Illinois Institute of Technology

Radiation Biophysics
Lecture 8:
Modifiers of the Response to Radiation
Andrew Howard

Effectors of Radiation Sensitivity

- Biological
  - Cells go through life cycles & are much more sensitive to radiation damage at some stages than at others
- Chemical
- Physical

Some problems to consider

- 1. Problem 1, Chapter 9, Alpen: see next slide
- 2. A human has a fever such that her body temperature is 39ºC. This fever is accompanied by an increase in white-blood cells. How will these conditions affect her sensitivity to radiation?

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 (see next page).
- 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?

Alpen #9.1, Table

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

Note: the conversion from percentage to micromolar is roughly 11.3 μM / %.

Errata

- Page 205, in the EXAMPLE: 4.0 μM l^{-1} should be 4.0 μM.
- Page 206, last sentence: If the lesions produced by high LET radiation are predominantly of type II (irrepairable), then ***m*-1** will be disappearingly small and no oxygen sensitization will be detectable.
- Page 213, last paragraph: *cysteine*, not *crysteine*

Cellular Life Cycles (review)

	Phases	
◆ Mitotic	M	(short)
– Sensitive		
◆ Presynthetic	G_1	(variable)
– Radiation causes 1/2 h delay here		
◆ Synthetic	S	(4 - 8 h)
– DNA synthesis		
– Least sensitive		
◆ Postsynthetic	G_2	(usually short 1 - 2h)
– Radiation causes 3 - 4 h delay here		
– End of G_2 sensitive		
Overall Process		14 h

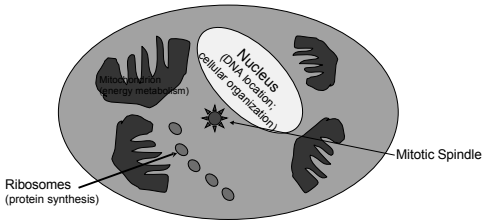
What happens in G1?

- Routine cellular metabolism
- Both buildup of new cellular structures and gathering energy to do so, i.e. both catabolism and anabolism:

Metabolism as a whole consists of:

Catabolism	*Anabolism*
Energy-producing	Energy-requiring
Breakdown of complex molecules into simpler ones, producing ATP	Build-up of complex molecules from simpler precursors, using ATP

A mitotic cell



Timescales of damage (fig. 4.1)

- Physics (~10^{-16} s)
  - Primary interaction event with biomolecule or H_2O
  - Excitations & ionizations
- Fast Chemistry (10^{-15} - 10^{-7} s)
  - Water radicals and other quasi-stable species form
  - Reactive species diffuse and cause damage
- Slower chemistry (10^{-7} to 10^{-3} s)
  - Further damage via diffusion
  - Chemical restitution & repair
- Biochemistry (msec-min): enzymatic repair of damage
- Biology (hrs-days): biological repopulation from surviving cells

Physical & Chemical Effectors

- Water
  $h\nu + H_2O$ → free radicals
  •OH, •H
  $•O_2^-$, etc
  ionized species

Pure water [redacted]

How does water matter?

- In a dry setting, the damage must be direct
- In a wet environment, water-derived free radicals are the source of much of the chemical damage
- Experiments that can eliminate secondary (radical-mediated) damage show much reduced radiosensitivity

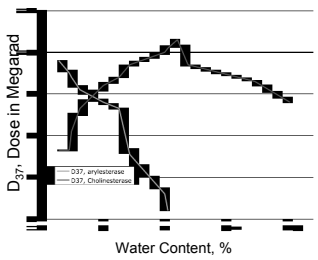
An example from my field

Protein crystals must be irradiated wet because they fall apart when they're dry.

- Protein crystals irradiated at 300K are destroyed:
  - In days on a conventional X-ray source
  - In minutes on a 2nd-generation synchrotron
  - In seconds on a 3rd-generation synchrotron
- They're essentially immortal at 100K except on a 3rd-generation synchrotron source, where they live for 5-100 minutes

Radiosensitivity of Enzymes

- Most enzymes are more radiosensitive in the presence of substantial hydrations than when dried
- Data can't readily be measured below ~4% hydration



Why does this work this way?

- Normal response
  - Arylesterase: less water means less damage
  - Dry conditions mean that radiosensitivity depends entirely on direct, not indirect damage
- Abnormal response: Cholinesterase
  - harder to explain
  - Augustinsson suggests: at low [H_2O], free radicals are detoxified by nonfunctional sulfhydryls in the protein
  - At higher water concentrations the sulfhydryls can't get to the damage in time

Do we care?

