Illinois Institute of Technology

Radiation Biophysics

Lecture 6:
Survival Models
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How do we define survival?

- It's not as easy at the cellular level as you might think.
- It takes a *lot* of radiation to destroy metabolism.
- It takes a lot *less* to compromise DNA replication badly enough to either:
  - Prevent replication after 1-4 generations
  - Produce large changes in morphology or function, again after 1-4 generations
- Therefore: we concentrate on *clonogenic* survival as a definition for cell survival

What kind of experiments do we envision here?

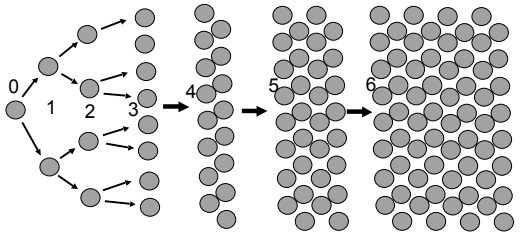
- Small number of cells placed on a growth medium
- Cells are exposed to a toxicant or to radiation
- Cells allowed to divide for some number of generations
- We compare the number of progeny in the treated cell group to the number in the untreated group
- Damage is said to be significant if the treated group produces fewer progeny than the control group

What do we mean by clonogenic survival?

- Clean definition: clonogenic survival is the ability to produce six generations of viable offspring
- This works well for prokaryotic cells and cultured eukyarotic cells, particularly immortalized ones
- It works less well for differentiated eukaryotic cells:
  - A respectable eukaryotic cell has a chromosomal component called a *telomere* that regulates the number of cell divisions before the cell undergoes programmed cell death (apoptosis)
  - If the cell you're studying is close to its natural cutoff point for cell divisions, it's clearly unfair to blame the treatment for its inability to produce five generations of progeny!

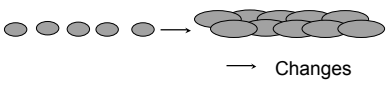
Six generations…

- Roughly corresponds to 50 surviving progeny



Contact inhibition

- Many cells change behavior when they come into contact with neighbors
- Often the change involves inhibition of replication
- That complicates the definition of clonogenic survival:
- If the cells stop dividing because they're getting too crowded, it's unfair to blame that on the treatment!

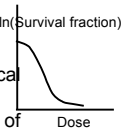


What's an immortalized cell line?

- Certain transformed cell lines lose their responsiveness to cell-cell communication and to the apoptotic count
- These cells can replicate without limit
- Often this kind of transformation is associated with cancer
- It's always questionable whether experiments on transformed cell lines are telling us anything useful about the behavior of untransformed cells
- But we're somewhat stuck with this kind of system

Mechanisms of Reproductive Cell Survival and Death

- Up until around 1970 there were two highly disparate lines of research surrounding these issues:
  - *Modelers,* who carried out mathematical studies of dose-response;
  - *Biologists*, who sought understanding of the mechanisms of the cellular response
    - Enzymatic
    - Molecular-biological
- Since 1970 there has been better communication between these two communities



Sorting out multiple causes

- … can be tricky.
- Ancient study of uranium mine workers:

Status	Smoking	Non-smoking
Miner	1	2
Non-miner	3	4

- Result:
  cancer(1) > cancer(3) >> cancer(2) ~ cancer(4)
- So the effect of mining is potentiated by smoking
- We'd like to know why!

