Week 6 lecture 11

Radiation biophysics: target theory, direct and indirect action of radiation.
Dosimetry, Biological effects of radiation
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The first few lecture slides cover some dosimetry concepts, basically different ways of measuring radiation, that developed over time. There is no evidence that any of the equations employed in this lecture slides were asked to be calculated with in any exam. But some of the dose concepts/dose units do appear on minimals – so the very least you should do is reach an understanding as to what they mean. Lets get to it.

Absorbed dose Slide #2; unit Gray (Gy) – This is the basic idea of dosimetry, measuring the amount of energy that was absorbed by the body (in Joules) by the radiation, over the mass of the body we are investigating (Kg). Unit is Gray (Gy) = J/Kg
The problem presented in the lecture slide, is that if we investigate a known lethal dose of radiation of 8 Gy (Gray), we see that the energy absorption is only equivalent to an increase of \(2 \times 10^{-3} \text{K}\) (Kelvin) which is the same as saying and increase of \(2 \times 10^{-3} \text{C}\) which we know to not be lethal, so this dosimetry concept is not quite what we're looking for to adequately quantify radiation absorption. (minimal #43)

Exposure Slide #3; unit Coulombs/Kg – we are now looking for a newer, better concept to quantify radiation absorption, we know that ionization causes radiation damage, so lets measure ionization in a certain body mass., or a specific volume.
So how do I measure ionization? I know that the basic event of ionization is the ejection of electrons from their orbitals, and being that electrons have electric charge, I should measure the change in this electric charge, in the mass under investigation.
But what I really need to do is measure the change in charge after all the electrons stop bouncing around, so I would get a good unbiased estimate of what happened. That is referred to in the lecture slides as “Electron equilibrium” - basically when all the electrons that were ionized lost their kinetic energy and came to a rest. (minimal #44)

The way it is done is depicted on the right (as best I could). The volume i'm investigating is the green mass, and the red arrow is the radiation hitting it. Now electrons (black dots) get ionized and are bouncing around. Some may leave the volume i'm investigating and some may stay, just bouncing all around in it. Some are shown to have bounced out of it, then ionizing another electron outside the volume that bounced right into it. That is why the change in charge is measured at electron equilibrium, when all of the electrons are resting, and I can sum up the total change in the volume.
**Kinetic Energy Released in Material** Slide #4 unit – Gray (Gy) – Looking at the previous two dose concepts, absorbed dose and exposure, we concluded that both a heat uptake and ionization occur, and we can measure each of them, but consider for a second the exposure concept – it does not tell me how much energy was absorbed as heat, only the energy absorbed as charged moved in and out of the unit volume. So some heat energy was liberated in the material the moment the ionizing radiation hit the unit volume.

**KERMA** simply states that we should measure the energy liberated in the material **the very moment** the ionizing radiation hits it, right before some is absorbed as heat and some causes all of those ionization events, that way I get all the energy of the radiation. KERMA is all of the sum of the initial kinetic energy released in my volume (mass unit) or - change in energy divided by the mass in question (J/Kg = Gy); minimal #143

*if this dose concept strikes you as weird, it did to me as well, since I could not imagine a possible way to measure energy transfer in such an impossibly small accurate time unit as soon as the radiation strike a volume. A little online research supported that this concept is mainly theoretical, but I cannot vouch for it, but I agree it's not very feasible.

**Equivalent dose** Slide #5 unit Sievert (Sv = J/Kg) – This is where it gets slightly interesting. The equivalent dose concept says that not all radiations are the same, some are more potent or efficient at causing damage than others, which makes sense, so we should consider that in our measurements. We can easily do that by adding a factor called the radiation quality factor or weighing factor (both relate to the same thing). This weighing factor of the radiation (denoted by $W_R$) is a characteristic factor to each type of radiation, based on its properties. Combine this weighing factor with the initial absorbed dose concept we talked about – and abrah kadabrah you have “Equivalent dose” (minimal #145)

Now being that we like to simplify things, lets look at the seemingly complicated (but in fact quite simple) formula we see on the lecture slide: $H_T = \Sigma R W_R D_{T,R}$

simplified we can read the formula out:
The equivalent dose $H_T$ is equal to the sum of all radiation ($\Sigma R$) multiplied by its weighing factor ($W_R$) multiplied by the absorbed dose In the given tissue from the radiation applied to it. ($D_{T,R}$)

“What the hell is the Sievert unit?" something I kept hearing. If you look at its units you will see that they are identical to Gy. In fact 1Sv=1Gy. But consider this – there is an “industry standard” called conventional X ray which is 250KeV whose effect on human tissues we know very well. 1Sv is the dose of a radiation that will cause the same damage a 1Gy absorbed dose of this conventional X ray will cause on humans. i.e. a radiation that has 3Sv will cause the same damage 3Gy of conventional X ray would cause on humans. (minimal #145)

Think it's overly complicated? Join the club.
**Effective dose** Slide #6 unit Sievert (Sv) – This dose concept also makes perfect sense, we mentioned in the last Equivalent dose concept that not all the radiation types affect tissues in the same way, and we should take under account the type of radiation we are dealing with.

