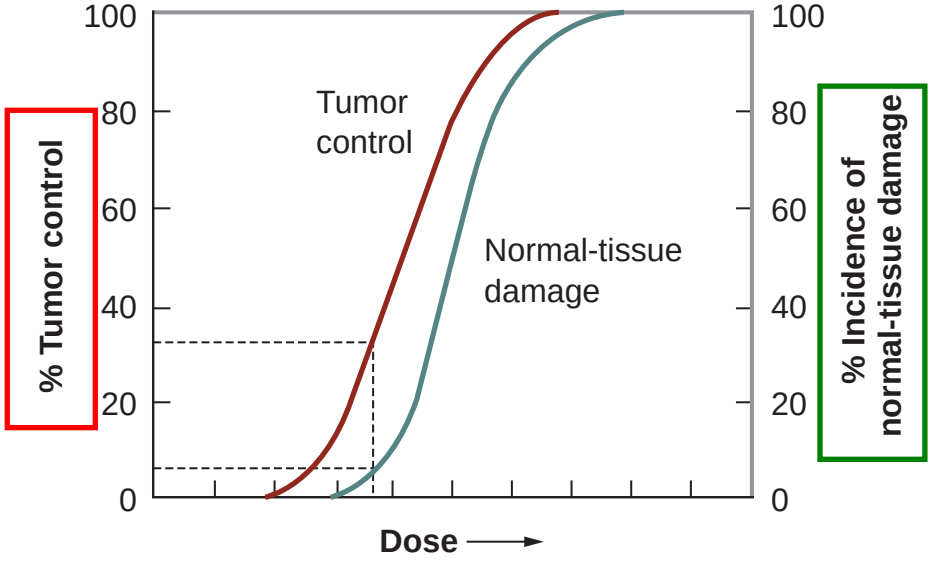
Chapter 19 – Dose-Response Relationship for Model Normal Tissue

11/14/2024

Outline

- **Dose-Response Relationship**
  - Therapeutic Ratio
- Mechanisms of Cell Death
- Assays for Dose-Response Relationship
  - Clonogenic Endpoints
  - Functional Endpoints
  - Inferring α/β from Multifractional Experiments

Dose-Response Relationship



Objective

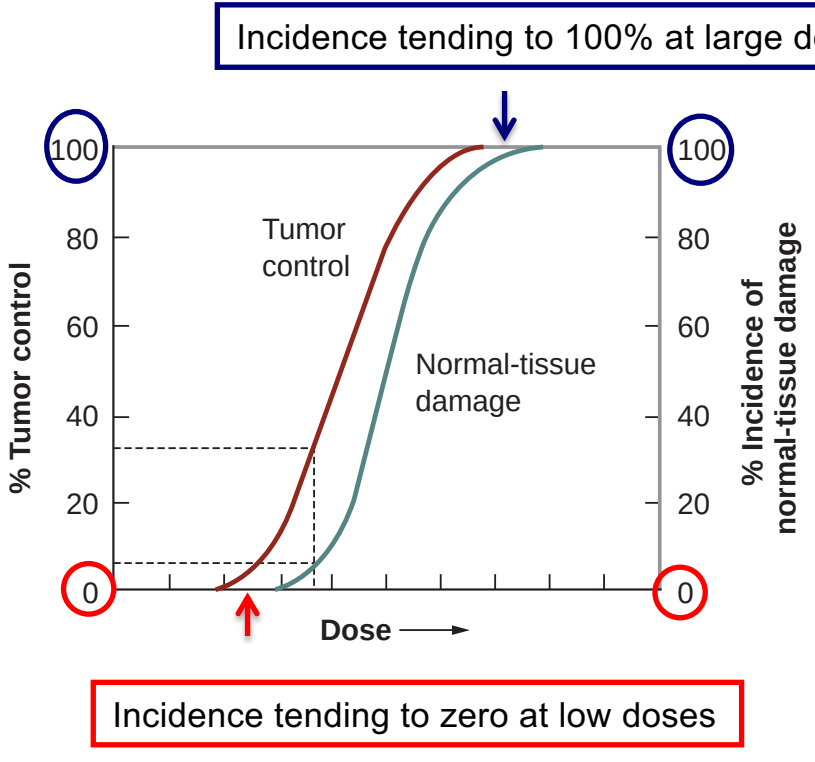
To study the relationship between a given **dose** and the consequent **biologic response**

The biologic endpoints of interest are

1) **Tumor control**

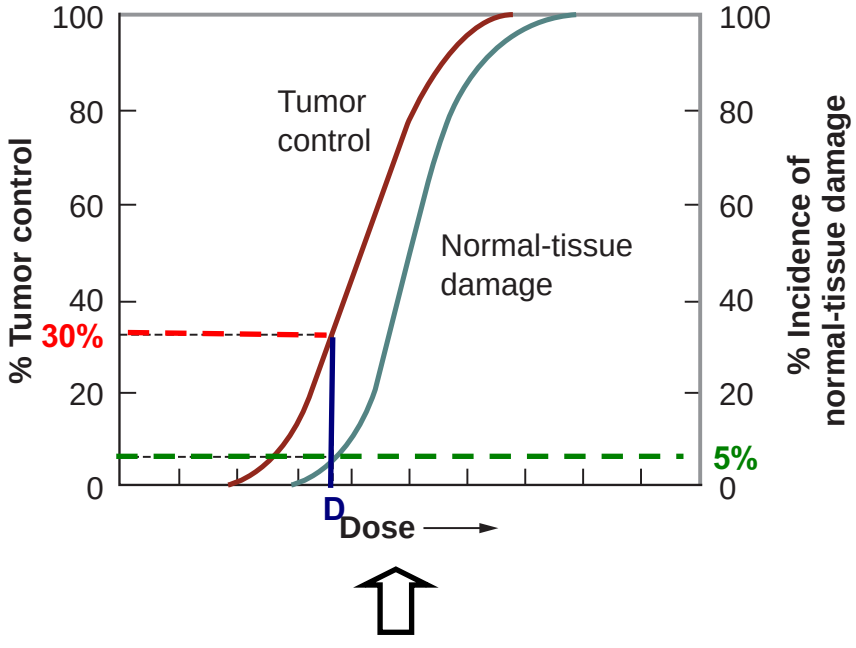
2) **Normal tissue damage**

Dose-Response Relationship



The dose-response curves typically have a **sigmoidal (S) shape** for both tumor control and normal-tissue complications

Therapeutic Ratio (Index)

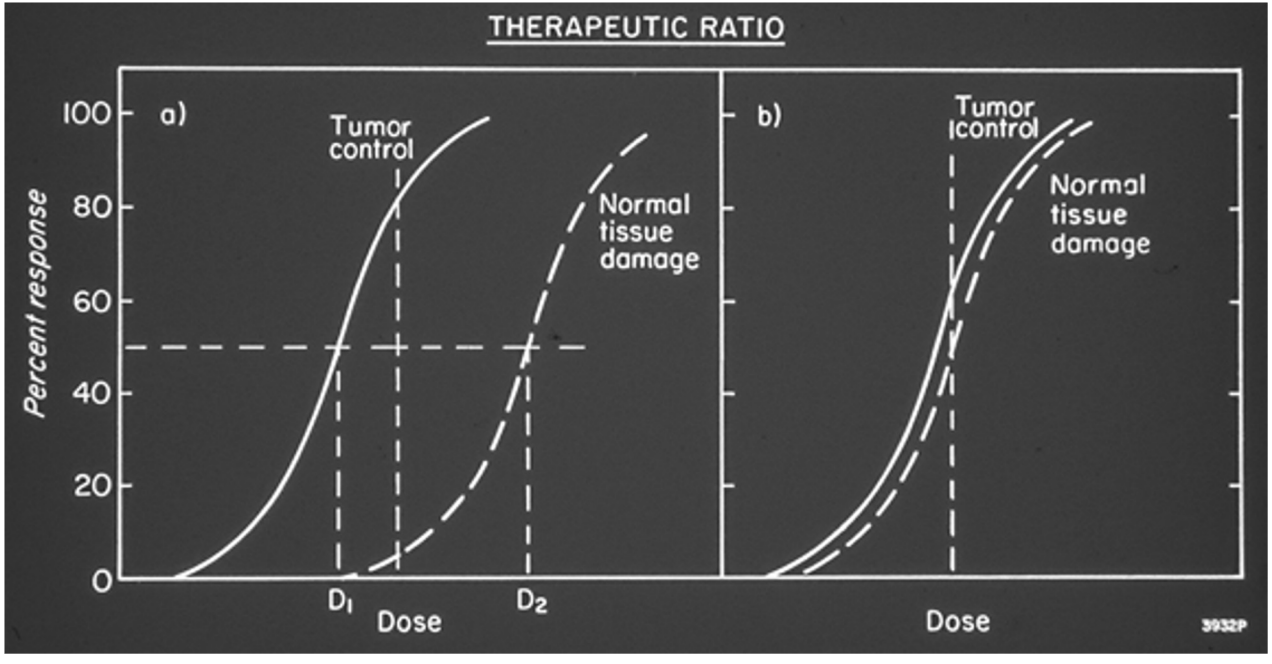


For a 5% incidence of normal-tissue damage, a 30% tumor control can be achieved.

