared:







Similarities and Differences:

- The models are similar in that they both do a reasonably good job fitting the data points!
- However, the linear-quadratic model does a better job in the low-dose, shoulder region of the survival curve, which historically, has been more important for the practice of radiation therapy.
- For very high radiation doses though, it could be that the target theory model is (slightly) better, which could have relevance for hypofractionation.

b) you may hear the physicians (and sometimes the physicists too) talking about α/β ratios; even though it was initially developed to fit cell survival curves, this model has also been adapated for clinical use as a way of predicting the sensitivity of normal tissues and tumors to changes in dose per fraction

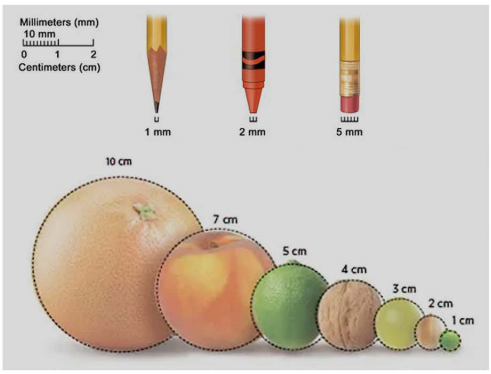
e. Putting Survival Curves into Perspective for Radiotherapy

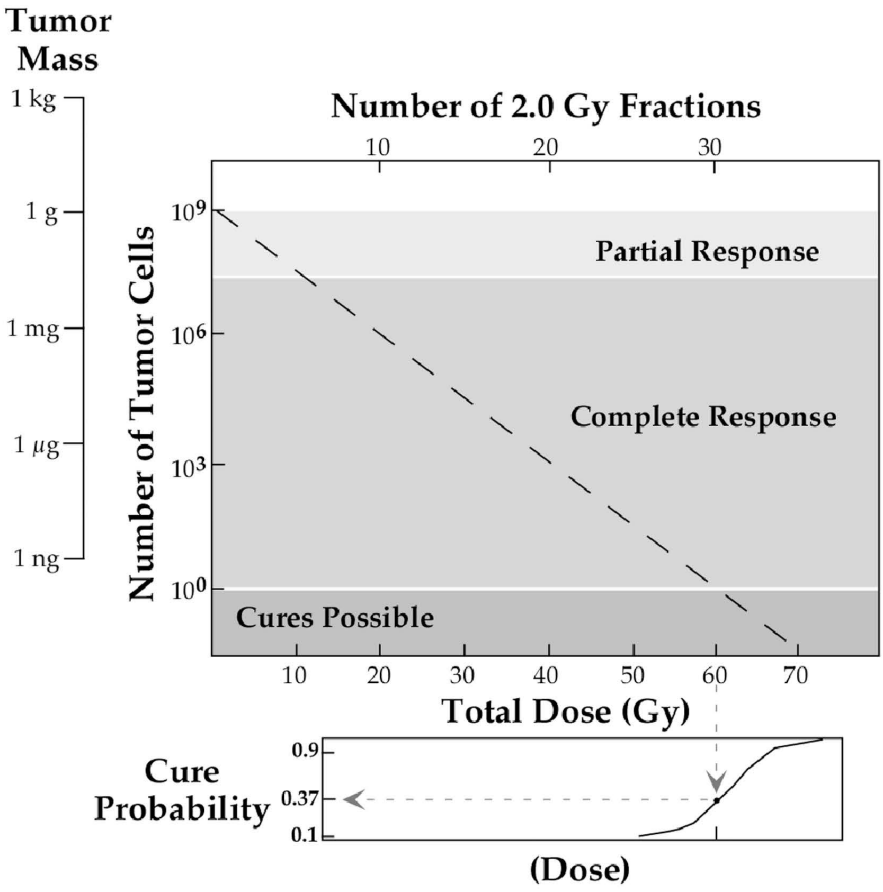
1. regardless of the particular survival curve model, the bottom line is: *THE KILLING OF TUMOR CELLS IS THE BASIS FOR HOW RADIOTHERAPY WORKS*

2. the problem is, however, that tumors have so many, many cells in them that it takes large radiation doses to kill every one of them (which is the only way to *guarantee* that the tumor won't grow back)

a) How many cells?

It has been estimated that a 1 cm tumor (1 gram - small, but not tiny) probably contains 10^9, or a billion cells; therefore, in order to get rid of such a tumor, the radiation dose would have to be high enough to kill at least 10 logs worth of cells!





2] don't be fooled though – tumors can start to shrink, and even disappear altogether, WAY before the dose is high enough to be sure they're gone for good

this is terminology borrowed from medical oncology

(a) for example, a **"partial response"** (tumor shrinks) occurs when the tumor has been depleted to only 30% (0.3) of its original cell numbers

(b) a **"complete response"** (tumor disappears) occurs when the tumor has been depleted to 1% (0.01) of its original cell numbers

3. on the other hand, the normal tissues in the radiation field that you *don't* want to cause too much damage to, can, at best, tolerate being depleted to 5% (0.05) of their initial cell numbers

Great, BUT…

…To what extent are these survival curves for cells in petri dishes applicable to what we <u>really</u> want to know, namely, what happens to intact tissues when they are irradiated?

