# Illinois Institute of Technology PHYSICS 561

Radiation Biophysics

Lecture 5:

Survival Curves

Andrew Howard





## Survival Curves

- We discussed models for cell survival last time
- We looked at various ln($S/S_0$) vs. dose models and the logic behind them
- Today we'll focus on the graphical implications and how we can look at the numbers
- Then we'll talk about cell cycles and other good solid cell-biology topics.
- (Warning: I'm more of a biochemist than a cell biologist, so don't expect high expertise here!)

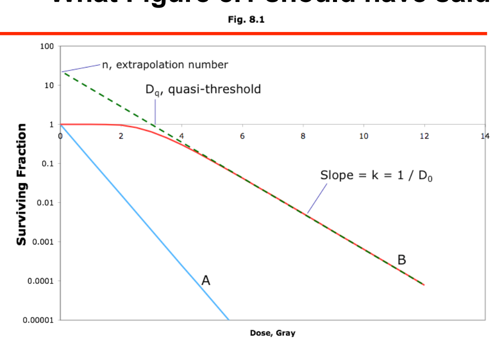


## Errata in Chapter 8

- Page 169, Paragraph 2, 1st sentence:
  Until the later 1950's it was *not* possible to use …
- Two sentences later:
  *Bacteroides* → *Bacillus* (at least I suspect so)
- Fig. 8.1, p. 173:
  The label that says $D_q$ is pointing at the wrong thing: it should be pointing at the place where the dashed line crosses the (Surviving faction = 1.0) value.



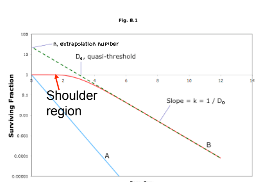
## What Figure 8.1 should have said






## Shoulder of the Survival Curve

- We recognize that with MTSH dose-response we have a region where the slope is close to zero. We describe that region as a shoulder:






## Slopes in the MTSH model

- Remember that the MTSH model says ln($S/S_0$) = ln(1-(1-exp($-D/D_0$))$^n$)
- Because $S/S_0$ = 1-(1-exp($-D/D_0$))$^n$
- So what is the slope of the $S/S_0$ vs. $D$ curve?
- … and … what is the slope of the ln($S/S_0$) vs. $D$ curve?
- In particular, what is the slope's behavior at low dose?
- Answer: calculate d$S/S_0$/ d$D$ and d(ln($S/S_0$)d$D$ and investigate their behavior at or near $D$ = 0.
- Note: we're looking here at the $n$>1 case.

## Slope investigation, part I

- For $S/S_0$ itself, $d(S/S_0)/dD = d/dD(1-(1-\exp(-D/D_0))^n)$
- The 1 out front doesn't affect the derivative: $d(S/S_0)/dD = -d/dD(1-\exp(-D/D_0))^n)$
- We'll do the rest of this calculation later based on the general formula $du^n/dx = nu^{n-1}du/dx$

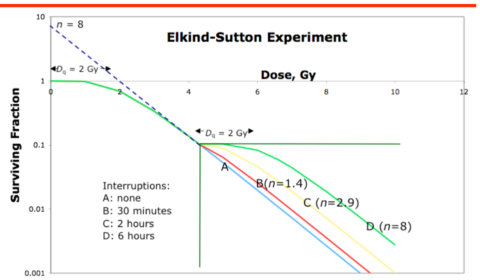


## The Elkind-Sutton Experiment

- Provides a way of probing repair functions in cells
- Procedure:
  - Irradiate and establish survival curve ("conditioning dose")
  - Take cells surviving at $S$=0.1 and subject them to further irradiation at varying time intervals after reaching $S$=0.1
- If repair is taking place, then the appearance of a curve similar to the original shoulder is indicative of full recovery



## Results






## Interpretation

- If slope and implied $n$ value are equivalent to the original curve, then repair is complete
- Smaller $n$ values indicate insufficient time has elapsed
- $n$=1 implies repair has not begun

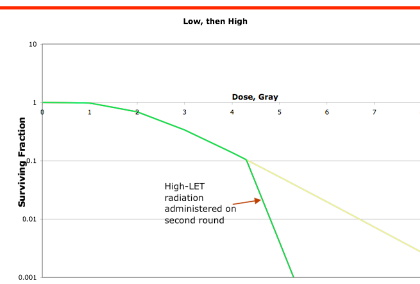


## Elkind-Sutton and LET

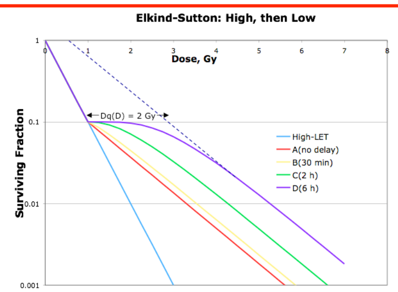
- We might expect more complicated results if we vary the LET for the two dosing regimens
- Low-LET first, high-LET second gives two lines of different slope, independent of the time interval
- High-LET first, low-LET second gives line followed by usual Elkind-Sutton distributions