- Not much:
  - We can't really alter the hydration of most cell systems without destroying them
  - We definitely can't influence the hydration states of whole organisms without doing damage that is probably much more significant than that of the radiation
- But these studies may help us understand mechanisms of damage, and that *could* be relevant

Temperature-sensitivity

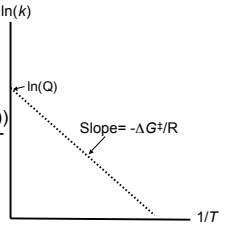
- Direct damage will be essentially temperature-independent
- Indirect damage should be temperature dependent because it relies on diffusion of radicals and ions from the site of their production to the macromolecule
- Ionizations and excitations may display different temperature dependencies because once the molecule is excited, its chemistry may depend on thermal interactions

Arrhenius plots

- We can examine temperature dependence via the Arrhenius plot, where we expect $k = Q\exp(-\Delta G^{\ddagger}/RT)$
- Thus $\ln k = \ln Q - \Delta G^{\ddagger}/RT$, so if we plot $\ln(k)$ as a function of $1/T$ then the slope will be $-\Delta G^{\ddagger}/R$, i.e it will be proportional to the activation energy $\Delta G^{\ddagger}$.
- If multiple processes are involved we may get a non-log-linear response.
- Over temperature ranges typical of the internals of homeothermic organisms, we're not going to see much effect!

Arrhenius plots: details

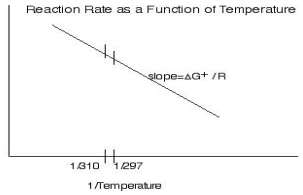
- We said $k = Q\exp(-\Delta G^{\ddagger}/RT)$
- Take the natural log of both sides: $\ln(k) = \ln(Q\exp(-\Delta G^{\ddagger}/RT))$
- But $\ln(x*y) = \ln(x) + \ln(y)$, so $\ln(k) = \ln(Q) + \ln(\exp(-\Delta G^{\ddagger}/RT))$
- Therefore $\ln(k) = \ln(Q) - \Delta G^{\ddagger}/RT$
- The relationship between $\ln(k)$ and $1/T$ is therefore linear with Y-intercept $\ln(Q)$ and slope $-\Delta G^{\ddagger}/R$



Radiation & Temperature

Kinetics

37°C = 310K
27°C = 300K



p199
T < 100K
100 < T < 170 K
170 < T < 420 K

Radiation and Temperature

- Effects in various temperature ranges:

T_{min}	T_{max}	Effects
0K	100K	temperature-insensitive; no charge migration, target must be hit.
100K	170K	excitation localized at site; exciton migration is crucial
170K	420K	disruption of disulfides, ionization

- Does this matter much with biological systems (esp. in homeotherms), where T ~ 310K almost all the time? Probably not, other than for understanding mechanisms.

Oxygen and Radiation

- FACT: O_2 is a radiation-sensitive molecule e.g. $O_2 + e^- \rightarrow O_2^{-}\bullet$ (superoxide) → interactions with macromolecules
- It's tempting to think that all biology occurs in the presence of 19% O_2, but it doesn't!
- H_2O free-radical chemistry in the presence of O_2 is different from H_2O free radical chemistry in the absence of O_2.
  (recall Fricke dosimetry story)
- $P(O_2)$ in tissue varies widely
  - Hemoglobin transports O_2
  - Myoglobin stores O_2

Damage Fixation by Oxygen

$R\bullet + O_2 \rightarrow RO_2^{\bullet}$
semi stable
10^{-7} - 10^{-3} sec?

Mitigators of { O_2 fixation; radical presence }

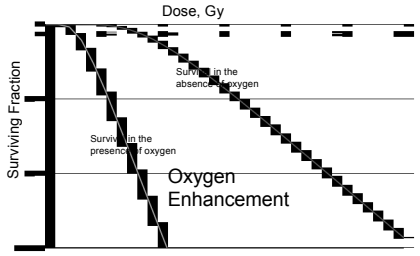
$R\bullet + R'SH \rightarrow R\text{-}H + R'\text{-}S\bullet$
$R'\text{-}S\bullet + R'\text{-}S\bullet \rightarrow R'\text{-}S\text{-}S\text{-}R'$ (dimerization)

Minor linguistic point

- Beware of the word *fixation*
- It doesn't mean correction: it refers to stabilization
- Stabilizing a radical doesn't make it less dangerous; it makes it more dangerous!
- So if we say that oxygen is involved in fixation of damage, we mean that it makes the damage worse, not better!

