Illinois Institute of Technology

Radiation Biophysics
Lecture 10
Radiation Biology of Normal Tissues
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Plans For This Class

- *In vivo* assays of normal tissue
- Acute lethal response
- Teratogenesis

} ch 10

- Nonstochastic effects (chapter 10)
- Late effects on normal tissue (chapter 11)

Homework problem 9.1

- See next few slides for specifics
- General point: you don't actually need the MTSH n and D_0 values to solve the problem—just the OER values and the recognition that in this situation the OER at high $[O_2]$ values is the m value (see eqn. 9.5)
- Then if you plot OER against $[O_2]$, find the point where $S/S_N = (m+1)/2 = 1.905$. That point is at $[O_2] = 0.61\%$.
- Ratio of type 1 to type 2 damage = $n_1/n_2 = m-1$.

Alpen, Chapter 9, Problem 1

- A mammalian cell line has a D_0 of 120 cGy and an extrapolation number of 12. The OER is found to be of a dose modifying character with a value of 2.81. What is the value of m in the Alper-Howard-Flanders equation? The following data have been found for this cell line for OER as a function of oxygen partial pressure:

Problem 9.1, continued

$[O_2]$, %	OER	$[O_2]$, %	OER
0.1353	1.10	0.7788	2.19
0.223	1.20	1.00	2.59
0.2865	1.33	1.75	2.77
0.3679	1.64	2.50	2.81
0.6065	1.88		

•What is the value of K in the same expression?
•What is the ratio of type 1 to type 2 damage in this cell line?

Remember how this works:

- Alpen, p. 205:
  When $[O_2]$ is very much larger than K,
  $S/S_N = (m[O_2] + K) / ([O_2] + K) = (m[O_2]) / ([O_2]) = m$
- Therefore we can compute m by finding the asymptotic value of S/S_N (for $[O_2]$ very large)
- Then we recognize that if $K = [O_2]$, then our general formula becomes
  $S/S_N = (m[O_2] + [O_2]) / ([O_2] + [O_2]) = (m+1) / 2$
  So we find the value of $[O_2]$ for which $S/S_N = (m+1)/2$ and that value of $[O_2]$ will be K. Shazam.

Computing n_1/n_2

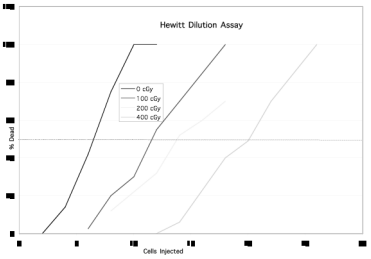
- Remember that the ratio of type-1 to type-2 lesions is $n_1/n_2 = (m - 1)$ and $K = k_{rep} / k_{fix}$
- So once we know m we can compute n_1/n_2.

Today's problem to consider

- Alpen, chapter 10, problem 1:
  Hewitt Dilution Assay with mortality rates listed for doses of 0, 1, 2, 4, 5, 6, and 8 Gy. Construct the curves for animal lethality versus dose of injected cells for each of the irradiation doses given. Construct the derived survival versus dose curve for the irradiation of the line of lymphoma cells. Estimate D_0 and n for an MTSH model. Then plot the survival curve in LQ format using the linearized form of the linear-quadratic expression. Which fits better?

Homework Hints

- 2. Hewitt Dilution Assay: doses 0-4 Gy shown below. Last 3 dose-levels are farther to right. Dotted red line corresponds to LD_{50}.



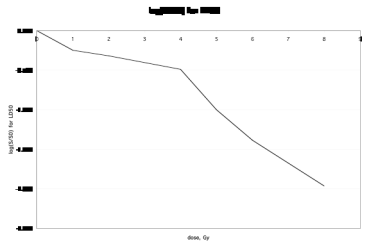
Homework Hints, Continued

2. Hewitt dilution assay:
from curves shown, we can estimate the LD_{50} for each radiation dose:

Dose, Gy	LD_{50}
0	6.5
1	11.6
2	13.8
4	20.1
5	65
6	159
8	598

So S/S_0 = (LD_{50} at a given dose)/ (LD_{50} at Dose=0).