## Low-LET followed by High-LET



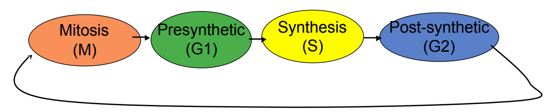
## High-LET, then Low-LET






## The Cell Cycle

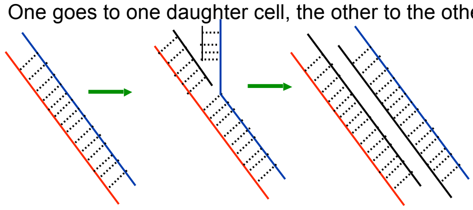
- Cells have a definite cycle over which specific activities occur.
- Particular activities are limited to specific parts of the cycle
- Howard and Pelc (1953) characterized four specific phases:
- M (mitosis, i.e cell division)
- G1 (growth prior to DNA replication)
- S (synthesis, i.e DNA replication)
- G2 (preparation for mitosis)






## What happens in S phase?

- DNA is replicated; thus, we have twice as much DNA at the end of S as at the beginning.
- During mitosis the two duplexes of DNA can separate
- One goes to one daughter cell, the other to the other





## How much time do these segments take?

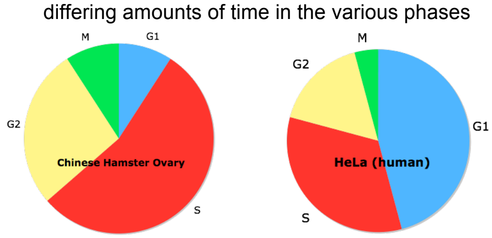
- Depends on the overall mitotic rate and the type of cell:
- Cell Cycle Times in hours:

| *Segment* | *CHO* | *HeLa* |
|---|---|---|
| M | 1 | 1 |
| G1 | 1 | 11 |
| S | 6 | 8 |
| G2 | 3 | 4 |
| TOTAL | 11 | 24 |



## Pie charts of cycle percentages

- The point is that different kinds of cells spend differing amounts of time in the various phases



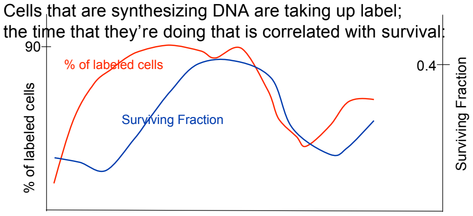



## Phase Sensitivity

- Many cells are much more sensitive to radiation in some parts of the cell cycle than they are in others.
- Why?
  - Repair is more vigorous in some stages
  - Unrepaired damage has more opportunity to manifest itself as clonal alteration close to mitosis
  - Access of repair enzymes to damaged DNA is sometimes influenced by how organized the DNA is.

## What phases are sensitive?

- In general, cells are radioresistant when they are synthesizing DNA.
- Cells that are synthesizing DNA are taking up label; the time that they're doing that is correlated with survival:

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## Survival Curves in Various Phases

- See fig. 8.8:
  - Late S is least radiosensitive
  - Early S next least
  - G-1 somewhat sensitive
  - G-2 and M most radiosensitive
- M and G2 curves are essentially straight lines (log-linear dose-response), suggesting that repair is unavailable or of little influence

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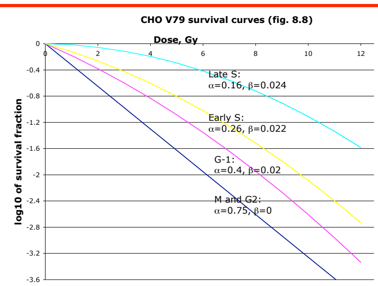
## Figure 8.8, reimagined with LQ model

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## Radiation-Induced Cell Progression Delay

- Note that various biochemical signals regulate progression from one phase of the cycle to another.
- To study this, you need synchronized cells . . .
- Sample study (Leeper, 1973):
  - CHO cells exposed to 1.5 Gy in mid-G1 experienced a delay of 0.5 h in cell division
  - 1.5 Gy in late S or early G2 caused a delay of 2-3 h
  - Dose-dependent: (4h for 3Gy, 6-7h for 6 Gy)

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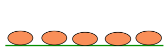
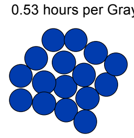
## Shape of the culture matters!

