Sublethal and Potentially Lethal Damage Recovery

A. Historical Perspective on "Cellular Repair" Phenomena

1] the study of cellular repair during the late 1950's and into the 1960's was hampered by the lack of assays both for DNA damage and its repair--as such, **all that could actually be measured as reflecting some aspect of "repair" was "increased survival of cells when allowed time for the supposed repair to occur"**; that is, an operational definition

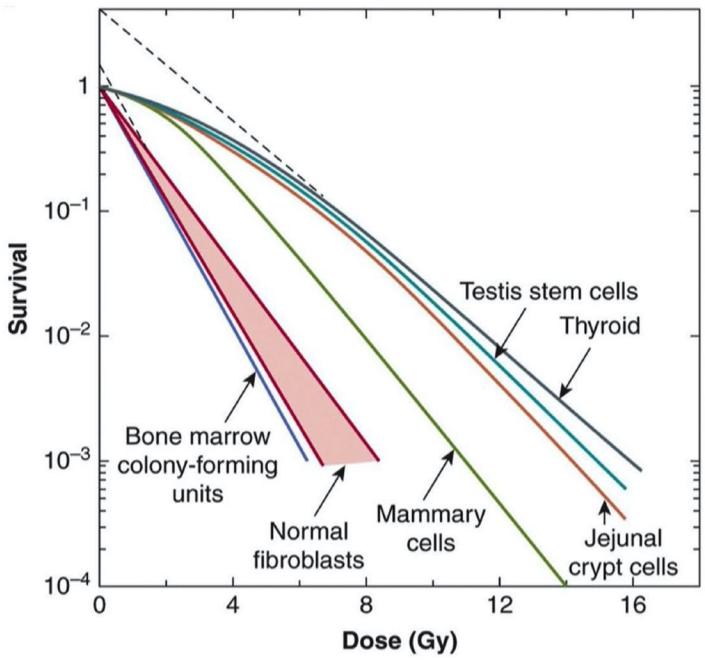
a. **lethal damage** - is irreversible, nonreparable, nonmodifiable and always causes cell death

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* (see below)

c. **potentially lethal damage** and repair (PLD/PLDR) - a spectrum of damage that may or may not be lethal depending on the post-irradiation environmental conditions; *assayed with a delayed plating experiment* (see below)

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 recovery capacity

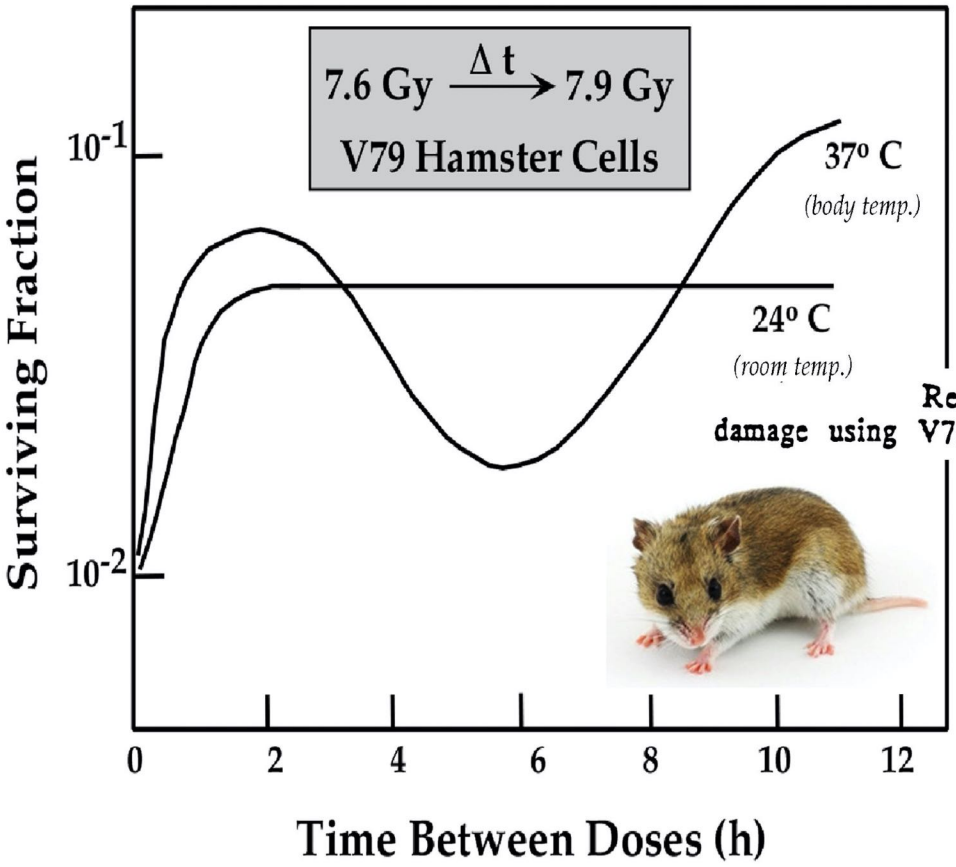


Survival curves for cells from some normal tissues. Most of the curves are for cells from rodent tissues, and the curves were produced using in vivo or in situ clonogenic assays. The range of values for normal human fibroblasts are for cultured cell lines.