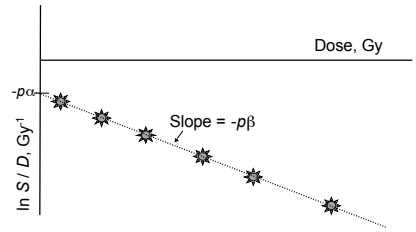
Homework Hints, Continued



Linearized Linear-Quadratic Model

- Recall that in the LQ model
  $S = \exp(-p(\alpha D + \beta D^2))$
- Note that this form is the one articulated in the "molecular model" section of the text (p. 146-151)
- The fudge-parameter p is the biological effectiveness factor for double-strand breaks (is that useful?)
- So $\ln S = -p(\alpha D + \beta D^2) = (-p(\alpha + \beta D))\, D$
- Therefore $\ln S / D = -p\alpha - p\beta D$
- Therefore a plot of $\ln S / D$ against D should have a slope of $-p\beta$ and a Y intercept of $-p\alpha$.

In principle this should look like this:



- But in reality in this problem the fit is pretty poor!

Non-text problem

Assume that ionizing radiation exerts its tumorigenic effects primarily through mutational events. Assume further the cigarette tar contains large numbers of cancer promoters. Which scenario would you expect would cause a higher incidence of cancer, and why?

- Irradiation followed by ten years of smoking
- Ten years of smoking followed by irradiation

… So what's the answer?

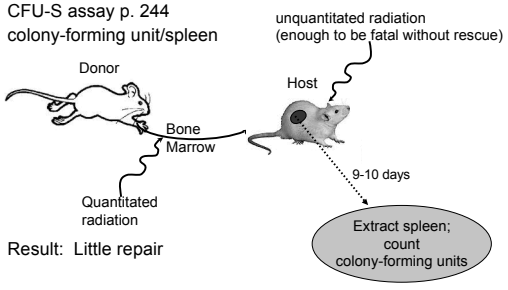
- Ionizing radiation can cause mutations
- It has no known role in promotion (at least, not to me)
- Cigarette smoke contains both mutagens and promoters
- Therefore ionizing radiation first and cigarette smoke later is somehat more likely to lead to cancer than doing it in the opposite order
- *However*:
  progression (to metastasis) often does involve further mutations, so the story is more complicated then.

So let's get back to chapter 10

- We've been discussing models for radiosensitivity of tumor cells
- This is important to oncologists *and* to general radiation biologists, but (perhaps) for different reasons:
  - The oncologist wants to know how to exploit differential radiosensitivity of tumor cells
  - The radiation biologist hopes that the results derived from studying tumor cells (which are relatively easy to culture) will help them understand normal cells
- So: now we move on to those normal cells.

In Vivo Assays of Normal Cells

- CFU-S assay p. 244
  colony-forming unit/spleen



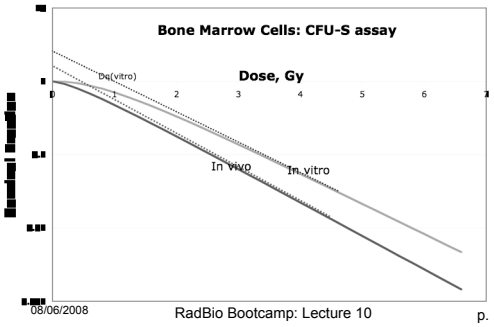
Result: Little repair

MTSH Model for Colony-Forming Units-Spleen (CFU-S)

	n	D_o, Gy
in vitro irradiation	2.5	1.05
in vivo	1.5	0.95

- Thus, the bone-marrow cells used here constitute a system with moderate inherent radiation sensitivity and virtually no capability of repair. Why is the *in vivo* repair capability smaller? Unclear.
- Response modifiers work as predicted.