- How the cells grow influences how much the cells' progression is altered by radiation
- Monolayers' progression is altered less than cells in a multicellular spheroid geometry

0.22 hours per Gray



0.53 hours per Gray



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## Is that such a big deal?

- Probably not:
- The cells in the spheroidal mass divide half as fast even in the absence of radiation, possibly due to contact inhibition.
- Therefore it may simply be that the whole mitotic clock has been slowed down, including the clock as it's been influenced by radiation.

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## Causes for these effects

- Why are cells more radiosensitive in M and G2?
  - Availability of repair enzymes
  - Repackaged DNA is hard to repair
- How is cell progression influenced by radiation?
  - Damage to protein kinases and cyclins involved in cellular checkpoints
  - Premature degradation of p21, maybe…



## Arithmetic & Calculus of Survival Models

- MTSH says $S/S_0 = 1 - (1\text{-e}^{-D/D_0})^n$
- What I want to investigate is the slope at low dose, I.e. for $D << D_0$,
- And at high dose, I.e. for $D << D_0$.
- But are we interested in the slope of S/S0 vs. D or $\ln(S/S_0)$ vs. $D$?
- Both!
- Slope = derivative with respect to $D$. So
- Slope = $d/dD(1 - (1\text{-e}^{-D/D_0})^n) = -d/dD(1\text{-e}^{-D/D_0})^n$



## MTSH slope, continued

- Recalling that $du^n/dx = nu^{n-1}du/dx$, for n>1,
- Slope = $-n(1\text{-e}^{-D/D_0})^{n-1}$ d/dD $(1\text{-e}^{-D/D_0})$ = $-n(1\text{-e}^{-D/D_0})^{n-1}$ $(-d/dD(e^{-D/D_0}))$
- But we know $de^u dx = e^u\, du/dx$, so $d/dD(e^{-D/D_0}) = e^{-D/D_0}(-1/D_0) = -1/D_0\, e^{-D/D_0}$
- Therefore Slope = $-n(1\text{-e}^{-D/D_0})^{n-1}$ $(-)(-1/D_0\, e^{-D/D_0})$
- i.e. Slope = $-n/D_0(1\text{-e}^{-D/D_0})^{n-1}e^{-D/D_0}$
- This formula is valid for all values of $D$, including $D << D_0$ and $D >> D_0$



## Slope at $D << D_0$

- For small $D$, I.e. for $D << D_0$,
- Slope is $-n/D_0\,(1\text{-e}^{-0/D0})^{n-1}e^{-0/D0}$ $= -n/D_0\,(1\text{-e}^0)^{n-1}e^{-0}$; for n > 1 this is $= -n/D_0\,(1\text{-}1)^{n-1}1 = 0$. Shazam.



## Slope of $\ln(S/S_0)$ vs $D$

- The behavior of the slope of the $\ln(S/S_0)$ vs $D$ curve is not much harder to determine.
- Recall d ln$u$ dx = $(1/u)du/dx$. We apply this here:
- $d \ln(S/S_0) / dD = (1/(S/S_0))\, d(S/S_0)/dD$.
- For very small $D$, $S/S_0 = 1$, so $d \ln(S/S_0) / dD = (1/1) * d(S/S_0)/dD = d(S/S_0)/dD$ .
- But we've just shown that that derivative is zero, so $d \ln(S/S_0) / dD = 0$.



## High-dose case

- We've covered the low-dose case.
- What happens at high dose, i.e. $D >> D_0$?
- What we'd like to show is that the slope of $\ln S/S_0$ vs. $D$ is $-1/D_0$. Let's see if we can do that.
- Slope = $d \ln(S/S_0) / dD = (1/(S/S_0))\, d/dD(S/S_0)$
- Thus slope = $(1 - (1\text{-e}^{-D/D_0})^n)^{-1} d/dD(1 - (1\text{-e}^{-D/D_0})^n)$
- For $D >> D_0$, $D/D_0$ is large and $-D/D_0$ is a large negative number; therefore $e^{-D/D_0}$ is close to zero.