Therapeutic ratio

- The ratio of the tumor response for a fixed level of normal tissue damage (text)
- The % of tumor control that can be achieved for a given level of normal tissue of damage (fig legend)
- The ratio of tumor response to normal tissue damage (summary)

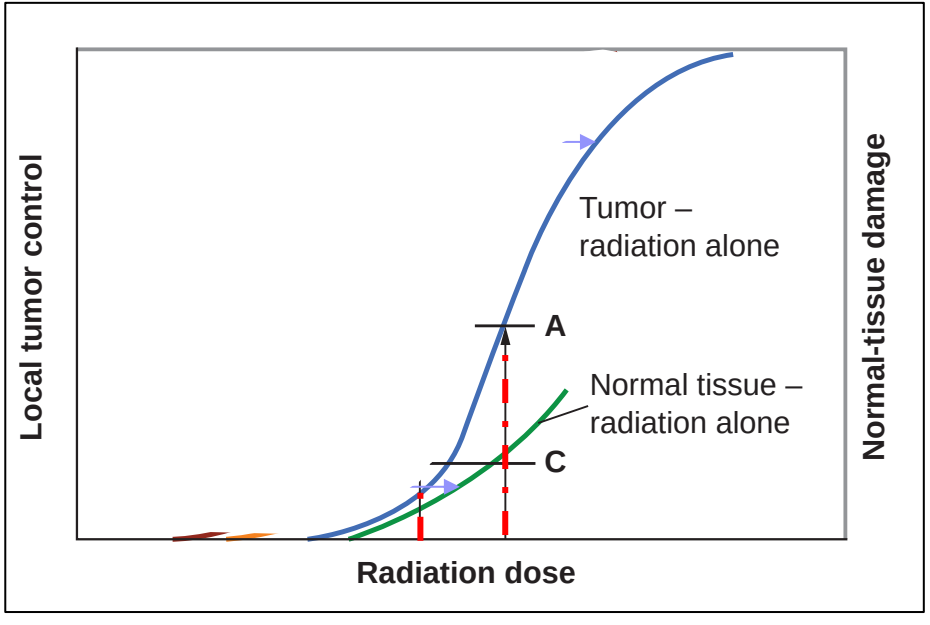
Therapeutic Ratio (TR)



Favorable TR

Unfavorable TR

Improving Therapeutic Ratio



Effect of Radiation Sensitizer

The addition of the drug moves both curves to the left (= **potentiation**)

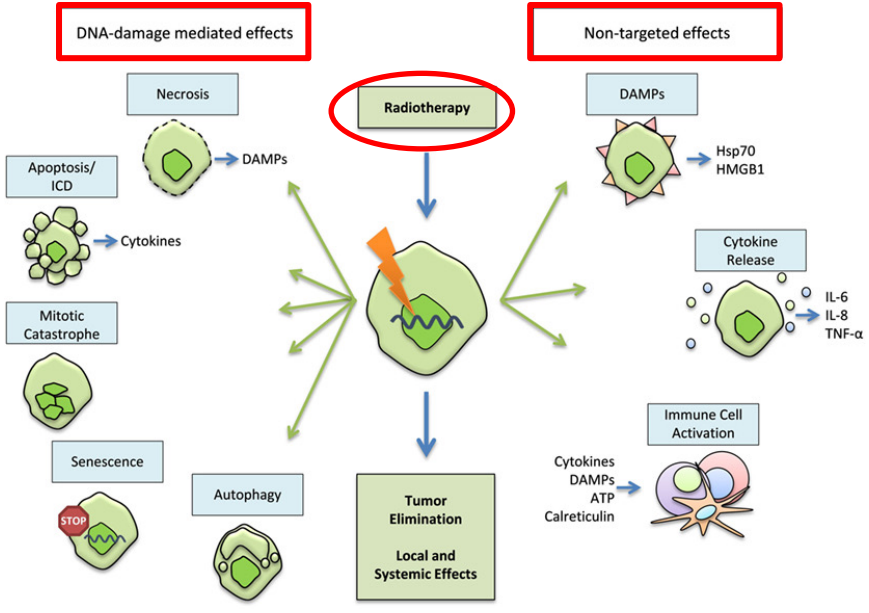
For the same level of normal tissue damage (C), a higher probability of tumor control is achieved (B) compared to without the drug (A)

As long as the drug increases tumor control **to a greater extent** than it increases normal tissue, damage, it will result in a **therapeutic gain**

Outline

- Dose-Response Relationship
  - Therapeutic Ratio
- **Mechanisms of Cell Death** **(in the context of Normal Tissue)**
- Assays for Dose-Response Relationship
  - Clonogenic Endpoints
  - Functional Endpoints
  - Inferring α/β from Multifractional Experiments

Types of Cell Death in Normal Tissues



Mitotic-linked cell death and apoptotic cell death are responsible for most cell killing by ionizing radiation

Implications for Radiation Therapy

IR also induces a form of **senescence** in which cells are still metabolically active

- e.g. fibroblasts are able to secrete growth factors and mitogens that promote the growth of tumor cells despite being growth arrested

Abscopal effect may also exist for normal tissues

- e.g., lymphopenia found after radiotherapy to nonlymphoid organs

Outline

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  - Therapeutic Ratio
- Mechanism of Cell Death
- **Assays for Dose-Response Relationship**
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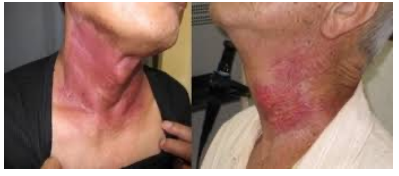
Normal Tissue

Radiation Response in Normal Tissues

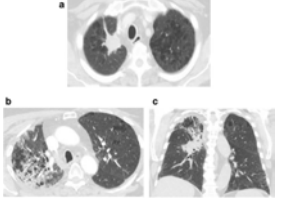
Early Responding

- Skin, intestines, epithelium, bone marrow, lymphoid tissues
- Respond early to the effects of radiation
- Rapidly dividing self-renewal tissues



Late Responding

- Spinal cord, lungs, kidney
- Expression of radiation damage occur at a later time point



Mechanism of Radiation Damage

Mechanism

Radiation damage is a result of depletion of the **critical parenchymal cells**

Timing of Damage Expression (Latency)

The difference in time at which early- and late-responding tissues express radiation damage is a function of **different cell turn over rates**

This has taken over the old model which ascribe the response of late-responding tissues entirely to vascular damage rather than depletion of parenchymal cells

Cytokines and Late Effects – Paradigm Shift

In the past (classical target theory) there was considerable discussion as to whether *parenchymal cell loss* or *vascular damage* was the main reason for late effects

It is more sensible to consider symptoms to result from **a dysregulated healing response**, the manifestations of which may change with time after RT and involve all cellular compartments

More on this in Chapter 20

Assays for Dose-Response Relationship

In its original place

Clonogenic Assay

End Point – **reproductive integrity** of individual cells (analogous to cell survival *in vitro*)

In some systems, the survival is observed ***in situ*** (e.g., regrowth of skin colonies, regenerating crypts in the jejunum)

In other systems, irradiated donor cells are **transplanted** into a recipient (e.g., spleen colony assay)

Assays for Dose-Response Relationship

Functional Assay

End Point – **functions of tissue or organ**

Examples – skin reaction in rodents or pigs (erythema and desquamation); pneumonitis or fibrosis of lung (breathing rate); myelopathy of the spinal cord (paralysis of hind limb)

The end points observed tend to reflect the minimum number of functional cells remaining in a tissue or organ, rather than the fraction of cells retaining reproductive integrity

Assays for Dose-Response Relationship

α/β Ratio Inferred from Multifractional Experiments

Developed by Douglas and Fowler

Linear-quadratic relationship is assumed, and a series of multifraction experiments performed

Used widely to infer values for α and β in the dose-response relationship for normal tissues in which the parameters cannot be measured directly