Tissue Dose Response Curves

A. Are cell survival curves generated from cells in petri dishes (*in vitro*) representative of what's going on in intact tissues and organs (*in vivo*)?

1) At first, nobody knew for sure!

2) therefore, *it became critically important to develop ways of estimating cellular radiosensitivity, but without actually taking samples of the patient's tissues (including the tumor) – enter the so-called* ***In Vivo Assay or Tissue Dose Response Curve***

(a) tissue assays have been developed for a variety of normal tissues and tumors that allow estimates of their relative radiation responses; there are two types of tissue assays:

1] *a clonogenic tissue assay is similar to a cellular survival assay in that it involves the counting of colonies...the only difference being that the irradiated subject serves as its own petri dish (instead of a glass or plastic vessel)*

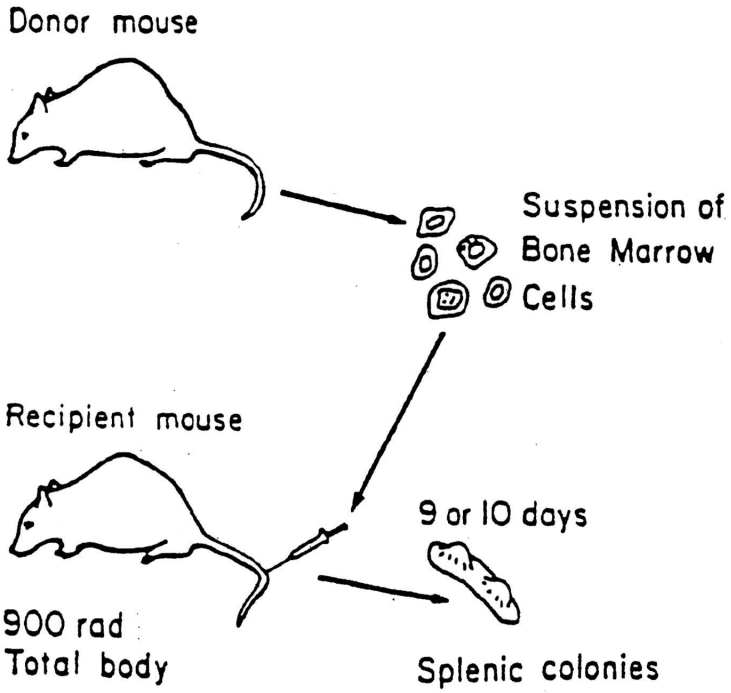
2} *a non-clonogenic tissue assay uses either structural or functional integrity of the tissue (i.e., is the tissue still intact and "working") as a surrogate endpoint for the survival of cells*

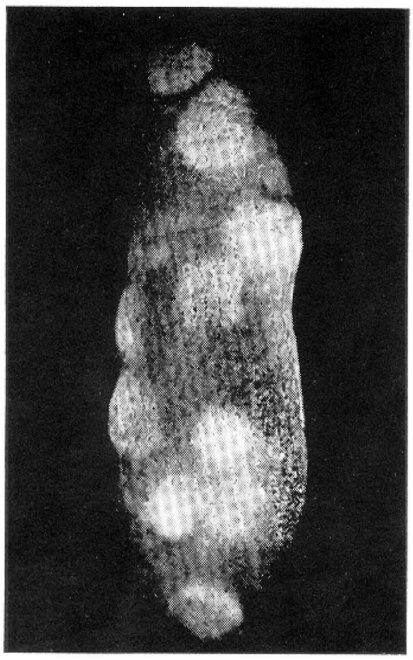
Dose Response Curves for Normal Tissues

Example of a Clonogenic Assay:

Spleen Colony Assay (Till and McCulloch, 1961) - could measure the survival curve for mouse bone marrow cells *in vivo*

1] the first step was to lethally irradiate (about 10 Gy single dose) a recipient mouse, and try to "rescue" it with injection of normal bone marrow from a syngeneic (genetically identical) donor mouse; the number of injected cells necessary to produce a visible nodule (of regrowing cells) in the previously sterilized spleen of the recipient was then determined and compared to how many colonies were obtained after the donated marrow was irradiated