b. with these concepts in mind, many investigators began to look for evidence of cellular "repair"

2] **Elkind and Sutton (1959) were the first to describe sublethal damage and repair (SLD/SLDR)**; this all sounds *really* primitive by today's standards, but was a really big deal at the time (published in *Nature*!)

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



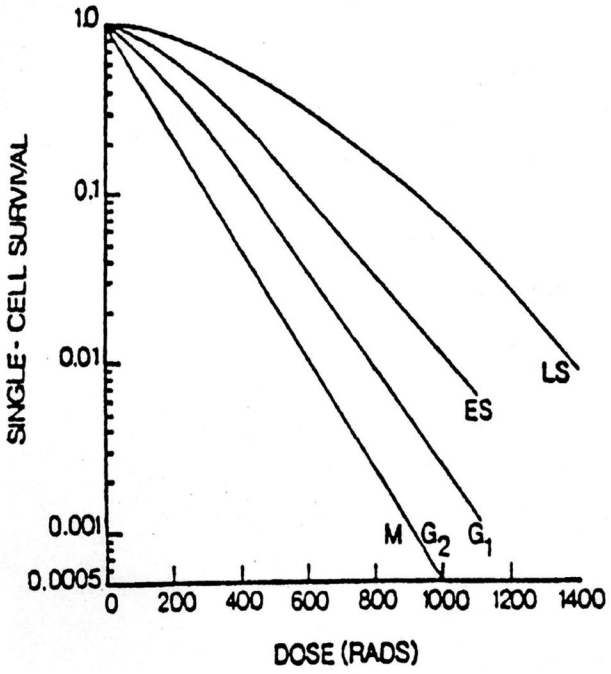
Recovery from sublethal x-ray damage using V79 Chinese hamster cells *in vitro*.

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

1) sublethal damage recovery

2) **progression of cells around the cell cycle between the two doses** and the celluolar "age response" for radiation

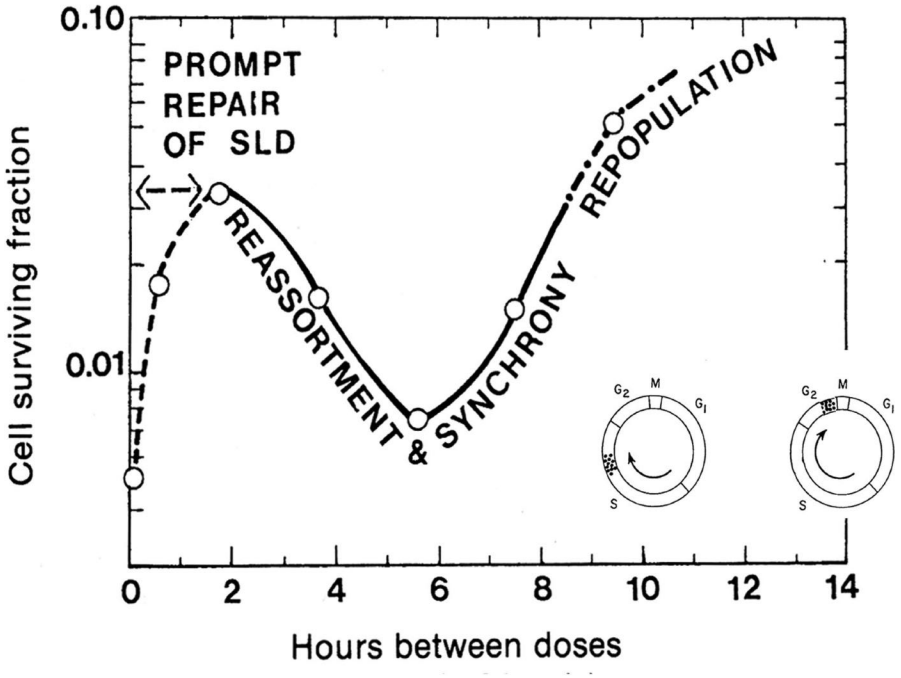
It turns out that cells in different phases of the cell cycle have different inherent radiosensitivities, i.e., survival curve shapes, with S phase cells most resistant (especially late S phase), and M phase cells the most sensitive (with G_2 cells a close second); this phenomenon was called the "**cell cycle age response**"



Survival curves illustrating the effect of cell cycle position on the response of a synchronous population of cells. Mitosis (M) and G2 are the most radiosensitive phases, whereas early S phase (ES) and late S phase (LS) are the most resistant. This is called the age response through the cell cycle. Movement of resistant cells into a more sensitive phase between the split doses accounts for the dip in the SLD recovery curve

(a) <u>How it all works</u>: The first dose partially synchronizes the surviving cells (because of different cellular sensitivities in different parts of the cell cycle--cells in the most resistant phases tend to survive preferentially), and as these then continue through the cell cycle, they necessarily move into more sensitive phases.