## High-dose case, continued

- Slope = $\lim_{D \to \infty}$ $\{(1 - (1-e^{-D/D_0})^n)^{-1}(-n/D_0)(1-0)^{n-1}e^{-D/D0})\}$
- That's messy because the denominator and the numerator both go to zero. There are ways to do that using L'Hôpital's rule, but there are simpler says that don't involve limits.
- The trick is to recognize that we can do a binomial expansion
- I'll do that in a separate set of HTML notes
- The result will be slope = $-1/D_0$ and ln $n$ is the Y intercept of the extrapolated curve



## Linear-Quadratic Model

- This is simpler. $\ln(S/S_0) = \alpha D + \beta D^2$
- Therefore slope = $d/dD\,(\ln(S/S_0)) = d/dD(\alpha D + \beta D^2)$
- Thus slope = $\alpha + 2\beta D$. That's a pretty simple form.
- At low dose, $|\alpha| >> 2|\beta|D$, so slope = $\alpha$.
- At high dose (what does that mean?) $|\alpha| << 2|\beta|D$, so slope = $2\beta D$.
- What constitutes a high dose?
  Well, it's a dose at which $|\alpha| << 2|\beta|D$, so $D >> |\alpha / 2\beta|$
- Thus if dose $>> |\alpha / 2\beta|$, then slope = $2\beta D$.

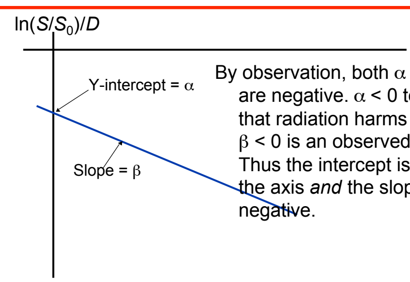


## Implications of this model

- At low dose slope = $\alpha$ is independent of dose but is nonzero; thus $\ln(S/S_0)$ is roughly linear with dose.
- At high dose slope = $2\beta D$, I.e. it's roughly quadratic.
- How can we represent this easily?
  We discussed this last time:
  $\ln(S/S_0) / D = \alpha + \beta D$,
- so by plotting $\ln(S/S_0) / D$ versus $D$, we can get a simple linear relationship.



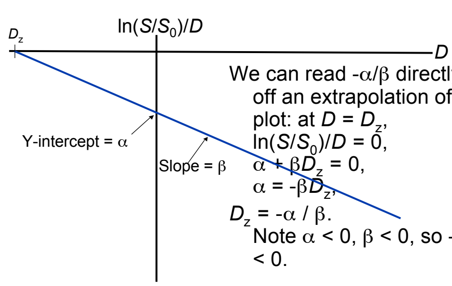
## LQ: Plot of ln(S/S0) / D versus D




By observation, both $\alpha$ and $\beta$ are negative. $\alpha < 0$ tells us that radiation harms cells; $\beta < 0$ is an observed fact. Thus the intercept is below the axis *and* the slope is negative.



## LQ graphical analysis: one step further




We can read $-\alpha/\beta$ directly off an extrapolation of the plot: at $D = D_z$,
$\ln(S/S_0)/D = 0$,
$\alpha + \beta D_z = 0$,
$\alpha = -\beta D_z$,
$D_z = -\alpha / \beta$.
Note $\alpha < 0$, $\beta < 0$, so $-\alpha/\beta < 0$.



## Where do linear and quadratic responses become equal?

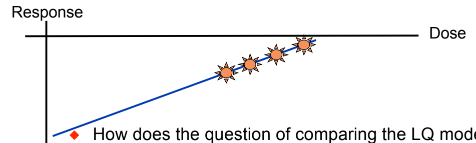
- At what dose does the linear response equal the quadratic response?
- At that dose, $\alpha D = \beta D^2$, $D = \alpha/\beta$
- So the value we read off the X-intercept of the previous curve is simply the opposite of the dose value at which the two influences are equal.

## How plausible is all this?

- Model studies suggest reasons to think that $\ln(S/S_0) = \alpha D + \beta D^2$ is a good approach.
- Much experimental data are consistent with the model
- Some of these LQ approaches allow for time-dependence to be built in.



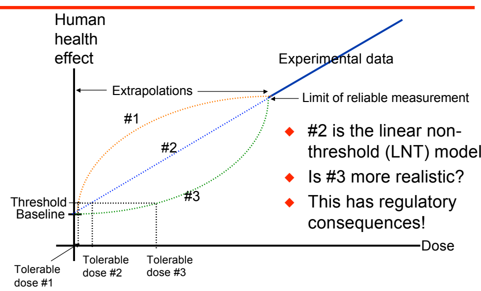
## LQ vs MTSH and thresholds




- How does the question of comparing the LQ model to the MTSH model relate to the question of threshold doses?
- A typical real-world question is a dose-response relationship for which the only reliable experimental results are obtained at high doses; at lower doses the confounding variables render the experiments uninformative.



## Dose-Response in Epidemiology




- #2 is the linear non-threshold (LNT) model
- Is #3 more realistic?
- This has regulatory consequences!