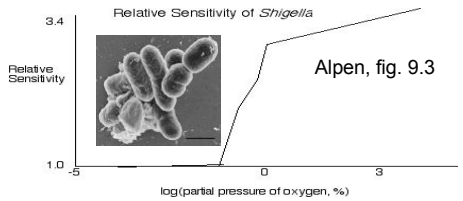
Experiments on Oxygen Sensitivity

- More cells are killed in air than in N_2:



Radiosensitivity as function of $P(O_2)$

- *Shigella* experiments show threshold in $P(O_2)$ below which few cells are killed

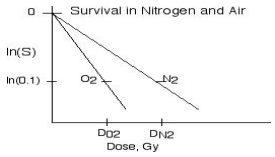


Alpen, fig. 9.3

Oxygen Enhancement Ratio

$$OER \equiv \frac{[\text{dose in } N_2 \text{ for surviving fraction } S/S_o]}{\text{dose in } O_2 \text{ for surviving fraction } S/S_o}$$

if $S = S_o/10$,
then $OER = D_N / D_O$
This definition is somewhat arbitrary,
but it works!



Quantitative Oxygen Sensitivity

- Paul Howard-Flanders and Tikvah Alper: Define "S/S_N" to be the ratio of the 10% survival dose under experimental conditions to the 10% survival dose in N_2, i.e. in the absence of oxygen
- "S/S_N" = $(m[O_2] + K) / ([O_2] + K)$
  - for any concentration of oxygen.
  - m and K are separately determined for each system
  - m is unitless, K is in concentration units
  - m represents maximum relative sensitivity so $m \geq 1$.

Extrema of "S/S_N"

- At $[O_2] = 0$,
  $S/S_N = (m*0 + K) / (0 + K) = 1$
- For $[O_2] >> K$,
  $S/S_N = (m[O_2] + K) / ([O_2] + K)$
  $= m[O_2] / [O_2] = m$
- Thus justifying description of m as maximum relative sensitivity

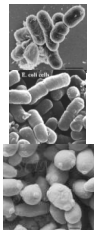
How to compute *m* and *K*

- m is available from asymptotic behavior
  - It's equal to S/S_N for $[O_2] >> K$.
  - In practice most systems are equally radiosensitive from about 1% $[O_2]$ on up
- To compute K, note that if $[O_2] = K$, then
  $S_K/S_N = (mK + K) / (K+K) = (m+1)/2$
- Therefore if we know m from asymptotic behavior, we can examine a curve like 9.3 to find the point where $S/S_N = (m+1)/2$ and read off K from the abscissa

Howard-Flanders Coefficients

- Coefficients *m* and *K* for various organisms:

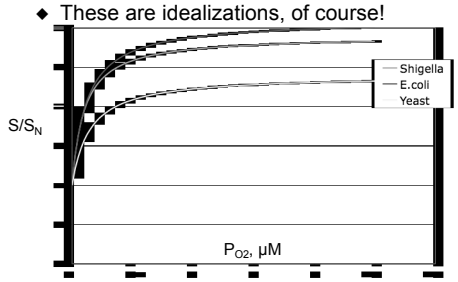
Organism	*m*	*K*, μM
Shigella flexneri Y6R	2.9	4.0
Escherichia coli B/r	3.1	4.7
Saccharomyces cerevisiae	2.4	5.8



- Can be read off curves like fig. 9.3

Curve-fits for Organismal Data

- These are idealizations, of course!



Why does this happen?

- Alper's model:
  2 types of damage from primary radiation event
  - Type I: lesion requires oxygen for lethality
  - Type II: always lethal, independent of oxygen
- Thus: type I can be chemically restituted
- Restitution competes with oxygen fixation
- Posit: n_1 type I lesions, n_2 type II lesions, k_{rep} = rate of repair, k_{fix} = rate of O_2 fixation, then:
- m-1 = n_1/n_2 and $K = k_{rep}/k_{fix}$

What happens with high-LET?

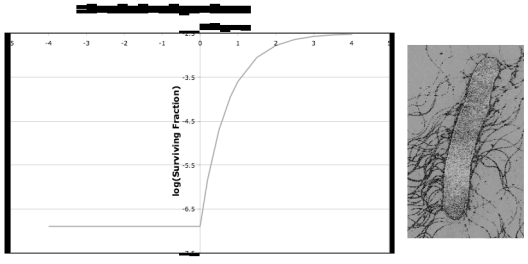
- Essentially all damage is type 2
- Nothing depends on restitution
- Therefore $n_1/n_2 << 1$, m-1=0, m=1
- Thus $S/S_N = ([O_2] + K) / ([O_2] + K) = 1$
- The fact that high-LET produces this behavior is mildly supportive of the appropriateness of Alper's model, although it's far from conclusive

Time-Dependence

- We can study the kinetics via short bursts of radiation
- We look to see whether providing O_2 at a certain time-point before or after irradiation sensitizes the cells
  - Result: Oxygen sensitizes the cell if present before irradiation
  - If available up until about 3 msec after irradiation, it still matters somewhat
  - If oxygen is made available later than a few msec post-irradiation, it doesn't sensitize the cell
- This suggests that the oxygen-dependent free radicals have lifetimes shorter than 3 msec.