Lea's model for cellular damage

- Four basic propositions (1955):
  - Clonogenic killing is multi-step
  - Absorption of energy in some critical volume is step 1
  - Deposition of energy as ionization or excitation in the critical volume will give rise to molecular damage
  - This molecular damage will prevent normal DNA replication and cell division
- Alpen argues that this predates Watson & Crick. That's not really true, but it probably began independent of Watson & Crick

Lea's assumptions

- There exists a specific target for the action of radiation
- There may be more than one target in the cell, and the inactivation of n of these targets will lead to loss of clonogenic survival
- Deposition of energy is discrete and random in time & space
- Inactivation of multiple targets does not involve any conditional probabilities,
  i.e., P(2nd hit) is unrelated to P(1st hit)

Typos in Alpen

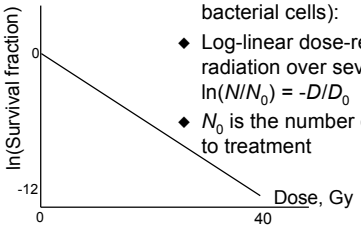
- Alpen seems to have replaced $\mathcal{D}$ with $\mathcal{D}$! several times
- Several terms have too many factorial signs in them;
  - Eqn. 7.3 should be $P(\rho,h,\mathcal{D}) = ({}_{\mathcal{D}}C_h)(\rho^h)(1-\rho)^{(\mathcal{D}-h)}(H(h))$
  - Eqn. 7.4 should be $S(\rho,\mathcal{D}) = \Sigma_{h=0}{}^{h=\mathcal{D}}\ P(\rho,h,\mathcal{D})$
- Axis labels are faulty sometimes too:
  Pp. 136-137: the lowest number on the Y axis should be 0.01, not 0.001

The role of DSBs

- We will eventually want to emphasize *unrepairable* DNA damage as the true bad actor in all of this
- We saw at the end of last class that double strand breaks are harder to repair with high fidelity
- So DSBs are likely to be the real issue here
- You can begin to see the utility of an interaction between the modelers and the biologists!

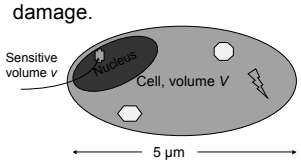
Log-linear response

- With cells that are distinctly deficient in DSB repair (e.g., bacterial cells):
- Log-linear dose-response to radiation over several logs $\ln(N/N_0) = -D/D_0$
- N_0 is the number of cells prior to treatment



The cellular damage model

- Cell has volume V; target volume is $v << V$
- Mechanistically we view v as the volume surrounding the DNA molecule such that absorption of energy within v will cause DNA damage.

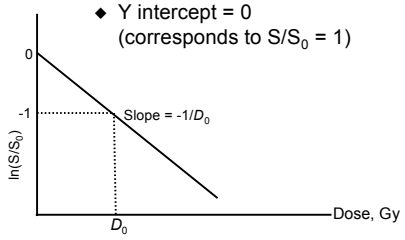


Single-target, single-hit model

- In this instance, each hit within the volume v is sufficient to incapacitate the cell
- Define S(D) as the survival fraction upon suffering the dose D. Define S_0 = survival fraction with no dose.
- Note that S_0 may not actually be 1: some cells may lack clonogenic capacity even in the absence of insult
- Then: $S/S_0 = \exp(-D/D_0)$
- D_0 = dose required to reduce survival by 1/e.

STSH model: graphical behavior

- Slope of curve = $-1/D_0$
- Y intercept = 0 (corresponds to $S/S_0 = 1$)