(b) the population as a whole becomes more sensitive by the time of the second dose, and a "dip" is noted in the SLD recovery curve; this is often called **reassortment** or **redistribution**



(c) Finally, if the time between the split doses was even longer, some cell begin to divide, causing the apparent surviving fraction to "overshoot". This is called repopulation.

(Adapted from Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB: Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). Radiat Res 25:359–376, 1965.)

A split-dose experiment only measures SLDR after a single dose (split into two parts), but what does SLDR look like when assessed in terms of whole survival curves?

SLD recovery manifests itself as a return of the shoulder region of the radiation survival curve, that is, that a cell that has repaired all of its sublethal damage will respond to a subsequent dose (or doses) as if it had never been irradiated in the first place

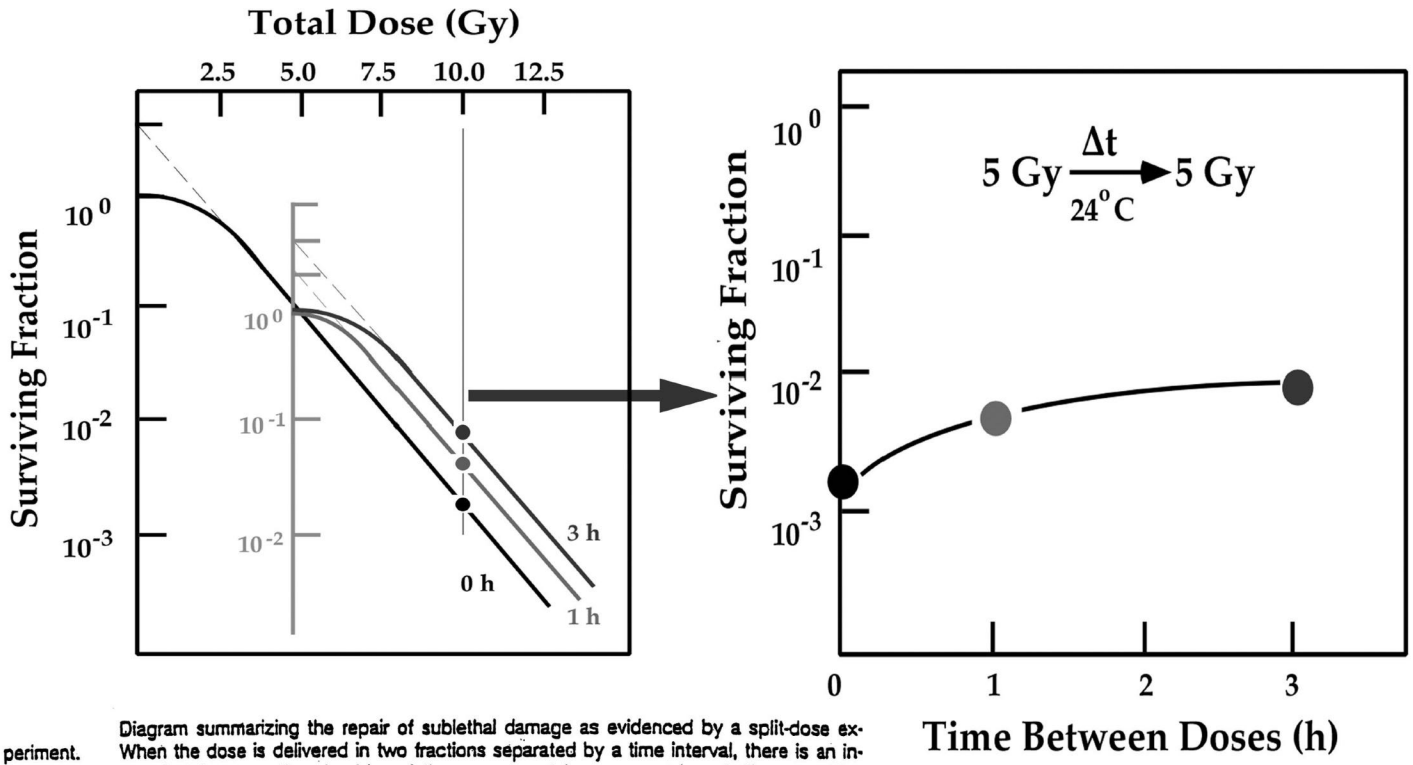


Diagram summarizing the repair of sublethal damage as evidenced by a split-dose experiment. When the dose is delivered in two fractions separated by a time interval, there is an increase in cell survival because the shoulder of the curve must be expressed each time. The fraction of cells surviving a split dose increases as the time interval between the two dose fractions increases. As the time interval increases from zero to 2 hours, the increase in survival is due to the repair of sublethal damage.