Here we also state that not all the tissues react in the same way to radiation, and some are more easily damaged than others, so we should also take into account the *tissue weighing factor* ($W_T$).

Now let's keep on simplifying, and consider the equation shown on the lecture slide:

$$ E = \sum_{T,R} W_T W_{R} D_{T,R} $$

Reading out the formula:
Effective dose ($E$) is the sum of all radiation hitting the tissue ($\sum_{T,R} W_T$) multiplied by the weighing factor of the tissue being radiated ($W_T$) multiplied by the weighing factor of the radiation in question ($W_R$) multiplied by the absorbed dose of the radiation in the tissue ($D_{T,R}$). Done.

**Dose dependence of radiation effects, dose-response curves** Slide #7 – Welcome to the most important graph of the lecture. This graph came up in SCTs and finals dating back, you need to understand it, and know how to draw it and label the axes.

Let's understand it. The $y$ axis represents the fraction of surviving individuals in the sample ($N/N_0$ where $N$ is the amount of surviving individuals at time of inspection and $N_0$ was the initial amount of undamaged individuals I had in the sample before it was radiated) the lower I am on the $y$ axis the less surviving individuals I have left in my sample. Being that the maximum $N/N_0$ value is 1 (all of our individuals are undamaged), that's where we would start the graph on the $y$ axis.

The $x$ axis represents the dose. The more right I am on the $x$ axis the more dose I have applied on my sample of individuals.

We can see in the graph that as I start radiating the sample with dose, I have less surviving individuals in my sample – the more radiation I apply, more individuals in my sample are damaged. This should make sense.

Now we get to the two main theories discussed in the presentation, the *target theory* and the *molecular theory*.

There is a little bit of statistics thrown into the target theory, but don't worry it's not too bad. Let's get to it.
The target theory – This theory states that a molecule has a target in it, that is the most sensitive part of that particular molecule. When the target is destroyed – that particular molecule is destroyed, this is also referred to as inactivation. A target may need 1 hit to inactivate, or more than that, but when that target is hit with enough hits to inactivate it, that molecule is inactivated. Obviously being that there are many molecules in a sample, the question of which are hit – is a question of probability.

Now lets get in the probability part of the deal and try and figure out this whole thing. Which probability distribution best describes the situation we have? Well, hits come in discrete integer packets. Meaning I can have 1, 2, 3, 4...k hits, but I can't have anything in the middle, like 2.4 hits. That is a discrete random variable. This is exactly the definition of the poisson distribution. So if anyone asks you what kind of distribution characterizes the inactivated molecules as a function of hit per target (target theory) you would say “poisson distribution”. Good. Next.

Lets try and make sense of the math of it, looking at the formula from the lecture slide:

\[ P_n = \frac{(VD)^n}{n!} e^{-VD} \]

the probability of getting n hits to cause the inactivation of the molecule ( \( P_n \) ) is equal to the Volume (V) times the Dose applied (D), to the power of number of hits required for inactivation (n) over n!, times the exponent \( e \) to the power of the volume times the dose.

This formula is based on the statistics poisson formula which you will eventually have to memorize for the biostats exam anyways, and it is a minimal in biophysics as well (minimal #134) so you just need to memorize it.

Now if we consider a molecule whose target only needs to be hit once to be inactivated (n=1). In that case, if I have a bunch of these molecule in a sample volume V, and I irradiate them with a dose D I would have some fraction that was inactivated and some that are still surviving. In this case of “one hit one inactivation” I can calculate the amount of surviving molecules in my sample by plugging n=0 (the probability of not getting any hits, and surviving) in the formula and get the fraction of surviving molecules (\( N/N_0 \)).

in this case of n=0 (no hits) the whole expression \( \frac{(VD)^n}{n!} \) equals 1. So all that's left is to calculate \( e^{-VD} \) as shown in lecture slide #20. This is the explanation to minimal #136.
Now let's consider the dose response curve illustrated on slide #9 on the top left. What we see, other than the graph we know – that tells us that the more dose we have the less surviving molecules we have, we see something else. We see a shoulder on the graph.

Let's take a look at the pink line that has the biggest such shoulder. This shoulder, means that even if I start applying radiation dose, the line still remains on the value of $1/N0$ which means that my molecules are not dying right away, only after I apply even more dose do they start dying.