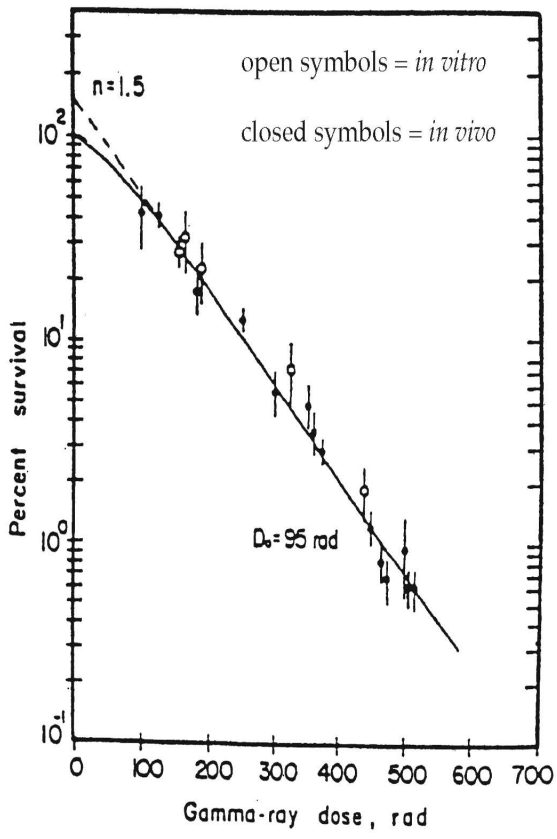




Colonies in the spleen of an unirradiated mouse which had been injected 10 days previously with 2 X 10^5 marrow cells

Science *144*: 8443, 1964.

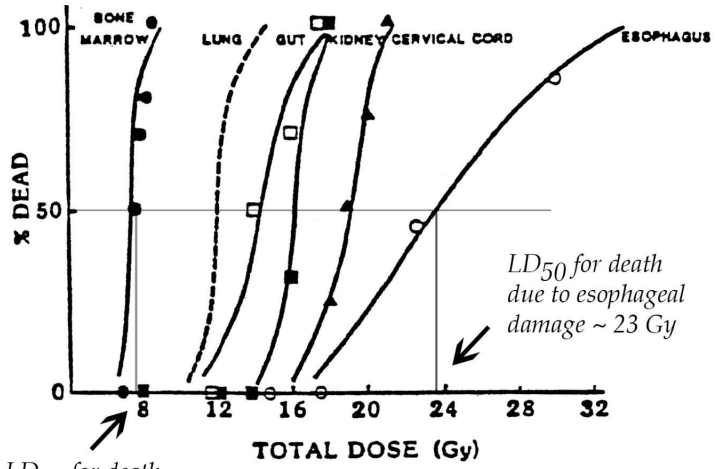
Survival curve measured in the mouse (*in vivo*) was identical to the one for the same cells grown in a petri dish (*in vitro*).



Gamma-ray survival curve for the colony-forming ability of mouse bone marrow cells. The cells are irradiated *in vivo* in the donor animal and grow into colonies in the spleens of supralethally irradiated recipient animals. (Redrawn from McCulloch EA, Till JE: Radiat Res 16:822, 1962)

Example of a Non-Clonogenic, Functional Assay:

LD_{50} Assays - an assay applicable for a number of different target organs where the endpoint evaluated is *"what dose does it take to kill half of the irradiated subjects due to a specific organ injury or failure?"*



Number of animals dead is a function of dose for six different normal tissues. In general, the curves are very steep, although they are displaced on the dose axis, bone marrow being most "sensitive," and esophagus, most "resistant." (Redrawn from Travis EL: Relative radiosensitivity of the human being. *Adv Radiat Biol* 1987; 12:205–238.)

LD_{50} for death due to esophageal damage ~ 23 Gy

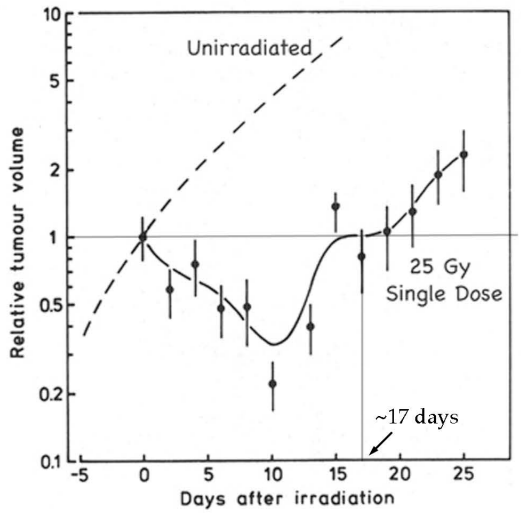
LD_{50} for death due to bone marrow destruction ~7.5 Gy

In this example, you can conclude that the bone marrow is more radiosensitive than the esophagus, based on the LD_{50} value

Dose Response Curves for Tumors

Two Examples of Non-Clonogenic, Functional Assays:

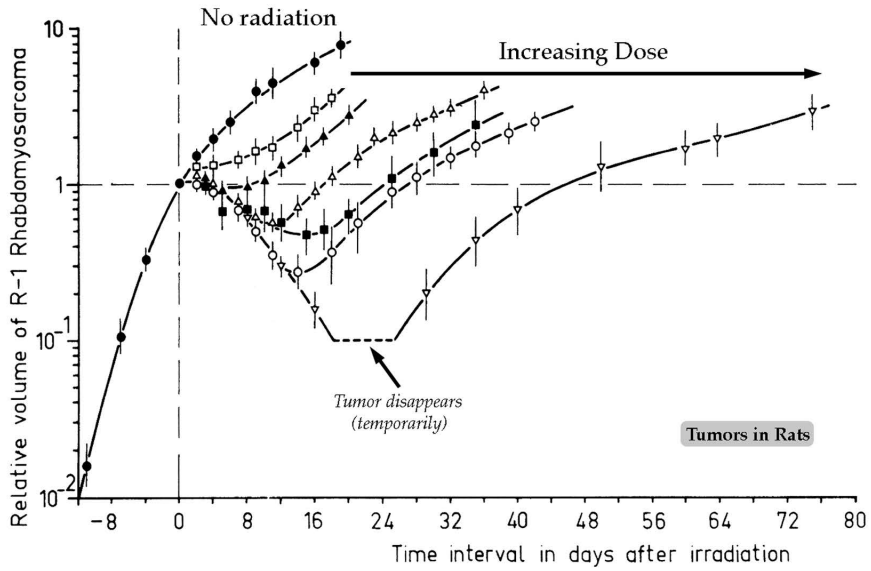
Tumor (Re-)Growth Delay Assay - uses changes in tumor size and/or growth rate during and after radiation therapy as an indirect indicator of the radiosensitivity of the tumor cells; for example, *a given tumor may slow its growth, stop its growth, regress in size (either slowly or quickly), or even disappear altogether during treatment or soon thereafter*



The longer the time it takes for a tumor to re-grow back to its pre-irradiation size, (its "regrowth delay"), the more radiosensitive the cells it contains...to a first approximation, anyway.

Growth characteristics of two, same-sized, mouse tumors, one irradiated with a large dose of X-rays (25 Gy) and the other unirradiated. The regrowth delay is around 17 days in this case.

From: Hall, *Radiobiology for the Radiologist, 5th Edition*, 2000.



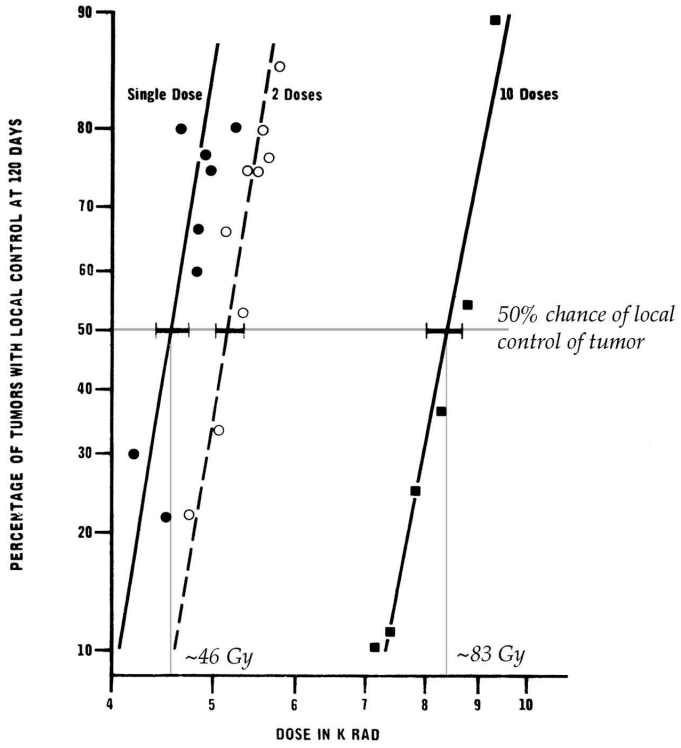
Another example of tumor regrowth delay for several different radiation doses of increasing size. Growth delay increases as the dose increases, implying that there is greater tumor cell kiling the higher the radiation dose.

50% Tumor Control Dose (TCD_{50}) - another non-clonogenic assay that measures how much radiation it takes to "locally control" a tumor (that is, for the main tumor mass to disappear and not grow back within a specified period of time); however, the presence of distant metastases is **not** taken into account in this type of assay, so it is not quite the same thing as saying "tumor cure"

...although tumor cure is itself a type of non-clonogenic assay!

Note in this example how the TCD_{50} increases with increasing fractionation of the total dose (total dose given as a single fraction, versus 2 fractions or 10 fractions), illustrating that protracting treatment over longer time intervals decreases the cell killing efficiency.

This is true for tumors as well as normal tissues.



Percentage of mouse mammary tumors locally controlled as a function of x-ray dose, for single exposures and for two different fractionation patterns.