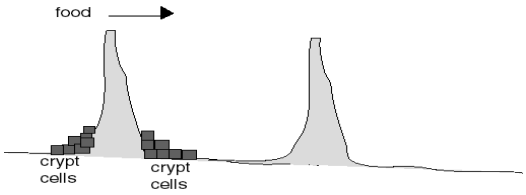
Results in vivo and in vitro



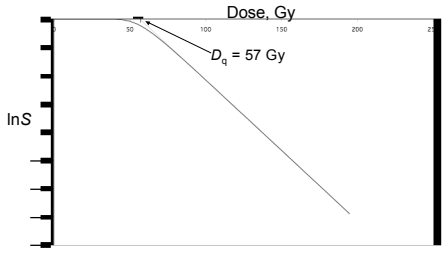
Gastrointestinal Crypt Cells

4 day turnover from base to tip.

Results: D_o ~ 10 Gy, n = 300 = exp(D_q/D_o)
large n value => rapid repair of sublethal damage

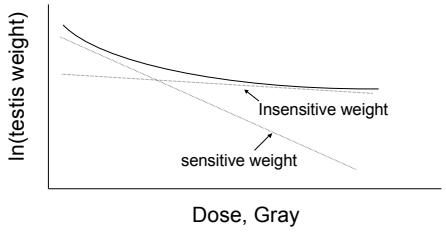


MTSH Model for GI crypt cells



Spermatogenesis

- Analyze radiation's effects via changes in weight of testes



Developmental Stages in Spermatogenesis in the Mouse

Cell type	Days to mature spermatozoon	LD_{50} (Gy)
Type A spermatogonia (type A_S, A_1-A_4)	35-45	> 2
Intermediate spermatogonia	32-35	0.2
Type B spermatogonia	30-35	1.0

Spermatogenesis, concluded

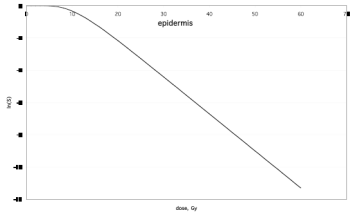
Cell type	Days to mature spermatozoon	LD_{50} (Gy)
Primary spermatocytes*	20-35	
Resting (preleptotene)		2
Leptotene, Zygotene		5
Pachytene		Unknown
Diplotene		8
Diakinesis		9
Secondary spermatocytes	20-22	10
Spermatids	7-20	15
Spermatozoa	0-7	500.

Testis Weight-Loss Assay

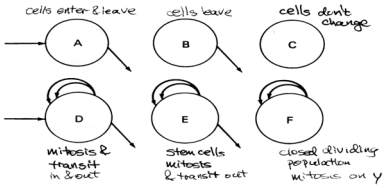
- Very early studies showed that a single exponential didn't describe the radiosensitivity of testes
- Endpoint studied was the change in weight of the testis as a function of radiation exposure
- Result:
  $W_D = W_s\exp(-k_sD) + W_t\exp(-k_tD)$,
  where $W_s + W_t$ = total pre-irradiation mass
- Notion is that testis contains two kinds of tissues, one of which is more radiosensitive than the other.

Skin

Exposure of epidermis can be modeled with MTSH kinetics. We find D_o ~ 4.35 Gy, n = 12.
Note $D_q = D_0 \ln n$ = 10.81 Gy.



Acute Lethal Effects (< 1 Month)



- Fig. 10.1 ⇒ Populations of cells
- D & E categories are responsible for most acute effects:
  - D: mitotic, translatable in & out
  - E: mitotic, translatable out (stem cells…)

How do humans die of acute radiation exposure?

- Acute lethal dose typically above 5 Gy
- Organ systems affected:
  - Blood-forming organs:
    sensitivity mostly dependent on cycle time

Cell type	*cycle time*	*sensitivity*
• Granulocyte	4 days	very high
• Platelet	12 days	moderate
• Erythrocyte	40 days	low

  - GI organs
  - CNS
  - Lymphocytes

Acute Lethal Effects (< 1 Month)

- Blood-forming effects
  - 3 weeks
  - Not very repairable
- GI - If not fatal within 10 days, recovery likely
- CNS
  - 1 Gy
    - Vomiting
    - Results from direct stimulation of neurons?
  - 100 Gy
    - Massive disorientation
    - Death