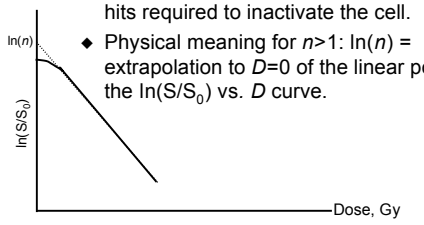


Multi-target, single-hit model

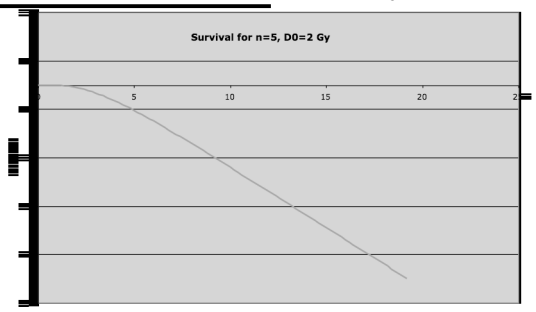
- Posits that n separate targets must be hit
- Probabilistic algebra given in Alpen
- Outcome: $S/S_0 = 1 - (1 - \exp(-qD))^n$, or for $D_0=1/q$, $S/S_0 = 1 - (1 - \exp(-D/D_0))^n$
- This model looks at first glance to involve a very different formula, but it doesn't, really:
- For $n = 1$, this is $S/S_0 = 1 - (1 - \exp(-D/D_0))^1$
- But that's just $S/S_0 = \exp(-qD)$, i.e. $\ln(S/S_0) = -qD$
- That's the same thing as STSH.

MTSH algebra

- Physical meaning of exponent *n*:
- Based on the derivation, it's the number of hits required to inactivate the cell.
- Physical meaning for *n*>1: ln(*n*) = extrapolation to *D*=0 of the linear portion of the $\ln(S/S_0)$ vs. *D* curve.



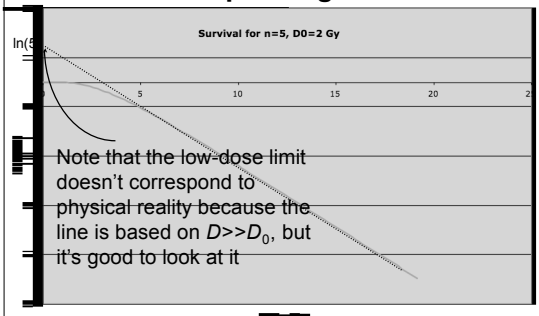
A semi-real case: n=5, D_0= 2 Gy



Behavior of this function for $D >> D_0$

- For $D >> D_0$, $\exp(-D/D_0) << 1$ so we can expand it:
- $(1- \exp(-D/D_0))^n = (1 - x)^n \sim 1 - nx$ for $x = \exp(-D/D_0) << 1$
- Therefore $1 - (1- \exp(-D/D_0))^n = nx = n\exp(-D/D_0)$
- Thus $\ln(S/S_0) = \ln(1 - (1- \exp(-D/D_0))^n)$ $= \ln(n\exp(-D/D_0) = \ln n - D / D_0$
- So the behavior for high doses is log-linear
  - with slope = $-1/D_0$, just as in the STSH model,
  - But with Y intercept = ln *n* rather than 0.

Extrapolating to D=0



Low-dose limit for MTSH with $n > 1$

- At *exactly* D=0, $S/S_0 = 1$ as we would expect
- Curve departs from linearity, though
- Slope of $\ln(S/S_0)$ vs. D curve at low dose: $\ln(S/S_0) = \ln(1 - (1 - \exp(-D/D_0))^n)$
- Remembering that $d(\ln(u))/dx = (1/u)du/dx$, $d/dD\ [(\ln(S/S0))] = (1-(1-\exp(-D/D_0))^n)^{-1}*$ $(0 - (1 - \exp(-D/D_0))^{n-1})*(-1/D_0)*\exp(-D/D_0) =$ $(1-(1-\exp(-D/D_0))^n)^{-1}(- (1 - \exp(-D/D_0))^{n-1}))*$ $(-1/D_0) \exp(-D/D_0)$. For D = 0, this is
- $d/dD[\ln(S/S_0)] = (1-(1-1)^n)^{-1}(-(1-1)^{n-1}))(-1/D_0)1 = 0$.

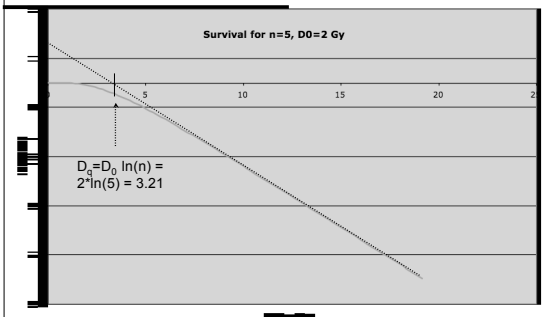
So what if the slope is zero?