(From Suit H, Wette R: Radiation dose fractionation and tumor control probability. Radiat Res 29:267–281, 1966.)

Cell Cycle Effects of Radiation

The movement of the mammalian cell through its cell cycle is perturbed by exposure to ionizing radiation; these "perturbations" occur because of three cell cycle-related phenomena:

Cell Cycle "Age Response": cells in different positions in the cell cycle have different radiosensitivities

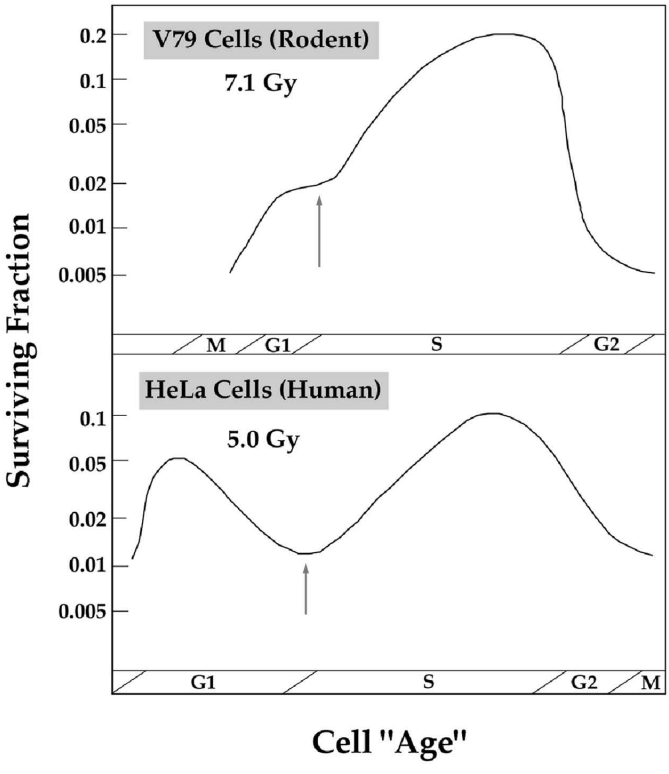
Division Delay: irradiated cells experience blocks and delays in their continued movement through the cell cycle

Compensatory Proliferation (Repopulation): a tissue preservation strategy in which the production of new cells is increased in response to damage to existing cells; discussed later in the course

Age Response Through the Cell Cycle

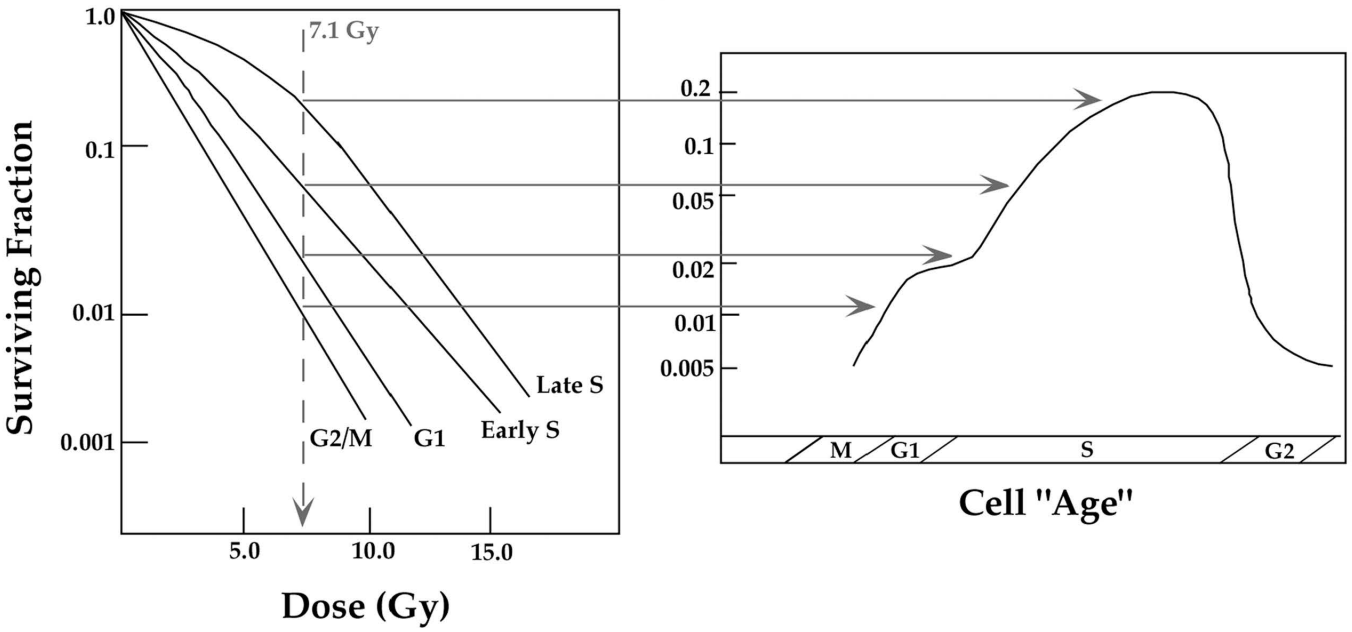
1) cells at different positions in the cell cycle have different sensitivities to radiation--this was first noted by Terasima and Tolmach (1960's), and even today, it is still not clear why this is the case