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  - **Clonogenic Endpoints**
  - Functional Endpoints
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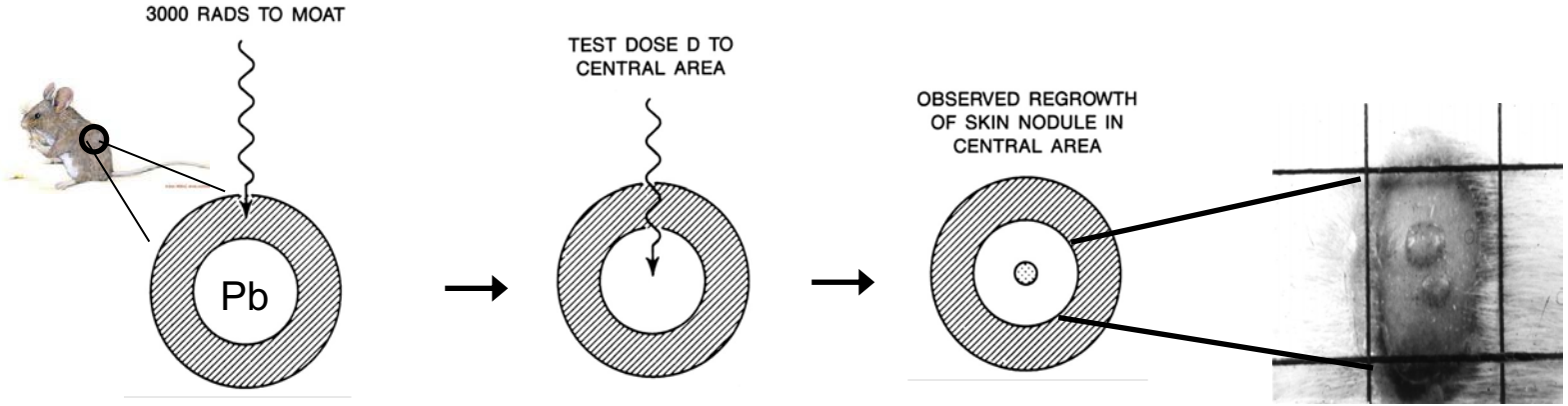
Clonogenic End Points

- **Clones Regrowing *in Situ***
  - Skin Colonies
  - Crypt Cells of the Mouse Jejunum
  - Testes Stem Cells
  - Kidney Tubule

  } Withers *et al.*
- Cells Transplanted to Another Site
  - Bone Marrow Stem Cell → Till & McCulloch
  - Mammary Cells
  - Thyroid Cells

  } Clifton & Gould

Skin Colonies



Hair was plucked

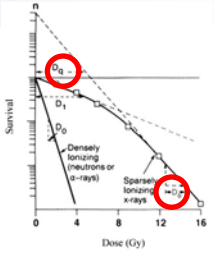
An annulus of skin treated to 30 Gy to produce a “moat” of dead cells

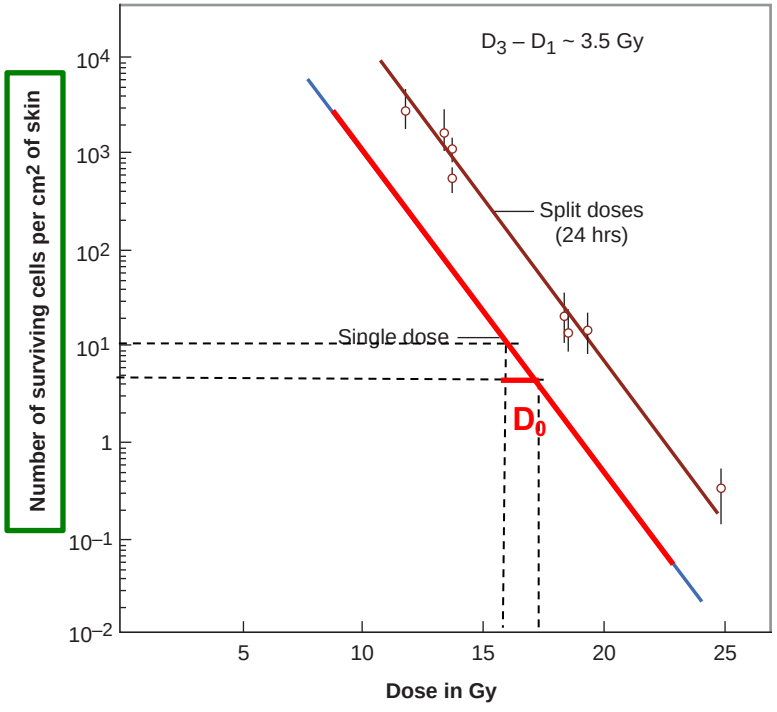
Protected intact skin cells in the center were treated with a test dose

Each nodule regrows from a surviving stem cell

A range of dose is necessary to construct a dose response curve

Skin Colonies





D_0 = 1.35 Gy

Note that this is very similar to D_0 of mammalian cells irradiated *in vitro*

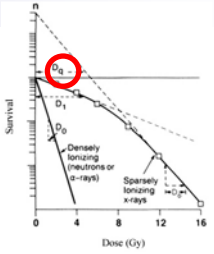
Limitation of the Assay

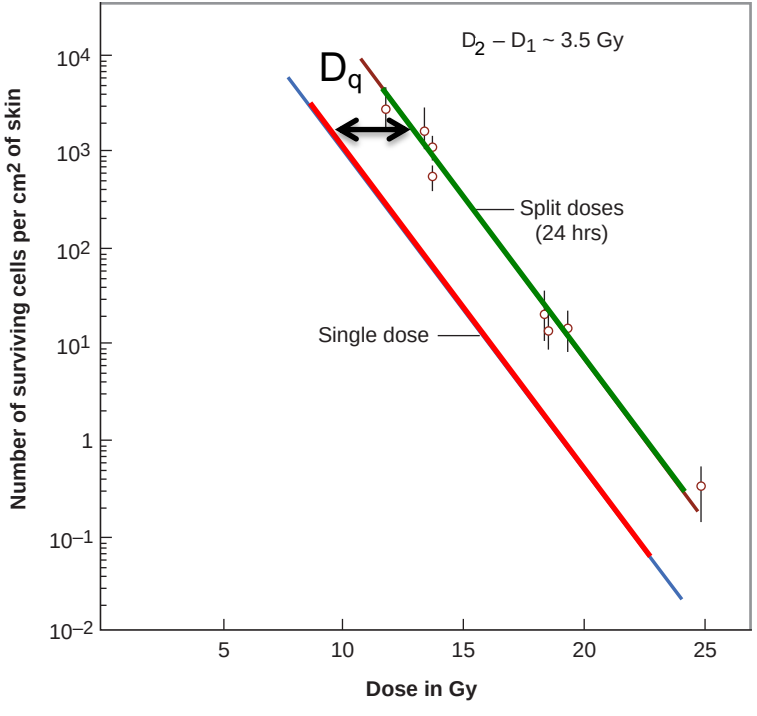
At too low a dose, it is impossible to count individual skin survival colony
At too high a dose, a very large area needs to be radiated

⇩

For single-dose survival curve data is available from 8-25 Gy

Skin Colonies





If the dose is split into 2 equal fractions separated by 24 hours, the shoulder is repeated

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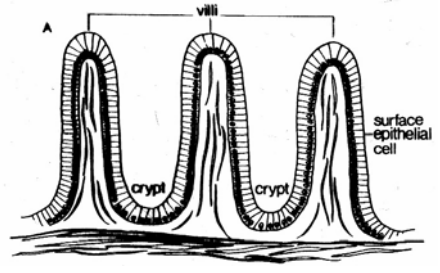
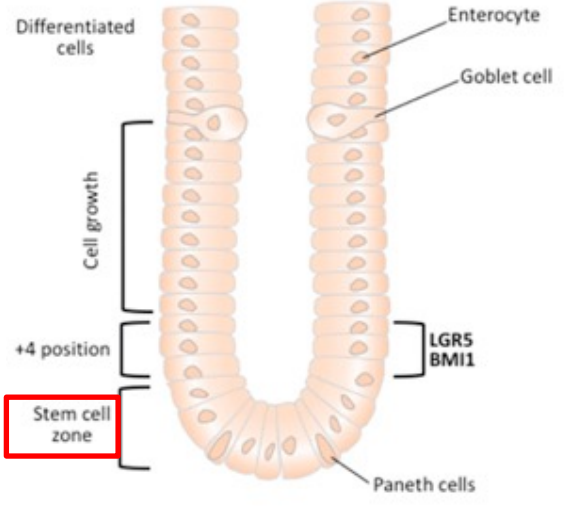
The separation of the 2 curves is a measure of the width of the shoulder, or D_q (quasi-threshold dose)