- It's been routinely claimed that the flat slope at low dose is a deficiency in the MTSH model:
- It implies that at very low dose, the exposure has no effect
- That's politically unpalatable, and it flies in the face of some logic.
- BUT it is consistent with the notion that there might be a "threshold" dose below which not much happens
- There are a number of circumstances where that appears to be valid!

MTSH Quasi-Threshold Dose

- We note that the curve stays close to linear until we get to fairly low doses.
- We describe D_q = dose at which the linear extrapolation hits $\ln(S/S_0) = 0$, i.e. $S=S_0$:
- Since the line is $\ln(S/S_0) = \ln n - D/D_0$,
  $0 = \ln n - D_q / D_0$, so $D_q = D_0 \ln n$

Quasi-Threshold Dose Graphically



Deficiencies in the MTSH model

- Zero slope at zero dose (is that really bad?)
  We can tweak this if we need to:
  $S/S_0 = \exp(-q_1 D)(1 - (1-\exp(-D/D_0)^n)$
  $\ln(S/S_0)$ has slope $-q_1$ at D=0.
- High-dose behavior:
  - Does it remain truly linear at $D >> D_0$?
  - Some suggestions that it doesn't:
    maybe D_0 gets bigger, i.e. the slope gets steeper, at very high dose (saturating repair mechanisms?)
- Derivation may or may not match realities

What do we do about this?

- Maybe we need to set aside MTSH!
- Late 1970's through today: other more explicitly *repair-based* models were concocted.
- Most wind up proposing linear-quadratic solutions, i.e.
  $\ln(S/S_0) = \alpha * D + \beta * D^2$
- The logic behind this varies from derivation to derivation, but the final results are hauntingly similar

Repair-based models

- Introduction
  - Poisson statistics
  - 2-term Taylor Expansions
- Linear-Quadratic Models
  - Molecular Model
  - Dual Radiation Action Model
  - Repair-misrepair model
  - Lethal-Potentially Lethal model
- Graphical Implications
- Limitations of Applicability

Poisson survival

- Alpen's comment:
  A cell can be killed only once, and further action on remaining cells is constrained to that smaller number of cells.
- This is equivalent to saying
  $dN = N * d(f(D))$, I.e. $dN/N = d(f(D))$, or
  $\ln(N) = f(D)$ $\ln N_0$, I.e. $\ln(N/N_0) = f(D)$
- But $S = N/N_0$, so we have a basic formalism:
  $\ln(S) = f(D)$, where $f(D)$ is some function of dose.
  Let's seek out the appropriate functional form.

Linear-Quadratic Model: Generalized Form

- Back away temporarily from mechanistic approaches, and say that given Poisson statistics for lethality ln(S) = f(*D*), where f is some function
- For an arbitrary function f(D), we Taylor expand in *D*:
- $\ln(S) = a_0 + a_1 D + a_2 D^2 + \ldots + a_n D^n + \ldots$
  Where a_i are the Taylor coefficients (including the factorials in the denominator)
- But we take $a_0 = 0$ because at $D = 0$ the survival fraction is 1, i.e. ln(S) = 0
- Thus the second-order expansion is $\ln(S) = a_1 D + a_2 D^2$

Molecular Model

- This emphasizes double-stranded breaks in DNA as a source of lasting damage
- Distinguishes between single hits causing DSBs and pairs of hits causing DSBs:
  Ultimately, the pairs of hits give rise to the quadratic dependency on *D* in the formulas
- The derivation in Alpen is okay, but we wind up with a few parameters that aren't independently determinable