Other useful factoids concerning sublethal damage recovery:

1. Survival curve model quirks

a) *when using the target theory model, the magnitude of SLDR depends on the size of the survival curve shoulder (Dq)*; the broader the shoulder, the greater the capacity for SLDR, and vice versa

1] therefore, **cell types with strictly exponential survival curves with no shoulder do not exhibit SLDR**

a. examples: for X-rays, bone marrow or types of cells with certain DNA repair defects (e.g., AT), and for high LET radiations, all cell types

b) however, *when using the linear-quadratic model, the concept of a "shoulder" doesn't exist strictly speaking, so in this case the capacity for SLDR is governed by the β term*, that is, the component of cell killing that involves an interaction between two different lesions

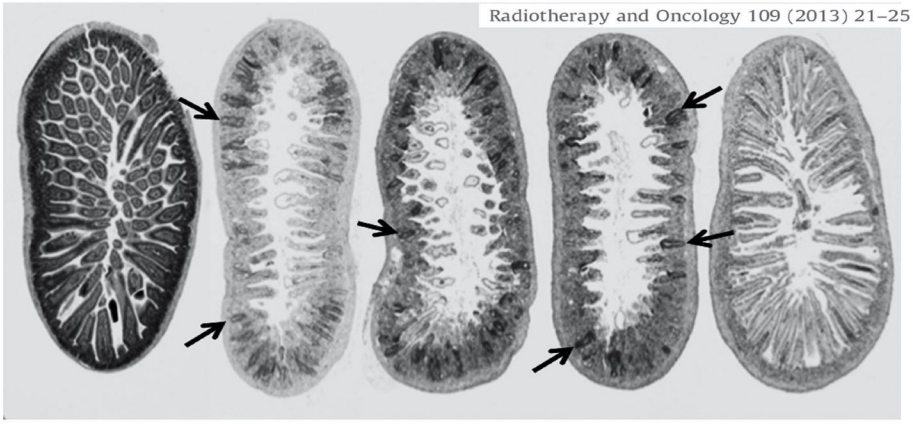
a. Note that this seems counterintuitive...which is why it sometimes shows up as a boards question!

2. SLDR occurs readily between about 10° and 40° C, but not for cells maintained on ice (at or below about 5° C), or at hyperthermic (above 40° C) temperatures

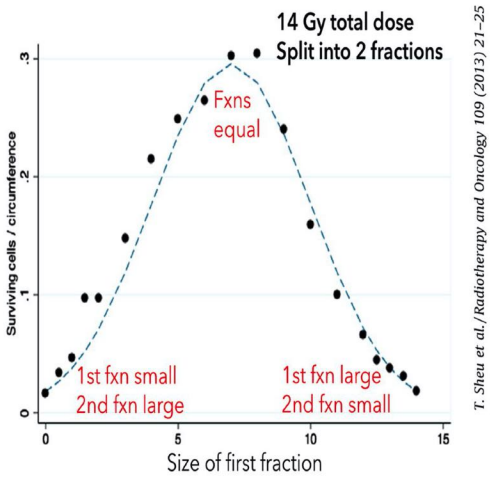
3. there is a reduction or elimination of SLDR if cells are kept (severely) hypoxic between the split doses

4. SLDR progressively decreases as the LET of the radiation increases, because more of the damage registered is lethal, rather than sublethal

5. *the maximum amount of recovery occurs when the two doses are nearly equal in size*, that is, 4 Gy + 4 Gy, as opposed to 2 Gy + 6 Gy or 6 Gy + 2 Gy. **This leads to the important conclusion that all treatment fields in radiotherapy should be treated daily, in order to maximize SLDR in normal tissues!**