- ☛ Cells in Mitosis (M-phase) are the most sensitive, with G2 cells a very close second
- ☛ Cells in S phase (especially, late S-phase) are the most resistant
- ☛ Cells in G1 phase are of intermediate radiosensitivity
  - ☛ sometimes however, another period of relative sensitivity occurs at the border of G1 and S-phase; this is most pronounced in cells that have a long G1 phase (like HeLa), compared to those with a short G1 phase (like V79 cells)



Forms of age response for cells with short G_1, represented by hamster cells (*A*), and cells with long G_1, represented by HeLa cells (*B*). The time scales have been adjusted so that *S* has a comparable length on the figure for both cell lines. (From Sinclair WK: Dependence of Radiosensitivity Upon Cell Age. In Proceedings of the Carmel Conference on Time and Dose Relationships in Radiation Biology as Applied to Radiotherapy, pp 97–107. BNL Report 50203 [C-57], 1969)

2) complete survival curves for synchronized cells show the following features:



Survival curves for Chinese hamster cells at different stages of the cell cycle after synchronization by both mitotic selection and tritiated thymidine treatment (after Sinclair & Morton 1966)

a. G2/M cells have steep survival curves (low D_0's) and little or no survival curve shoulders

b. late S cells have somewhat shallower survival curves (higher D_0's), but mainly, have very broad shoulders in the low dose region

To summarize:

- ***the main reason for the age response through the cell cycle is that more resistant phases have broad shoulders on their survival curves, and more sensitive phases have little or no shoulders at all; the survival curve slopes don't change that much***

- ***it also follows that if a mixed population of cells of different "ages" is irradiated, the most resistant ones (S phase, plus some G1) would be most likely to survive, and this will change the overall cellular mix...unless redistribution occurs, in which case the cell population goes back to its original mix***

Radiation-Induced Division Delay

☞all cells, regardless whether they are destined to live or die, experience a radiation-induced perturbation in their movement through the cell cycle termed "division delay"

☞a good approximation of the amount of delay experienced per unit of dose delivered is about 1-2 hours/Gy (X-rays); (in other words, the larger the dose, the greater, and longer, the delay period)

But what is division delay caused by?????

controlled by so-called "checkpoint genes"

☞ *We now know that, in response to having experienced damage to DNA, cells "pause" during G2 phase of the cell cycle in order to check everything over...*

Is there any damage left?
Did repair occur?
Did repair occur correctly?
Is anything else wrong with the cell?

...prior to making the commitment to enter mitosis

Cellular "Repair" and Fractionation/Protraction

A. Historical Perspective on "Cellular Repair" Phenomena

1] back in the 1950s, it was still unclear that the repair of DNA damage (or lack thereof) was the basis for cellular radiosensitivity, so radiobiologists came up with more generic, whole cell-level descriptions of what they considered "repair" (a better term is "recovery" since actual repair wasn't measured)

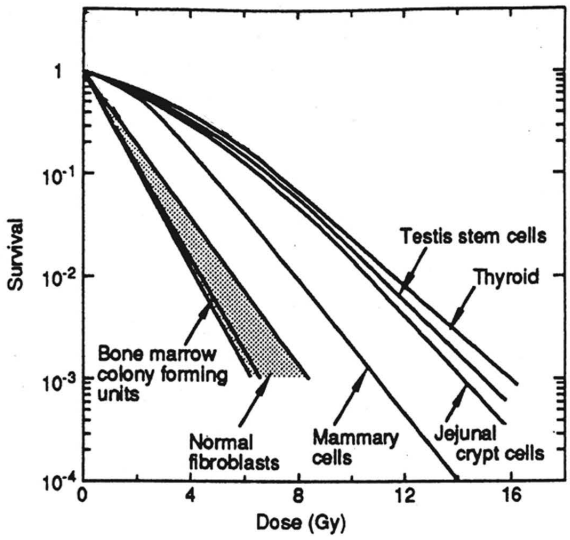
2] because of these reasons, correct terminology is very important

a. **lethal damage** - is irreversible, nonreparable, nonmodifiable and always causes cell death

b. **sublethal damage** and repair (SLD/SLDR) - is normally reparable within a few hours unless more sublethal damage is added; if so, these can interact to form lethal damage; *assayed using a split dose experiment*

B. **Sublethal damage and repair** (SLD/SLDR) - a type of damage that is normally reparable within a few hours, unless more sublethal damage is added; if so, these can interact to form **lethal damage**