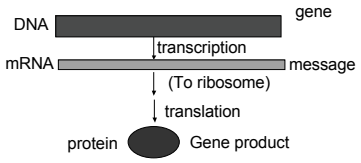
1 - 2 - 5 Gy
Lethality

Lymphocytes: a special case

- Mature lymphocytes are fairly radiosensitive
- This is unusual for a nondividing, terminal cell type
- Some kind of "interphase death"
- There's a lot of p53 gene product produced in lymphocytes: maybe they're being stimulated into dying with moderate radiation exposure

A detour into molecular biology

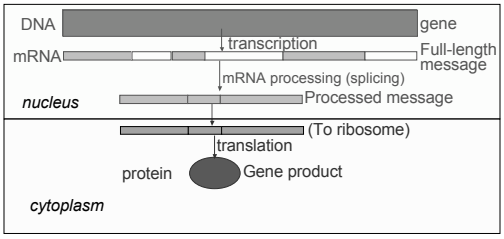
- The "central dogma" of molecular biology involves:
- Transcription (DNA codes for messenger RNA)
  Translation (mRNA codes for protein synthesis in the ribosomes)
- So in prokaryotic (anuclear) organisms, it's simple:



How this works at the base-pair level

- DNA Duplex:
  DNA strand: 5'-A-T-T-C-C-G-3'
  DNA strand: 3'-T-A-A-G-G-C-5'
- DNA-RNA hybrid, produced by transcription of DNA:
  DNA strand: 5'-A-T-T-C-C-G-3'
  RNA strand: 3'-U-A-A-G-G-C-5'
- Resulting RNA strand that can code for protein:
  RNA strand: 3'-U-A-A-G-G-C-5'
- Note that DNA bases are dA, dC, dG, dT;
  RNA bases are A,C,G,U (thymine is methyluracil)

In eukaryotes, it's more complex

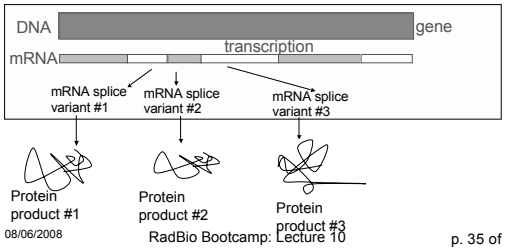


Numbers matter, too!

- Suppose the original gene was 2000 base-pairs long.
- The full-length message will therefore be 2000 bases.
- The truncated (processed) mRNA might be 720 bases.
- The resulting protein would be 720/3 = 240 amino acids long.

Can it get messier? Yes.

- One full-length message can be spliceosomally processed in multiple ways to produce several viable protein products



Bcl3: an example

- Bcl3 is an important gene in regulation of apoptosis and therefore in carcinogenesis and other developmentally-related pathologies.
- Exists in multiple splice variants, all derived from a single gene.
- Some variants stimulate apoptosis, others inhibit it!
- See Gil Ast, "The Alternative Genome," *Scientific American*, April 2005, pp. 58-65.

Problem to consider

- Using, in part, the information in fig. 10.10, summarize which systems in a mammal are radiosensitive at various stages of fetal development.

Gestational Radiosensitivity

- Alpen's Fig. 10.10 provides a long list of radiosensitivity data for various organs and organ systems
- In some cases the maximum sensitivities are early, in others much later

Teratogenesis & Fetal Development

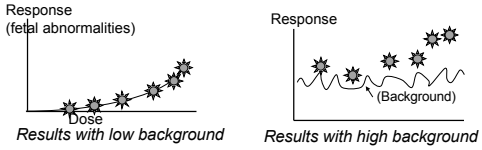
- Teratogenesis - Embryological abnormality
  monster
- It's traditional to argue that the fetus is highly radiosensitive, but it actually isn't, compared to other rapidly dividing cell systems.
- We need to distinguish among:
  - Damage to gametes before fertilization
  - Somatic damage to growing organism
  - Damage to mother that influences development

Measurement Problems associated with Teratogenesis

- Confounding effects
  (how can one tell these phenomena apart?)
  - Damage to embryo/fetus
  - Genetic damage before fertilization
  - Damage to placental system
- High background
  - High incidence of birth defects in unexposed subjects
  - 5% of all births involve some significant abnormality

Why does a high background matter?