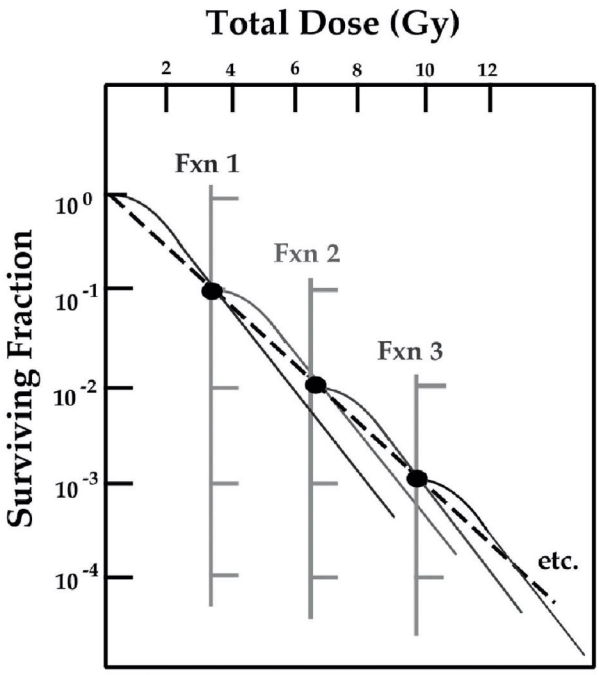


Dose:	0Gy	7 + 7Gy	4 + 10Gy	10 + 4Gy	14Gy
Crypts:	161.3 ± 1.4	41.6 ± 2.4	32.9 ± 2.5	24.5 ± 1.7	2.9 ± 0.4



T. Sheu et al./Radiotherapy and Oncology 109 (2013) 21–25

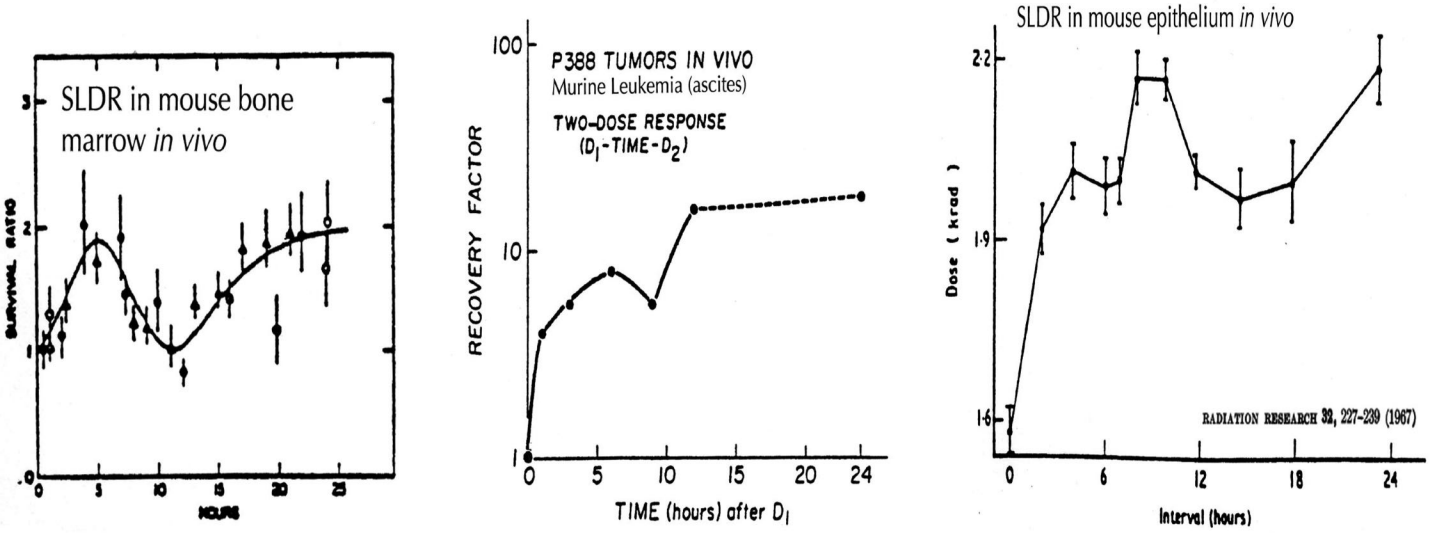
6. **SLDR occurs time after time with undiminished magnitude if many doses are given**



Repeated rounds of sublethal damage recovery explain almost single-handedly why radiation therapy given as one or a few large dose fractions is so much more damaging than giving multiple, small dose fractions spread out over protracted time periods.