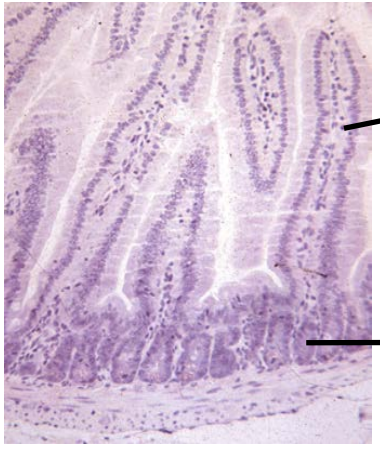
$D_q = 3.5$ Gy

Crypt Cells of the Mouse Jejunum



Scanning EM of jejunal villi

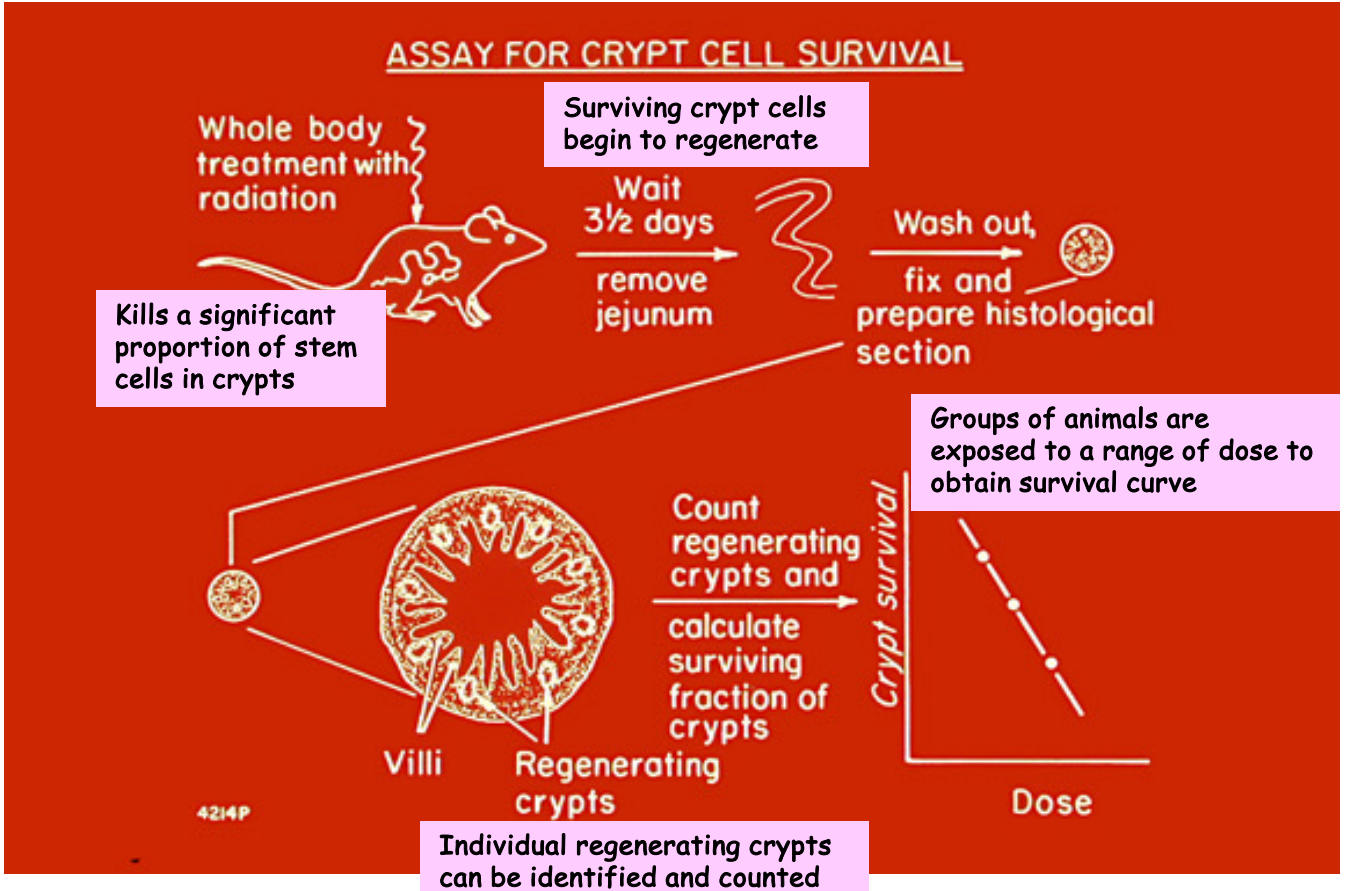




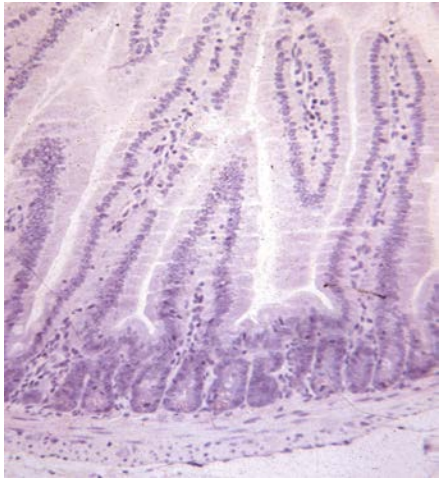
Stem cells differentiate from the **+4 position** upward to the villi
Transit time 4 days for small intestine and 5 days for large intestine

Like skin cells, the lining of the jejunum is a classic example of self-renewal system

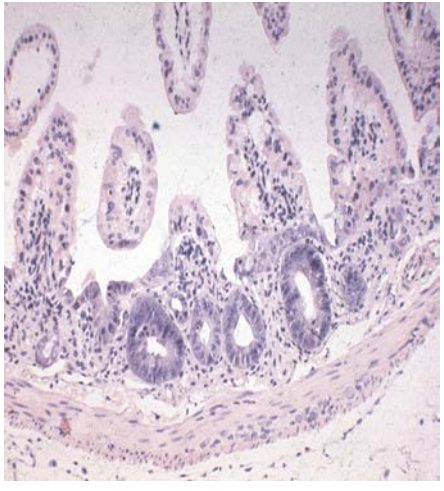
Crypt Cell Survival Assay



Crypt Cells of the Mouse Jejunum



Unirradiated jejunum

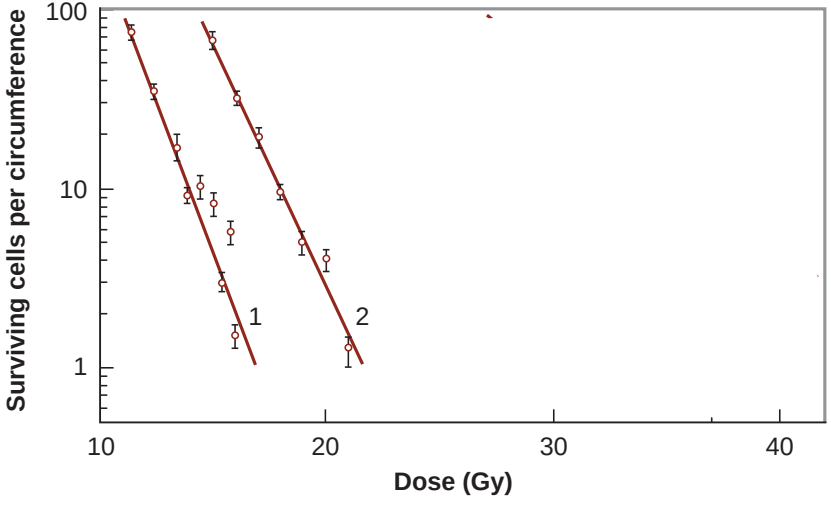


Regenerating crypts seen at 3.5 days following irradiation

The number of regenerating crypts per circumference of the sectioned jejunum as a measure of radiation damage

Crypt Cells of the Mouse Jejunum

Note this is not SF



D_0 = 1.3 Gy

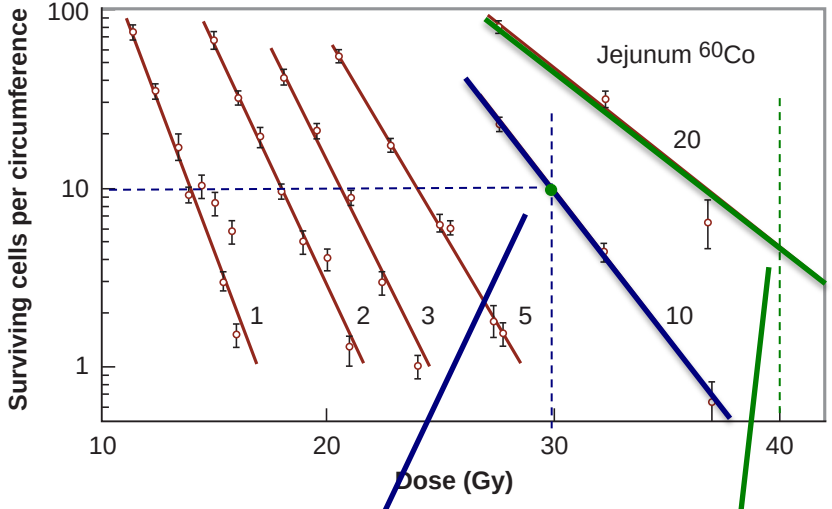
D_q = 4 - 4.5 Gy

Indicates substantial repair

Caveat – a dose of 10 Gy or above is necessary to cause sufficient damage so that individual regenerating crypts can be identified

How to obtain dose-response curve at dose-response at low dose region?