- With low background, a small dose-response effect can be discerned even in the presence of some experimental error
- With high and nonuniform background, it's hard to pick the signal out of the noise.



Stages of Development

- Single cell → rather sensitive
- Conceptus
  - 2 cell: Rather resistant:
    both cells are totipotent, i.e. capable of differentiation into all necessary tissue types
  - All cells remain totipotent in the first few cell divisions; differentiation begins just before implantation
  - Severe damage to embryo can prevent implantation
- Early differentiation: Onset of abnormalities

Types of fetal damage

- Severe mental retardation
  - Occurs in 1-5 Gy range?
  - Is this really teratogenic or does it involve damage to mother at higher doses?
- Microcephaly
  - Might occur even in 1 Gy range
  - Data supporting that are disputable
- Other types of damage described in mouse studies

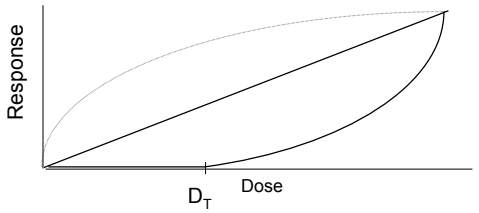
Stochastic Effects of Radiation

- One of two overarching categories of damage, particularly as it produces long-term effects:
- Percent of population affected by the exposure may be dose-dependent

  -BUT-
- Severity of condition in an affected individual is independent of dose
- Cancer is traditionally regarded as stochastic, but that may be an oversimplification
- *Nonstochastic* damage is damage that does display dose-response relationships in an individual

Nonstochastic Effects on Normal Tissue

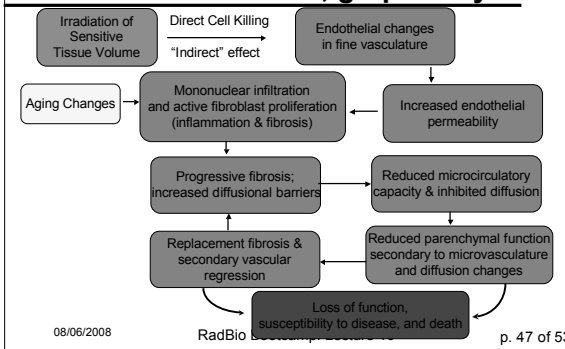
- Severity of condition *does* show dose dependence
- Possible threshold dose (no effect below D_T)
- Most important mechanism: disruption of vascularization (Casarett model, Fig. 11.1)



Casarett model for microvasculature

- Sequence of events beginning with irradiation and ending with loss of function, susceptibility to disease, and death
- Shown in Fig. 11.1
- Note cyclic effect of fibrosis and secondary vascular regression

Casarett model, graphically



Other types of late damage

- The assertion: If vascular damage were the whole story for the late effects of radiation, then the time of onset of late damage should be more or less the same for all organs. That's false!
- Stromal and parenchymal damage
  - parenchymal cells are those involved in the actual function of an organ, e.g. the cells in the liver that actually filter out damaging chemicals
  - Stromal cells are the support cells that undergird and provide morphological support for the parenchymal cells

Vascular endothelium as target

- Endothelial cells lining capillaries are a cell-renewal system, so damage there will hurt the organ that those capillaries supply with blood.
- Types of damage:
  - Direct: interphase death of cells in wall (DNA damage leading to apoptosis)
  - Indirect: interference with cell renewal

Functional Subunits

- Concept: The fate of an organ depends on individual functional subunits (FSUs).
- When all the stem cells that give rise to the functioning cells in a functional subunit die, then the functional subunit can't continue to operate
- Examples:
  - In the kidney: the nephron
  - In the lung: the alveolus
  - In the pancreas: a single islet of Langerhans
  - In the small intestine: a gastrointestinal crypt
- Can we generalize this to all tissues? Maybe not.