This was figured out the hard way during the early years of radiation therapy...the biological underpinning, sublethal damage recovery, wasn't "discovered" for another 40 years

7. SLDR has also been demonstrated for normal tissues and tumors *in vivo*; the actual repair rates ("half-times for repair") are a matter of importance for radiotherapy, particularly in the case of altered fractionation schedules using multiple fractions per day



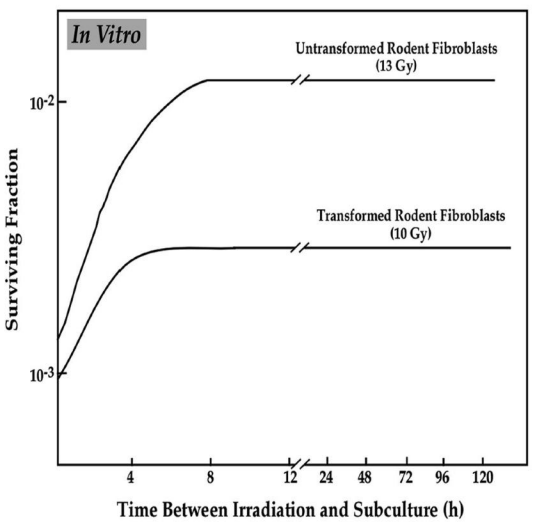
Adapted from: Radiat Res *41*: 450, 1970

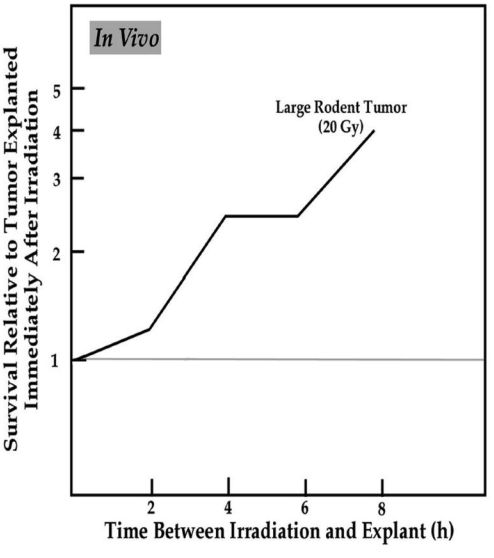
C. **Potentially Lethal Damage Recovery (PLD/PLDR)** - a spectrum of radiation damage that may or may not be repairable by the cell, depending on the environmental conditions in the hours after irradiation:

- ***Conditions that favor cell growth and division prevent PLD from being repaired*** - such as, plenty of oxygen and nutrients, proper temperature (body temp), lots of room to grow, etc.

- ***Conditions that discourage cell growth and division allow PLD to be repaired*** - such as, nutrient and/or oxygen deprivation, low temperature, overcrowding, too acidic or too basic conditions, etc.

1) *potentially lethal damage is measured using a technique known as "delayed plating"*; in this experiment, cells in an overcrowded petri dish or tumors in an animal are first irradiated, and then either harvested immediately, or else, left in place for different periods of time before being harvested and assayed for clonogenic survival





Demonstration of the effect of "delayed plating" on cell survival in the hours after irradiation. For both overcrowded cells in a petri dish (left) and for tumors *in vivo* (also overcrowded, right), cell survival increased the longer the cells were left in place prior to stimulating them to divide. **This phenomenon was termed "potentially lethal damage recovery"**. Note that untransformed cells – that are practically all quiescent – are better able to perform PLDR than tranformed cells, many of which are still going through the cell cycle.

2) What is it about continued cell growth that PREVENTS this type of repair from occurring?

Answer:

The molecular controls over the processes of DNA repair and cell cycle transit share some signaling pathways, i.e., the two processes regulate each other to some extent

this is a good thing because it helps safeguard the DNA from replicating while it may still contain damage (same idea as cell cycle checkpoints)

Doing both repair and proliferation would likely deplete the cell's energy stores

Sublethal and potentially lethal damage recovery - what are they, really?

A. Both are descriptive phenomena discovered 50+ years ago (with PLDR probably another manifestation of SLDR, which is why it's typically considered less important), but by today's standards, do they have known mechanistic underpinnings?

1) Answer: sort of, but not quite...

a] The time courses for SLDR and PLDR match the time courses for the rejoining of chromosome breaks

b] Chromosome breaks occur secondary to "open" DNA double strand breaks, which may or may not then go on to rejoin, although not necesarily correctly

1. If the cell has enough time to rejoin them and they rejoin *correctly*, that would properly be termed "repair" and the cell would survive, which probably accounts for SLDR/PLDR; if they don't rejoin or rejoin incorrectly, that would usually be lethal to the cell

c] But which repair pathways are involved? NHEJ? HR? MMEJ? All of them? Does it vary with the exact conditions, the exact type of lesion, etc.? And what about the kinds of DNA damages that create double strand breaks as part of their repair process, e.g., some crosslinks?

This is where things get kind of murky!