Michael's results with *Serratia*

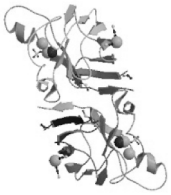
- Time constant ~ 0.5 msec for sensitization



What radicals are involved?

- The short time-span shown in this experiment suggests that free radicals are involved:
  but which ones?
- Michael suggests that superoxide ($O_2^{-}\bullet$), hydroperoxyl (H-O=O•), and e_{aq}^{-} have shorter lifetimes than is consistent with 0.5 msec timing
- Other researchers continue to plug for superoxide and hydroperoxyl as candidates
- OH• doesn't interact with O_2 that much except via $CO_2^{\bullet -}$:
  $OH\bullet + HCO_2^{-} \rightarrow CO_2^{\bullet -} + H_2O$
  $CO_2^{\bullet -} + O_2 \rightarrow CO_2 + O_2^{-}\bullet$

Superoxide



Human Cu-Zn SOD

- We're still not sure if superoxide is a major actor in oxygen-mediated damage
- But this is how superoxide is detoxified by superoxide dismutase (SOD):
  - $2\ O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2$ (catalyzed by SOD)
  - $H_2O_2 \rightarrow H_2O + (1/2)O_2$ (catalyzed by catalase)
- Exogenous SOD doesn't help alleviate damage (but maybe that's because it isn't tied to catalase, so peroxide can build up to toxic levels?)
- Manipulating constitutive levels of SOD by genetic means gives muddy results

Thiol Mitigators

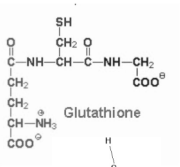
We expect that oxygen competes with thiols, such that the more thiol mitigation is involved, the less oxygen-dependent fixation of damage can occur:

1. [macro]-R• + R´SH → [macro]-R-H + R´S•
   2R´S• → R´-S-S-R´

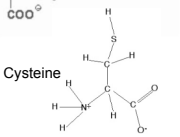
2. [small]• + R´SH → [small]-H + R´S•
   2R´S• → R´-S-S-R´

Glutathione as mediator

- Glutathione can readily dimerize under oxidizing conditions:
  R-SH + HS-R → R-S-S-H
- Reasonably prevalent in cells
- In principle cysteine could also operate this way, but its cellular concentration is too low



Glutathione



Cysteine

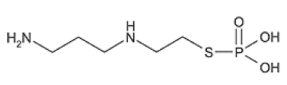
Are thiols *really* important?

- Some argument about that
- Revesz's results support the competition model
- Maybe only *exogenous* thiols really influence radiosensitivity
- Introducing cysteine or synthetic thiols does definitely mitigate damage:
  $RSH + OH\bullet \rightarrow RS\bullet + H_2O$
  $RS^{-} + OH\bullet \rightarrow RS\bullet + OH^{-}$

. . . And then these RS• radicals recombine as disulfides

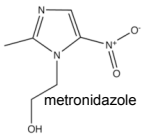
Dose reduction factors

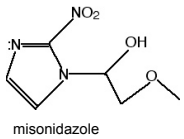
- We envision the effects of these exogenous thiols as involving dose reduction
- The quantitation is equivalent to reducing the available dose to influence the biological system
- Example, WR2721 or amifostine:
  - Works well in vitro
  - Limited utility in vivo
  - Fairly high toxicity



Sensitization by Nitroaromatics

- How do we make the cells in the rapidly growing tumor as rad-sensitive as they would be if P_{O_2} were higher?
- Nitroaromatics react with radicals to "fix" (stabilize) the damage

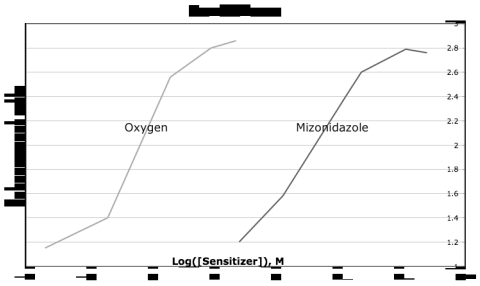




Nitroaromatic action

- Like oxygen, nitroaromatics react with short-lived radicals to produce longer-lived and therefore more reactive radicals
- Concentrations required to enhance radiosensitivity are much higher than with O_2
- These compounds have other applications, but they can be used therapeutically as potentiators of radiation damage

Oxygen vs. Mizonidazole (fig. 9.5)



5-Halogen-substituted pyrimidines

- These are molecules that resemble thymine
- The halogen at the 5- position looks like the methyl group in thymine and can be incorporated in place of thymine in DNA
- Most common: 5-bromodeoxyuridine
- Sensitization produced by ready reaction with the aqueous electron

The 5-BrdU radical

- Semi-stable radical is actually resident in the DNA and can influence chemistry in neighboring bases
- Other mechanisms are probably acting too.