Dual Radiation-Action Formulation

- Emphasizes that a single interaction between a high-LET radiation event and a cell produces a DSB, whereas low-LET radiation requires pairs of events
- Gives rise to a linear-quadratic model:
- The one-event DSB (linear) coefficient predominates for high-LET radiation
- The two-event (quadratic) coefficient predominates for low-LET radiation

Tobias: Repair-Misrepair Model

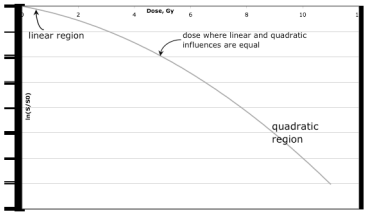
- Posit: linear and quadratic mechanisms up front for repair, with explicit *time*-dependence
- Time-independent formulas arise at times that are long compared with cell-cycle times
- In those cases
  $S = \exp(-\alpha D)(1+\alpha D/\varepsilon)^{\varepsilon}$
  where $\varepsilon = \lambda/k$ is the ratio of the repair rates of linear damage to quadratic damage.
- This gives roughly quadratic behavior in ln S.

Lethal - Potentially Lethal Model

- Sets up a three-state system:
- Undamaged cells (A)
- Potentially-lethally-damaged cells (B)
- Lethally damaged cells (C)
- Eurepair returns state B to state A
- B automatically becomes C at long times
- Gives rise to explicit quadratic formulation
  $\ln(S) = \alpha D + \beta D^2$
  with α and β having explicit time-dependence

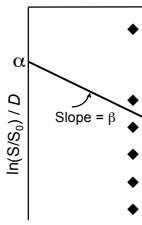
LQ Graphical Analysis

- At low dose the linear dependence predominates; at higher doses the quadratic dominates



$\ln(S/S0) = \alpha D + \beta D^2$
Can we assign physical significance to α and β, or perhaps to β/α?

How do we linearize the relationship?



- Pretty simple, actually: $\ln(S/S0) = \alpha D + \beta D^2$ means $\ln(S/S0) / D = \alpha + bD$
- So: plot $\ln(S/S0) / D$ versus D.
- Y-intercept = α
- Slope=β
- By convention $\alpha < 0$ (radiation kills!)
- β is generally < 0 also

What are the units of α, β, and α/β?

- In order for αD to be unitless, α must be measured in terms of inverse dose, e.g. α is in Gy^{-1}
- In order for βD^2 to be unitless, β must be measured in terms of inverse dose squared, e.g. β is in Gy^{-2}.
- Therefore α/β must be in units of dose, e.g in Gray

Modeled significance of α/β

- Suppose we expose a cell line to a dose equal to α/β.
- Then $\ln(S/S_0) = \alpha D + \beta D^2$ $= \alpha(\alpha/\beta) + \beta(\alpha/b)^2 = \alpha^2/\beta + \alpha^2/\beta$
- Thus at dose $D = \alpha/\beta$, influence from linear term and influence from quadratic term are equally significant
- Thus it's the crossover point:
  - Linear damage predominates for $D < \alpha / \beta$
  - Quadratic damage predominates for $D > \alpha / \beta$

Clonogenic survivability

- Even unirradiated cells don't provide 100% survival;
- Survival for irradiated cells has to be normalized against what's happening to the controls
- This sets an upper limit on the accurace of the determinations
- You also need to set up a lot of plates (Poisson statistics)
- This limits one's ability to distinguish between two LQ models or between an LQ model and an MTSH model on the basis of the resulting muddy data

Another limitation on accuracy and applicability: feeder cells

- Often the treated cells survive poorly if they aren't provided with metabolites from neighboring cells
- So we irradiate a set of cells enough that they cannot divide but they can metabolize
- Plate out the cells you wish to study atop those
- This provides a feeder-cell layer that will supply the cells we wish to study
- This limits applicability because the feeders can be problematic
- Recent advances make this less of an issue than before