The reason for that is simple, we mentioned a situation in which we only need one hit to cause inactivation – in this situation as soon as I apply radiation some molecules are going to be hit and inactivated, thus my surviving curve is going to start dropping right away. But what if I need 2, 3, or 10 hits for inactivation? Then I will see a shoulder, meaning that even though I started blasting the sample with radiation, it takes a few shots even for the first few molecules to be inactivated. This shoulder on the survivability curve was asked about a few times in the past, and if you understand it, it's an easy answer. This is also explained in the blue box in that same slide.

**The molecular theory** (Slide #10)- A theory, like the target theory, is great, but another one exists, that says that damage to tissues cannot simply be attributed to radiosensitive targets in every molecule. And here are the reasons:

- In organisms there is a very distinct relationship between DNA damage and radiation damage and cell death
- The loss of biological functions in living organisms (eukaryotic cells) is strongly associated with DNA damage.
- Whenever cells lack or have problems with their DNA repair mechanisms their radiation sensitivity goes up

According to all of the above observations, a new theory emerged, saying that radiation damage is due to DNA damage, which is the one critical molecule whose inactivation cannot be recovered from. In other words if the DNA is destroyed, the cell will die. (minimal #150)

**DNA strand break** (Slide #11)– the DNA as we very well know, is an anti-parallel double helix molecule, a molecule with two backbones on each side of it, so to speak. Damage to those backbones would mean damage to the DNA molecule. If only one of the strands is broken, given enough time the DNA can repair itself. If both strands break the DNA is inactivated – thus a double strand DNA break is a non survivable event to a DNA molecule.
A double strand DNA break can occur as a result of one ionizing event, or two independent ionizing events.

Let's consider the DNA double strand damages shown in lecture slide #11. If we focus on the two left ones we see depictions of double strand breaks that are a result of one ionizing event. In the very middle depiction (the right one out of the two left ones) we can see an ionizing particle going straight through both backbones, damaging both of them as it travels through the material. The leftmost depiction shows us a scenario in which an ionizing particle breaks one strand of DNA first hand, and then causes ionization in the vicinity of the second strain, the ionized particle created by the ionizing radiation then ionized the second strand, and damages it. This is also considered to be the same ionization event that results in a double strand DNA damage.

The rightmost depiction shows us the double DNA strand damage caused by two independent ionizing events.

The equation shown on this lecture slide is basically calculating the fraction of survivability ($N/N_0$), taking into account the probability that a double strand break will occur due to a single ionizing event (denoted $\alpha D$) and the probability that a double strand break will occur due to two independent ionizing events (denoted $\beta D^2$) which is to the 2nd power since it's a less likely event (this may confuse you because a number to the second power will give you a higher number, which may correspond to a higher probability, but notice there is a negative sign before the whole expression, so in fact it's going to be a smaller number).

I don't think this equation is of any importance, but it is on the minimals (#153) so I made an effort to explain it. If you ask me if you need to know it I couldn't tell you. All I can tell you is that I didn't find any mention of it being asked at any SCTs or final, but that does not mean it would not appear in the future. I, myself, did not bother remembering it, just knowing that the likelihood of a double strand break due to two independent ionizing event is considerably smaller than it occurring due to one such event was enough for me.
**Direct and indirect effects of radiation** (Slide #12) – So far we discussed what happens if the radiation hits the molecule, either a radiosensitive target inside the molecule (the target theory) or hits the DNA strand directly (molecular theory). These are direct interactions of the radiation with the molecule.

So how can we indirectly affect a molecule? 
It just so happens to be that biological tissues are to a great extent contained in water solutions. Those H₂O molecules in the biological environment are essentially solvent molecules, and our radiosensitive molecules of interest are the solute. In other words our molecules are swimming in a pool of H₂O. So is it good or bad? Let's consider a process called “radical formation”.

Radical formation is when free radicals are formed from stable molecules. This can occur in our case by ionizing radiation, that causes ionization of stable molecules, turning them into radicals (a free radical is a highly reactive species due to it having unpaired electrons in its orbitals). Let's consider what may take place if a water (H₂O) molecule is turned into a radical.

The ionizing radiation interacts with the a stable water molecule and turns it into one of few possible radicals (minimal #140). This free radical now, which lets say is in the vicinity of the molecule of interest is “going crazy” violently looking for an electron to snatch, to make it more stable. If it snatches an electron from our molecule of interest, the radical will have ionized it, and damaged it.

So let's ask again, is it good to be surrounded in solvent (water) molecules? Well it's like being surrounded by molecules than could in any moment turn into radicals and harm our molecules of interest floating amongst them.