Crypt Cells of the Mouse Jejunum



3 Gy x 10 = 30 Gy

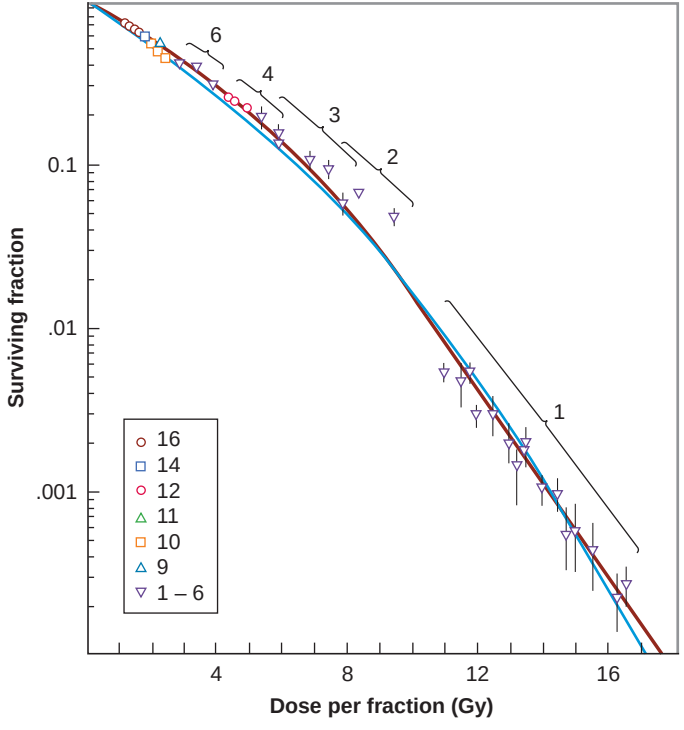
2 Gy x 20 = 40 Gy

Deliver dose in multiple fractions, and assume that in a fractionated regimen each dose produces the same amount of cell killing

⇩

The shape of the entire survival curve can be reconstructed

Crypt Cells of the Mouse Jejunum



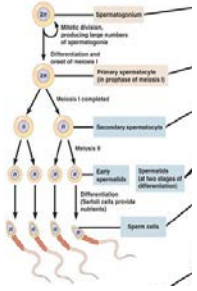
The numbers on the curve refer to the number of fractions used to reconstruct the curve

D_0 = 1.43 Gy
D_q = 4.3 Gy

Note that the data are equally well fitted by the linear quadratic equation

Effective single-dose survival curve reconstructed from multifraction experiments

Testes Stem Cells



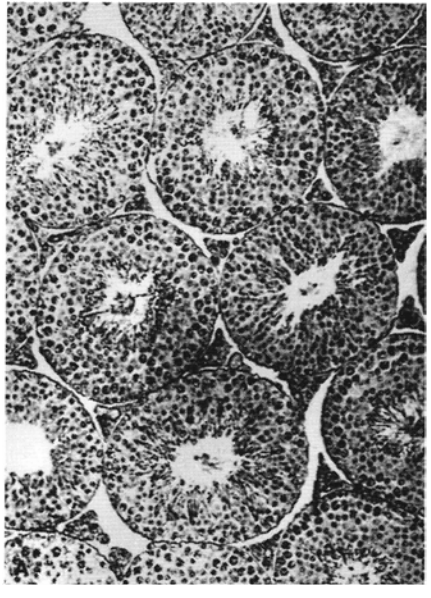
Irradiate mice

↓ 6 wks

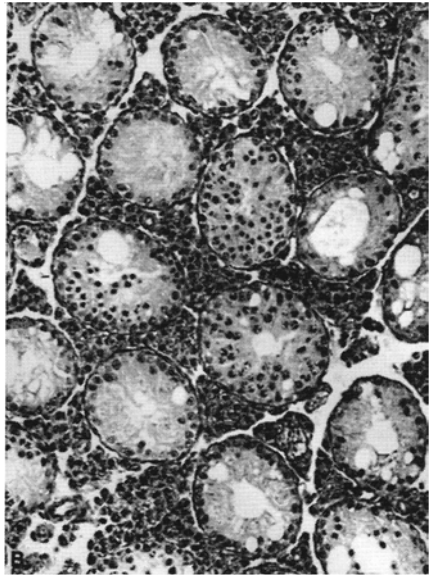
Section testes

↓

Count # tubules containing spermatogenic epithelium

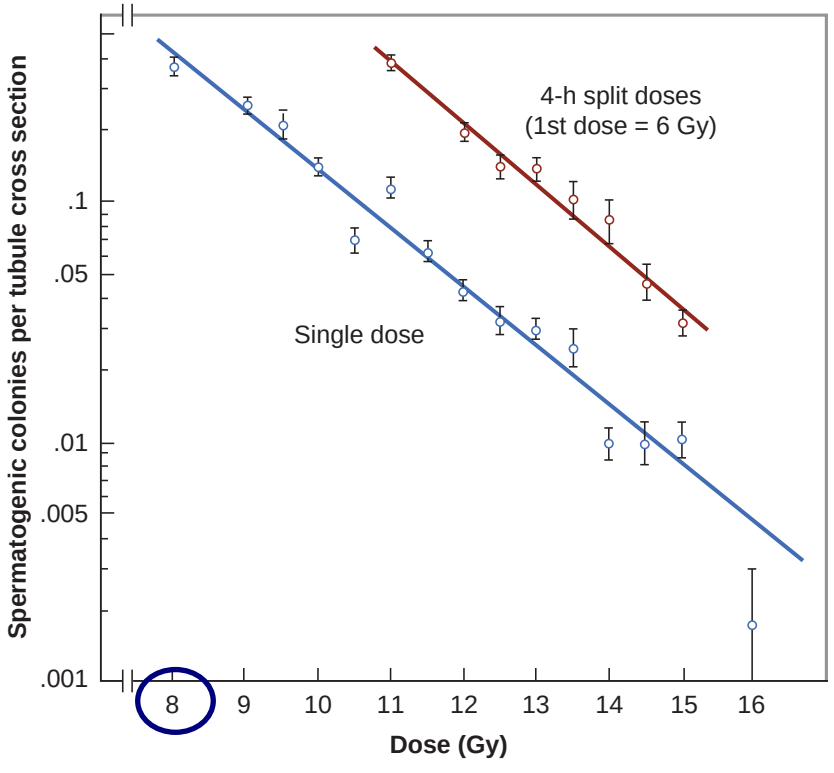


Normal testis



35 days after 9 Gy

Testes Stem Cells

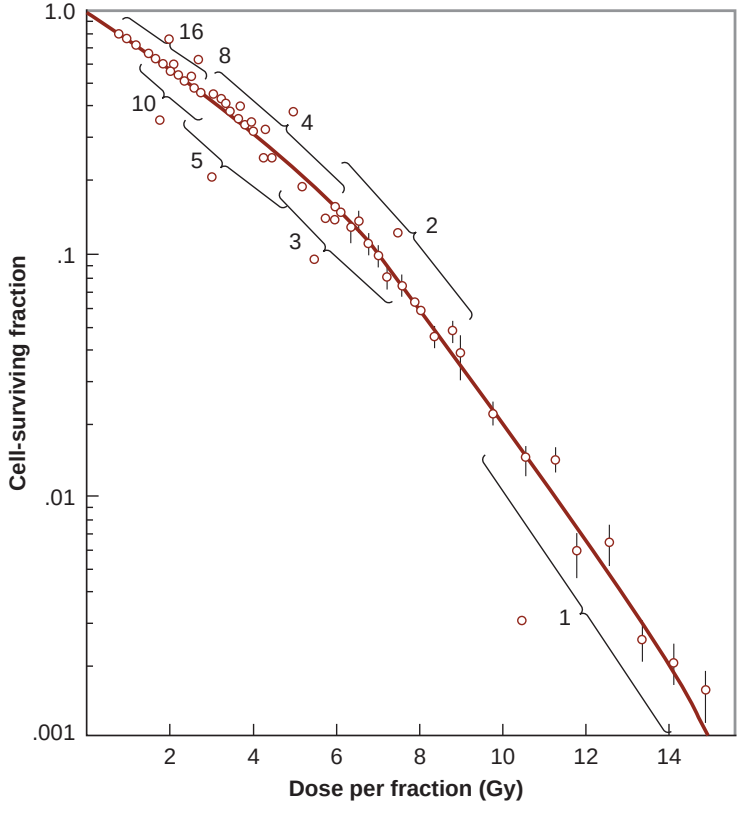


D_0 = 1.68 Gy

D_q = 2.7 Gy

⇦ Note that a relatively high single dose of 8-16 Gy are necessary to score individual surviving colonies