1. it was discovered that the shoulder region on radiation survival curves was a reflection of the cell's ability to repair SLD, i.e., a big shoulder meant a large repair capacity

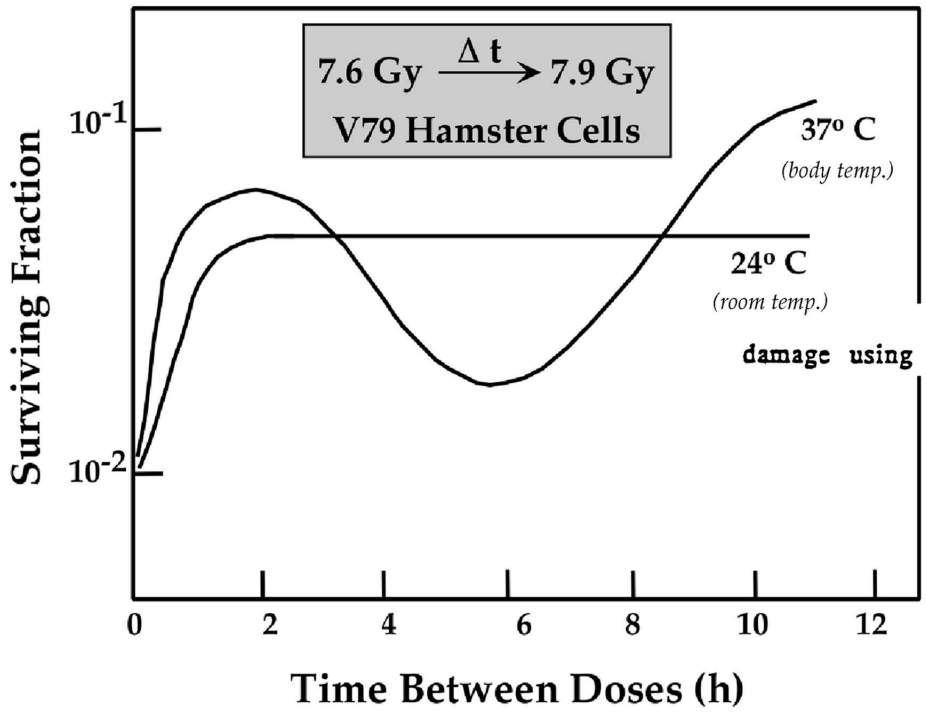


Survival curves for cells from some normal tissues. Most of the curves are for cells from rodent tissues, and the curves were produced using in vivo or in situ clonogenic assays

(Modified from Hall, 1988, and Fertil and Malaise, 1981.)

2] **Elkind and Sutton (1959) were the first to describe sublethal damage and repair**

a. SLDR is assayed using a **split dose experiment**, in which a single radiation dose is either immediately followed by a second dose, or a varying time interval is placed between the first and second dose; the fraction of cells surviving the two doses is then compared as a function of the time interval between them

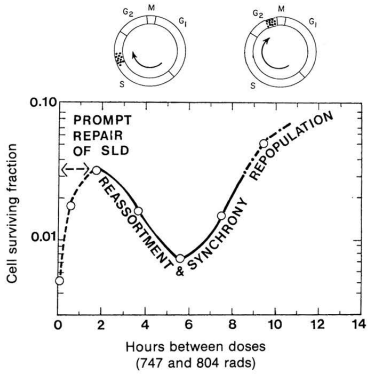


Recovery from sublethal x-ray damage using V79 Chinese hamster cells *in vitro*.

b. The shape of the recovery curve is due to two distinct effects:

1) **the intracellular repair of sublethal damage**

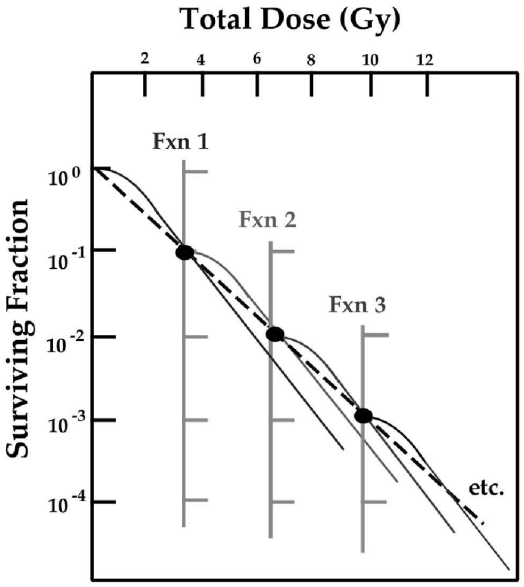
2) **progression of cells around the cell cycle between the two doses** and age response effects



Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at 37°C for various time intervals between the two doses. The survivors of the first dose are predominantly in a resistant phase of the cycle (late S). If the interval between doses is about 6 hours, these resistant cells have moved to the G2-M phase, which is sensitive. (Adapted from Radiat Res 25:359–376, 1965)

c. *What the repair of SLD means in terms of complete survival curves is that cells surviving the first dose act less and less like they ever were irradiated, the more time allowed before the second dose;* eventually, the survival curve for previously irradiated cells would be nearly identical to that for unirradiated cells, including **the reappearance of the shoulder**

d. **SLDR occurs repeatedly and with undiminished magnitude if multiple dose fractions are given; the same holds true for continuous, low dose rate irradiation** (but in this case, the repair occurs *during* the extended treatment time rather than in the radiation-free interval between fractions)



D. Cellular "Repair": Implications for Radiation Protection and Radiation Therapy

1] Without having to invoke any other radiobiological concepts or principles, ***the existence of sublethal damage recovery has profound implications for both radiation therapy and radiation protection***

a) because these types of cellular recovery can occur repeatedly (and with undiminished magnitude) each time a radiation dose is delivered, **it follows that either protracting or fractionating a total dose reduces its biological effectiveness...this is called the "dose rate effect", and occurs for a number of (radio-)biological endpoints following exposure to <u>low LET radiation</u>**

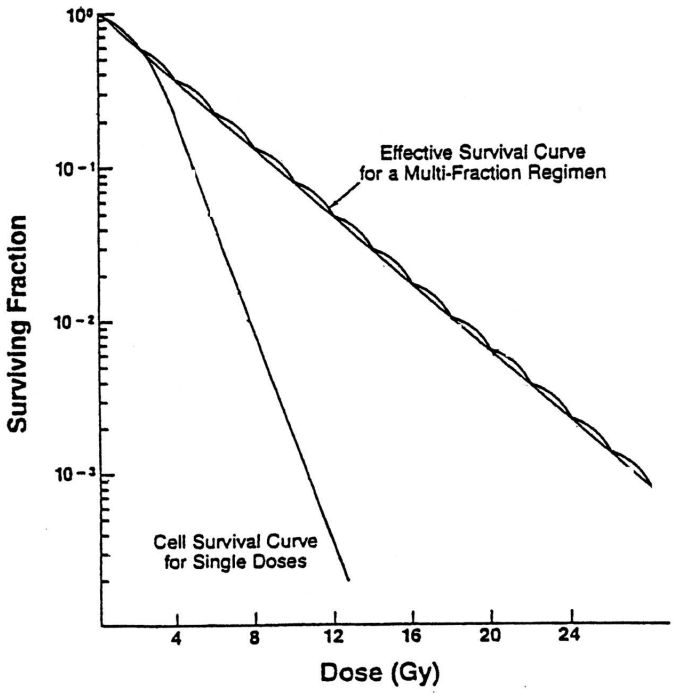
b) **cellular repair can explain almost completely why radiotherapy delivered as many, small dose fractions is much less damaging to patients than giving one or a few large doses** (we learned this the hard way in the early days of radiotherapy, well before there was any understanding of repair)

2] other effects of radiation (in addition to cell killing) are also reduced when the dose is delivered in increments over time

fewer mutations when dose is fractionated / protracted
fewer chromosome aberrations
lower frequency of neoplastic transformation of cells
reduced risk of carcinogenesis

3] Survival curves for fractionated or protracted irradiation:

a. *a survival curve generated from a series of repeated small doses is termed a "multifraction survival curve"*; note that in this case, the dose axis refers to "total dose delivered", not dose per fraction!



The concept of an "effective" survival curve for a multifraction regimen is illustrated. When the radiation dose is delivered in a series of equal fractions separated by time intervals sufficiently long for the repair of sublethal damage to be complete between fractions, the shoulder of the survival curve is repeated many times. The effective dose survival curve is an exponential function of dose, that is, a straight line from the origin through a point on the single dose survival curve corresponding to the daily dose fraction (eg, 2 Gy).

b. Multifraction survival curves:

- have no shoulder, i.e., the extrapolation number, n, is approximately 1.0
- have shallower slopes (larger D_0's) than for their corresponding acute dose survival curves

• **are constructed from the product of the surviving fractions of each subsequent dose;** thus, if the surviving fraction after a 4 Gy dose is 0.3, the surviving fraction after 4 doses of 4 Gy (provided they are separated by enough time to allow for maximum SLDR, and ignoring any possible reassortment effects) will be:

$$(0.3) \times (0.3) \times (0.3) \times (0.3) = (0.3)^4 = 0.0081$$

Take-Home Messages

☞*even with only slight differences in the capacities for SLDR among different types of tumor cells, a whole spectrum of overall radiocurabilities could result (because this small difference would be magnified for each successive radiation dose fraction delivered)*

☞*whether cellular recovery would be advantageous or disadvantageous for radiotherapy would depend on the recovery capacity of the dose-limiting normal tissue compared to that for the tumor (and this would likely vary on a patient-by-patient basis)*