So what happens when our molecules of interest are surrounded in solvent? It seems that even if the ionizing radiation does not hit and ionize the molecule of interest directly, causing radical formation around it may be enough to inactivate the molecule indirectly. This is an indirect effect of radiation. This is also commonly referred to as “the target gets bigger” (being that now I can hit solvent molecules around the molecule of interest and still inactivate it)

This is also shown in the left side graph on lecture slide #14.

Accordingly we can conclude that molecules of interest in dry samples, being dry – are not surrounded by solvent molecules, and will be generally less sensitive, since only a direct hit from the ionizing radiation may cause damage or inactivation.
Factors influencing radiation sensitivity (Slide #15) – There are a few factors, some were discussed and some are going to be reviewed now, that affect the radiation sensitivity of a given tissue to ionization by a given radiation.

Quality of radiation – this was discussed in the radiation weighing factor earlier in this document. The characteristics of the dangerousness (quality) of a given radiation is its LET value and its penetrability (discussed in the previous document about interactions with absorbing material). Intuitively the more LET and more penetrability an ionizing radiation has, the higher its “quality” and dangerousness.

Relative Biological Effectiveness or (RBE) is a measure of how effective a radiation is causing harm to us compared to a conventional (250KeV) X ray. The higher the RBE the more damage the radiation in question is going to cause human tissues.

Biological factors – this was discussed as the weight factor of the tissue. Basically the cell cycle (which we should know, but if we don't we can look at lecture slide #17) is the cell growth, replication of DNA and cell division (mitosis) cycle. Some stages in the cell cycle are more sensitive, and some are less. The most sensitive is the mitosis (M) phase and preparation for mitosis (G2) phase, a cell in these phases will be considerably more radiosensitive.

The least sensitive stage is the S phase, or more accurately, the late S phase (DNA replication); (minimal #154).

The above statement also means that cells that spend more time in mitosis are also going to be more sensitive, like cells that divide often (lymphatic cells, white blood cells etc.) a table showing the radisensitivity of cells by rate of division is on the bottom of slide#17. Please note – almost every SCT and final featuring questions about radiation biology features also a T/F or relation analysis question comparing a less sensitive and more sensitive cell according to what is shown in the table. Don't worry, you don't need to memorize it, just remember that cells that divide more often are more radiosensitive, it helps to memorize the top two most sensitive ones shown in the table (lymphatic cells and white blood cells).

An example to such a question could be T/F - “Nerve cells are more radiosensitive to ionizing radiation than lymphatic cells” - False.

The second factor is differentiation. This is a simple way of saying specification. Cells that are very differentiated are cells that are very specific, and are less radiosensitive, and cells that are less differentiated are more generic (like stem cells) and are more radiosensitive. e.g. generally speaking cancer cells are less differentiated than the tissue around them, thus they are harder to irradiate.
**Fractionation** – a very simple concept ode to the ability of DNA to repair single strand brakes, to repair itself after being hit, as long as it wasn't inactivated. If I spread the radiation dose over a longer time (take breaks when applying radiation doses) DNA can repair itself to an extent and lower radiation damage. This is shown in the graph on slide #18. This is only if the damage done to the DNA is a single strand brake.

**Effect of Oxygen** (slide #19) – The final issue we need to discuss is what tissue is more radiosensitive, a tissue with a higher oxygen level (hyperoxic) or lower oxygen level (hypoxic).
This is rather simple to answer. Oxygen can form free radicals, and we know that if a molecule that can form free radicals is in the vicinity of our molecules of interest it can be ionized and damage or inactivate our molecule of interest. Simply put, the higher the oxygen level in the tissue – the more the tissue will be radiosensitive. This is shown in the top left graph of slide #19.

The last lecture slide contains information that is as useless to you as the junkmail you receive daily in your mailbox.

**Minimum requirement questions**

**Minimals #130-136; 138 142-146; 149-156** were discussed in this document

**Minimal #137**

*What is D37?*

*D37 denotes the dose at which 37 % of the irradiated objects survive. If one ionization causes inactivation, D37 corresponds to one hit in a radiosensitive volume (V·D=1, that is D=1/V).*

This pertains to the target theory. The D37 is like an industry standard to quantify a certain dose that needs to be applied to kill 63% of the irradiated objects. Which is like saying a dose at which only 37% remain.
The rest of the minimal is just a relationship between the D37 and the volume in case of one hit one inactivation scenario, that you need to remember.

**Minimal #147**

*What is the smallest dose which can produce a biological effect?*

*Theoretically even a single quantum is enough to produce a point mutation, since any photon that is able to produce ionization is capable of breaking a chemical bond.*

This minimal simply states that any dose of ionizing radiation could potentially cause the inactivation of a DNA molecule by double stand break, and cause “point mutations” due to the ability to break chemical bonds between atoms that make up the DNA backbones.