Testes Stem Cells



Effective survival curve reconstructed from multifraction experiments

D_0 = 1.6 Gy

D_q = 3.92 Gy

Kidney Tubules

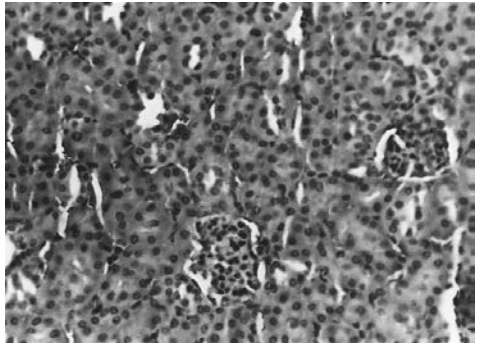
Irradiate one kidney of each mouse

↓ **60 wks**

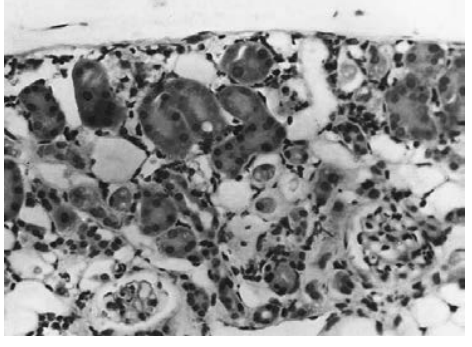
Section kidney

↓

Count # of regenerating tubules



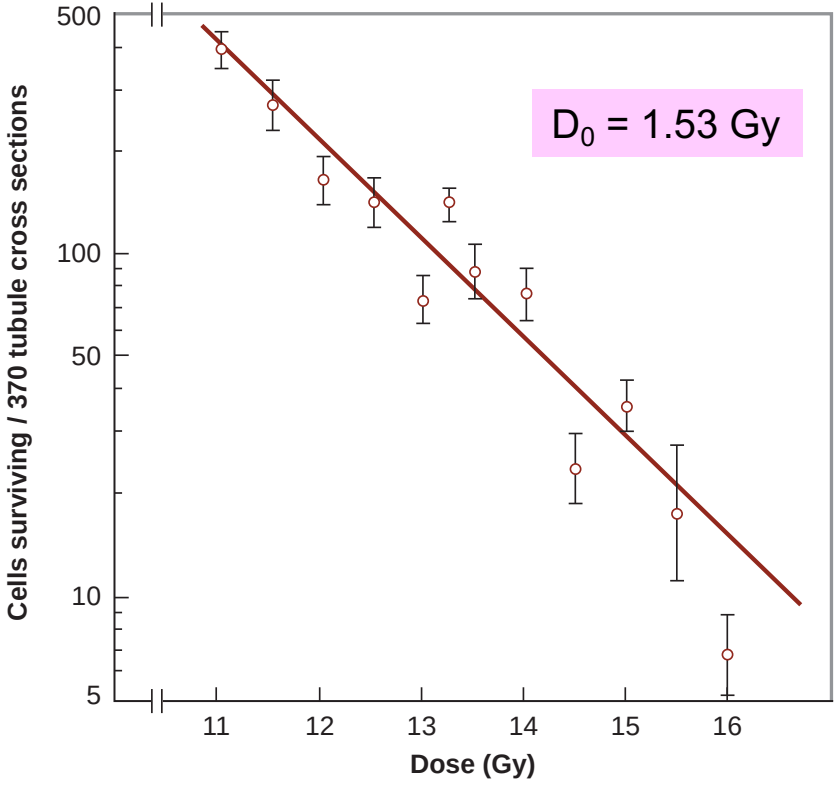
Normal kidney tubules



60 weeks after 13 Gy

This is the first clonal assay for a late-responding tissue!!!

Kidney Tubules



Note that the radiosensitivity (D_0) of the late responding tissue is not very different from that of early responding tissue

The ***rate*** of response, however is very different
This is a function of turnover of the cell population

Time required for depletion of epithelium after 14 Gy

Jejunum	3 days
Skin	12-14 days
Testes tubules	30 days
Kidney tubules	300 days

Clonogenic End Points

- Clones Regrowing *in Situ*
  - Skin Colonies
  - Crypt Cells of the Mouse Jejunum
  - Testes Stem Cells
  - Kidney Tubule

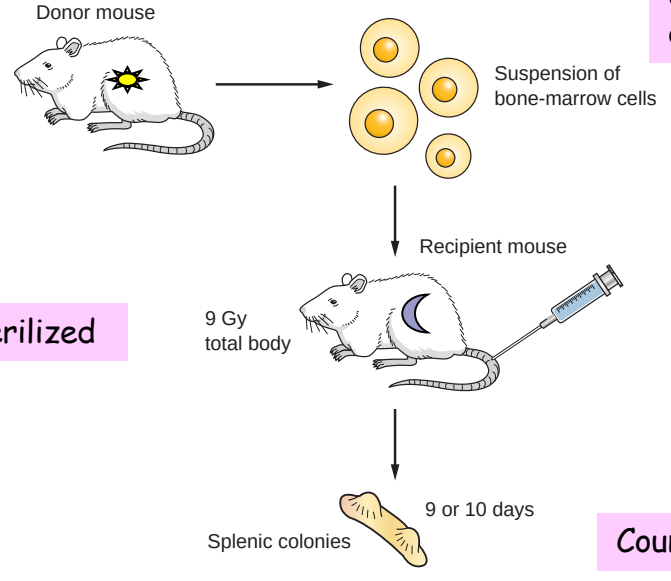
  } Withers et al.

- **Cells Transplanted to Another Site**
  - Bone Marrow Stem Cell → Till & McCulloch
  - Mammary Cells
  - Thyroid Cells

  } Clifton & Gould

Bone Marrow Stem Cells

Irradiated with to some test dose



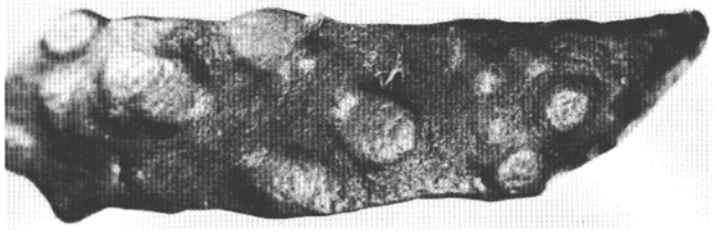
Contain a small proportion of stem cells