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 and potentially lethal damage recovery has profound implications for both radiation protection and radiation therapy…*

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 low LET radiation**

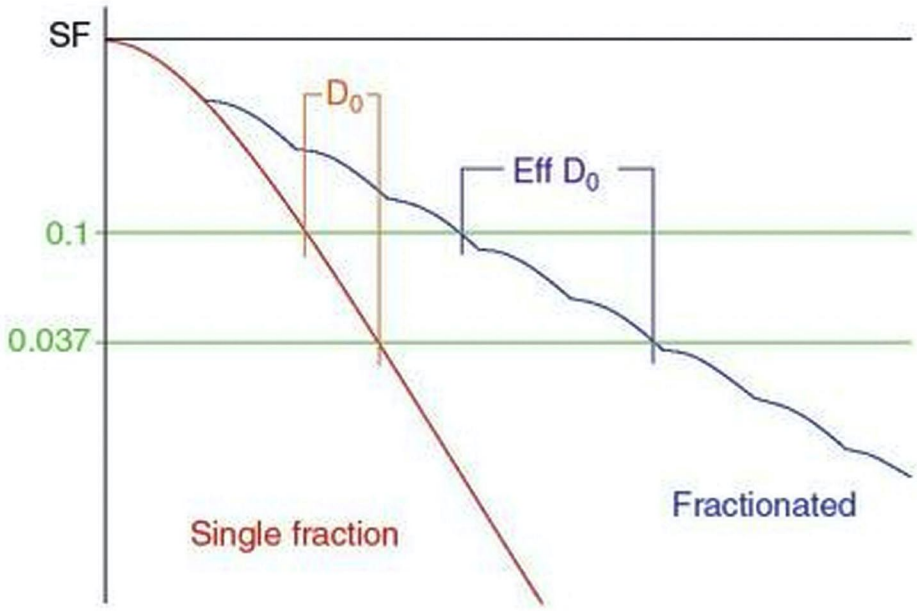
2] other effects of radiation (in addition to cell killing) are also reduced when the dose is delivered in increments over time

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!

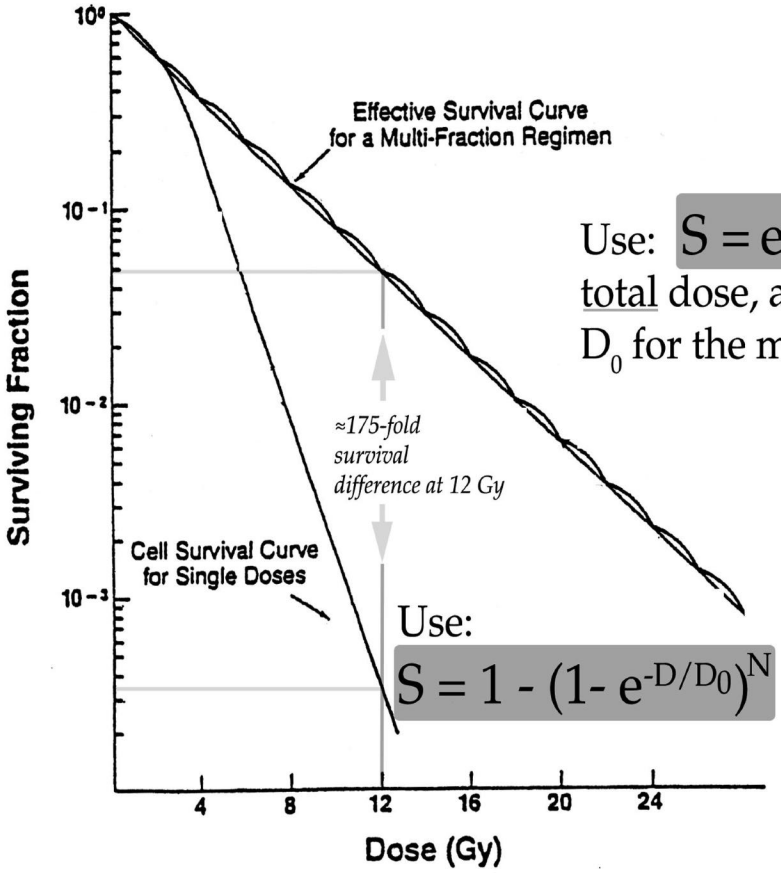
D_0 versus effective D_0. When radiation is fractionated, you need much more dose to achieve the same amount of cell kill. Therefore, effective D_0 is always larger than D_0.



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:

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



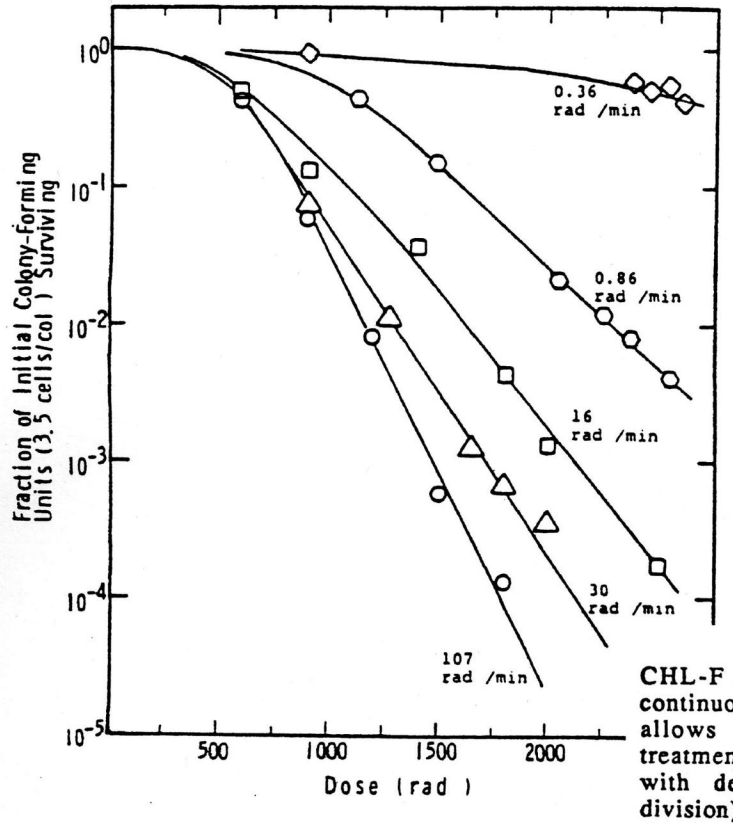
Use: $S = e^{-D/D_0}$ where D is the total dose, and D_0 is the effective D_0 for the multifraction curve

If using the linear-quadratic model instead, can use the equation:

$S = e^{-\alpha D}$

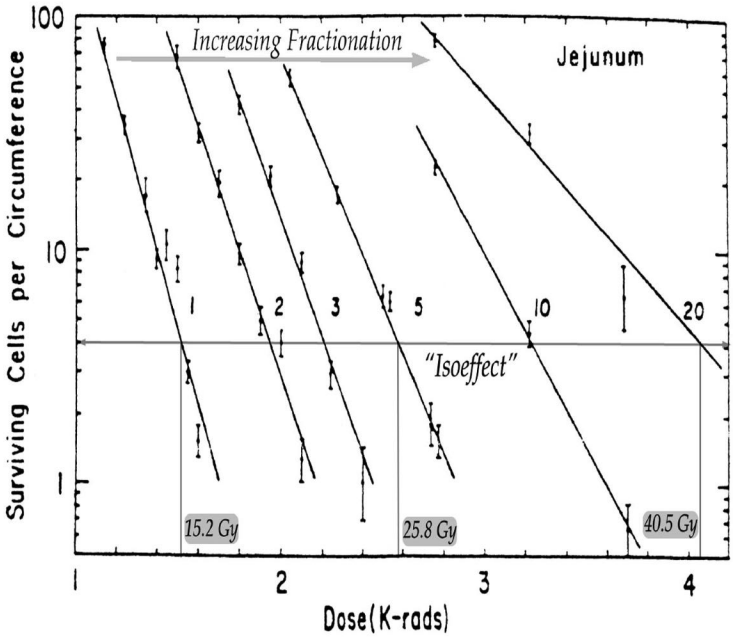
because the β term disappears when the survival curve is exponential

4] **low dose rate survival curves are similar to multifraction survival curves,** except in the case of low dose rate treatment, repair is occurring during the treatment instead of in the interval between treatments...but the same principles of SLDR and PLDR apply



Dose rate effects for asynchronous CHL-F cells *in vitro*. Delivering the dose continuously over an extended time period also allows cellular repair to occur during the treatment, so survival curves become shallower with decreasing dose rate. Repopulation (cell division) during treatment can also contribute to the increased cell survival at low dose rates.

5] **multifraction or low dose rate survival curves can also be generated for tumors and normal tissues *in vivo;*** the only problem is that other radiobiological phenomena could be occurring at the same time complicating interpretation of the results



Survival curves for crypt cells in the mouse jejunum exposed to single or multiple doses of γ-rays (1 to 20 fractions). The score of radiation damage is the number of surviving cells per circumference (ie, the number of regenerating crypts per circumference of the jejunum). This quantity is plotted on a logarithmic scale against radiation dose on a linear scale. The D_0 for the single-dose survival curve is about 1.3 Gy (130 rads).

From Withers, HR, Mason K, Reid BO, Dubrasky N, Barkley HT, Brown W, Smathers JB: Cancer 34:39–47, 1974)

Take-Home Messages about Cellular "Repair":

☞ *cellular recovery phenomena, first demonstrated in the 1950's into the 1970's, are a manifestation of the molecular repair of damaged DNA following irradiation*

☞ ***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 these cellular recovery phenomena would be advantageous or disadvantageous for radiotherapy would depend on the recovery capacity of the dose-limiting normal tissue*