Spleen cells sterilized

Count the # of colonies

Till & McCulloch's Spleen Colony Assay

Bone Marrow Stem Cells

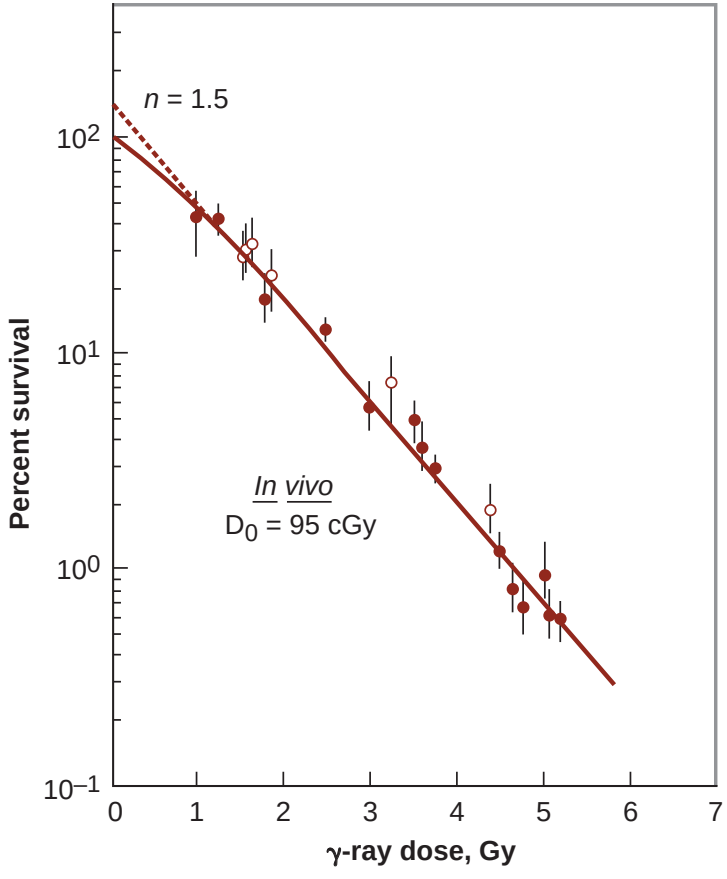


Spleen Colonies

About 10^4 must be injected into a recipient to produce 1 spleen colony, thus need to correct for plating efficiency

$$\text{Surviving Fraction} = \frac{\text{Colonies counted}}{\text{Cells inoculated x PE}}$$

Bone Marrow Stem Cells



D_0 = 0.95 Gy

Note that there is almost no shoulder

Mammary Cells

Irradiate donor mice

↓

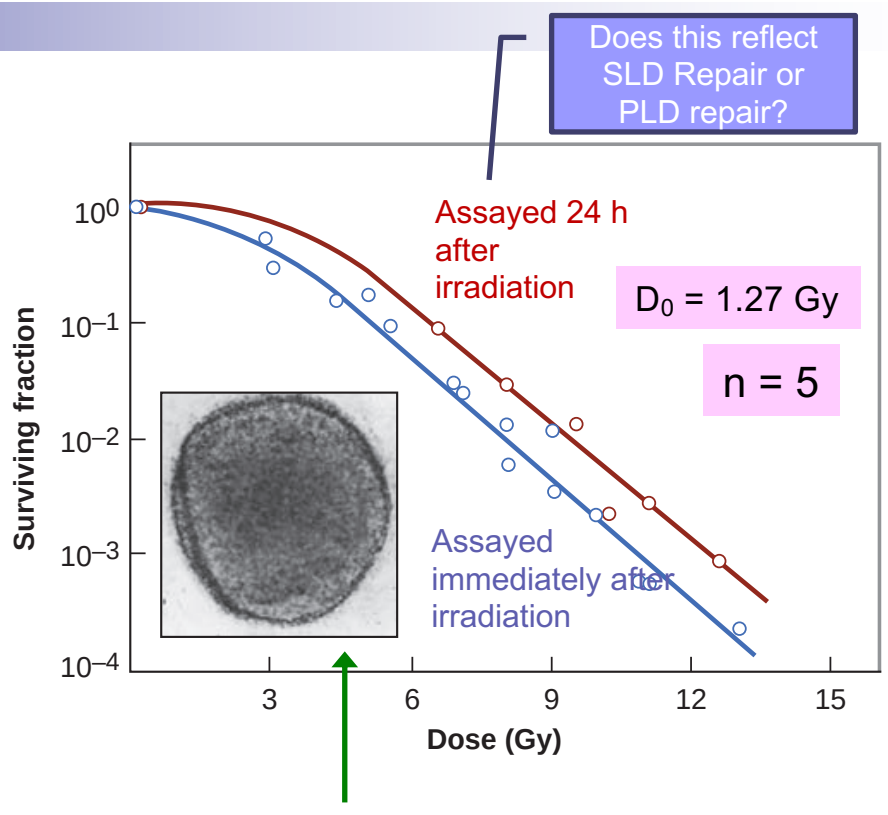
Remove mammary cells and disperse into single cells

↓

Implant into the **fat pads** of recipient animals

↓ 3.5 wks

Count # of mammary structures



Milk filled spherical alveolar unit developed from a single surviving transplanted cell

The initial motivation is to study carcinogenesis in epithelial cells in a quantitative manner

Thyroid Gland Cells

Irradiate donor mice

↓

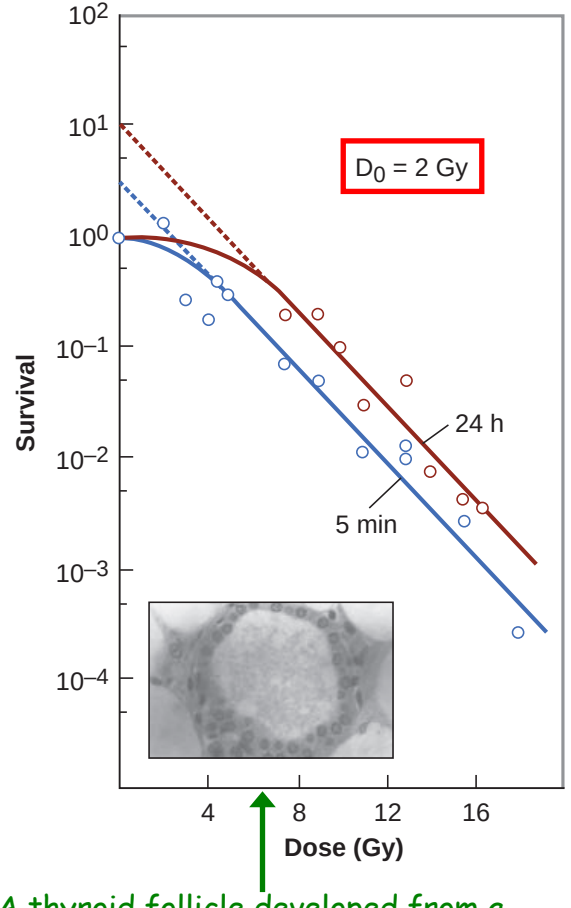
Remove thyroid cells and disperse into single cells

↓

Implant into the fat pads of recipient animals

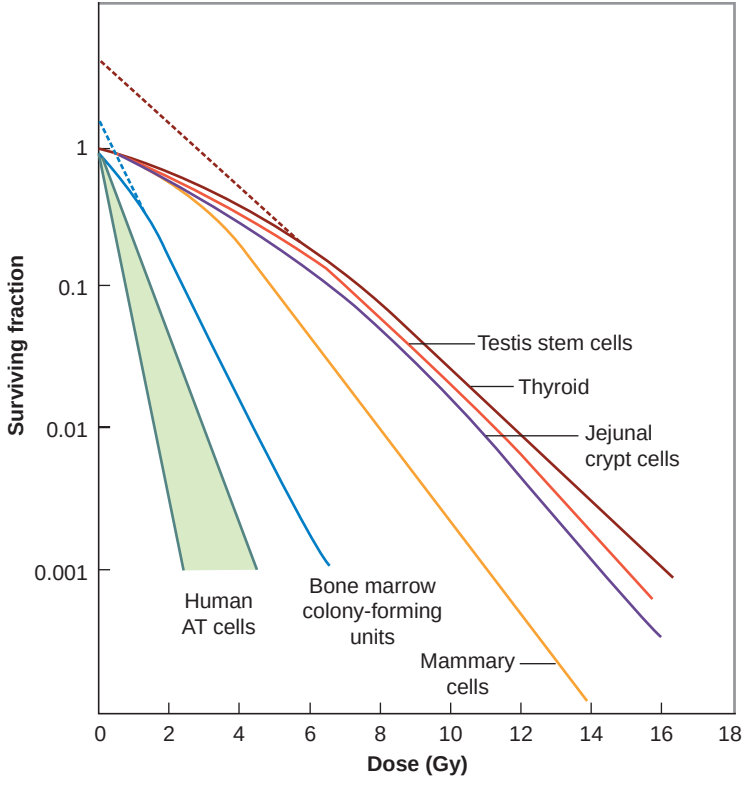
↓ 4 wks

Count # of follicle structures



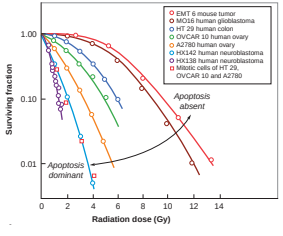
A thyroid follicle developed from a single surviving transplanted thyroid cell

Summary of Dose-Response Curves for Clonogenic Assays



Variation in radiation sensitivity is primarily due to difference in width of the shoulders

Notably, these are all ***in vivo* assays** obtained by irradiation of whole animals (as opposed to cells in culture)



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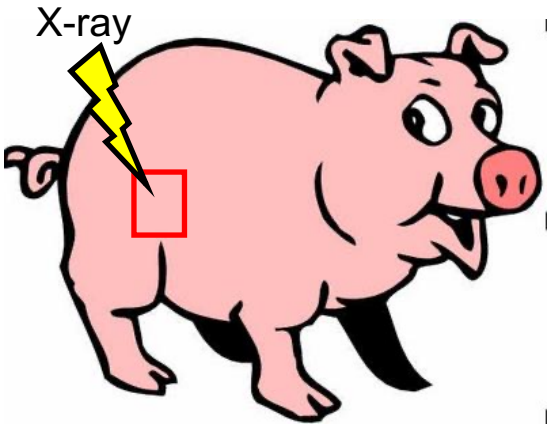
  - **Functional Endpoints**
  - Inferring α/β from Multifractional Experiments

Functional Assays

- Not a direct measure of cell survival, but direct relevance to clinical side effects
- Examples include
  - Pig Skin
  - Rodent Skin

  } Fowler
  - Early and Late Response of the Lung → Travis
  - Spinal Cord Myelopathy → van der Kogel

Pig Skin



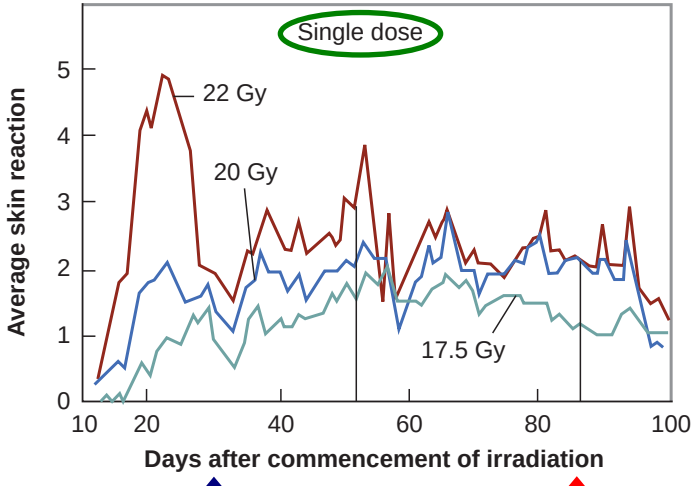
Pig skin shares many common features with human skin – color, hair follicles, sweat glands, a layer of subcutaneous fat

Response of pig skin to radiation resembles that of human skin, both qualitatively and quantitatively

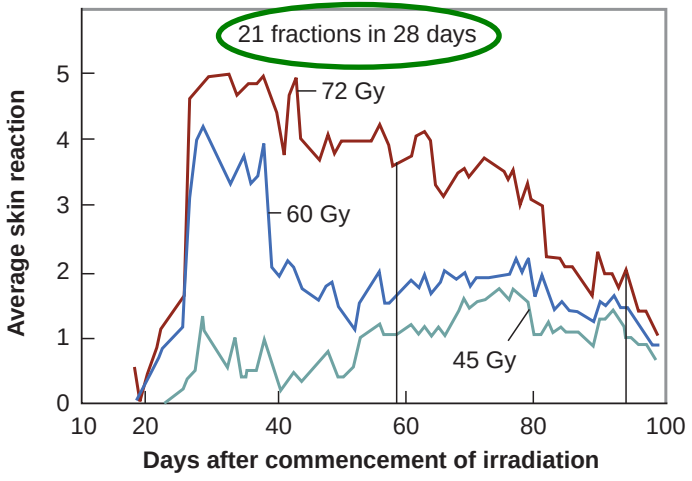
Arbitrary Score	Reaction
0	No visible reaction
1	Faint erythema
2	Erythema
3	Marked erythema
4	Moist desquamation of < ½ the irradiated area
5	Moist desquamation of > ½ the irradiated area

Skin reactions were scored daily

Pig Skin



Effects of Fractionation



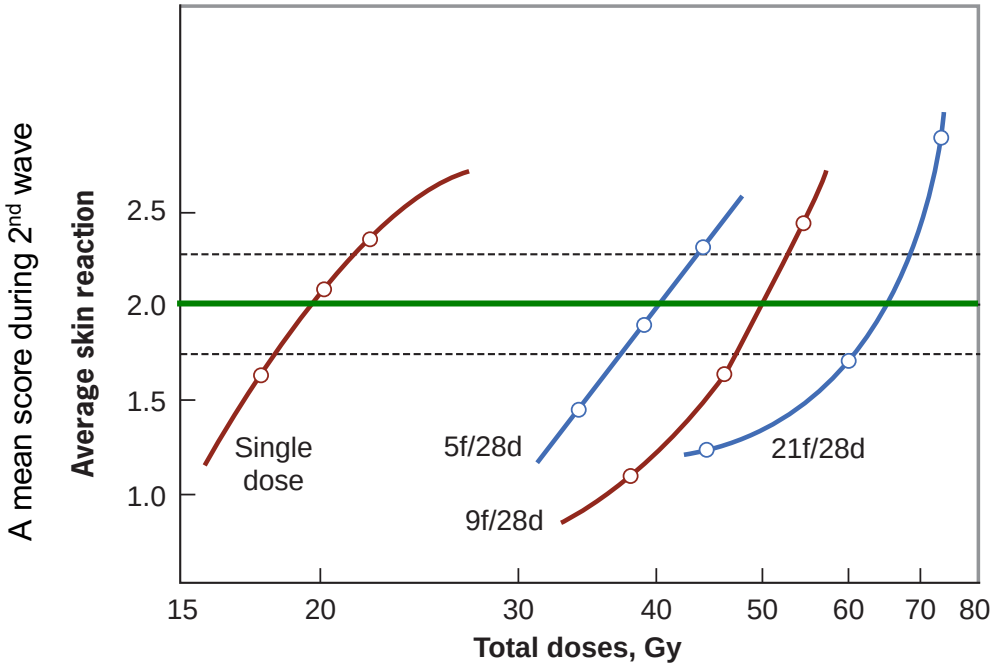
Early wave of erythema occurred at 10-40 days, representing "acute" reaction

A 2nd broad wave of moderately severe reaction took place at 50-85 days, which correlate well with late skin damage (up to 2 years) and with subcutaneous damage

Late effects may also be studied by measuring the contraction that results from fibrosis a year or more after irradiation

Pig Skin

Average skin reaction as a function of total dose



Note the skin sparing effect with fractionation

This is the subject of Chapter 23

Rodent Skin

Cheaper and less awkward to work with than pigs

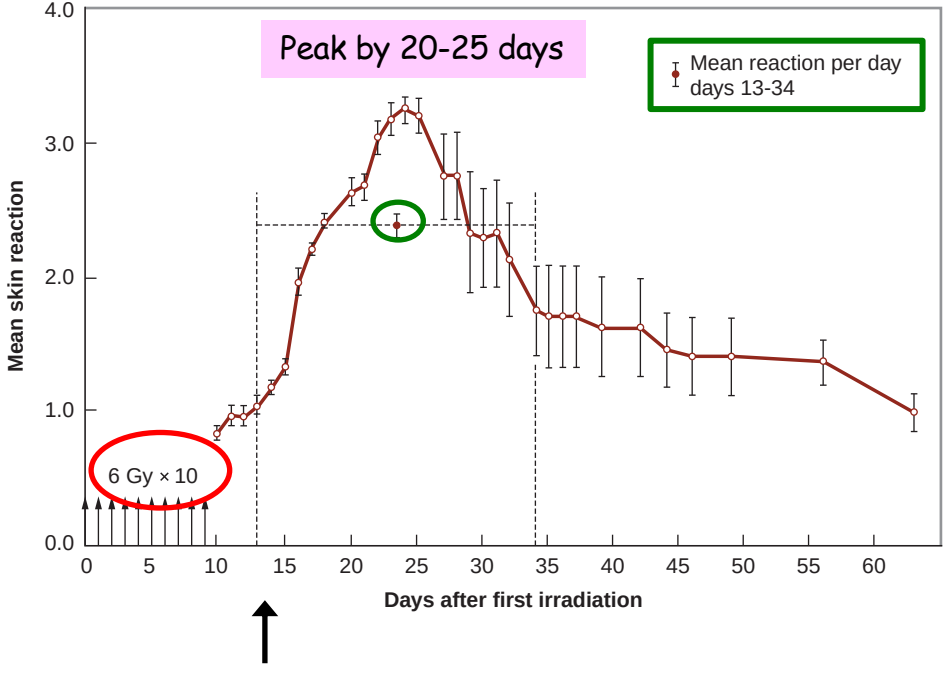
One hind leg is irradiated
The other serves as a control

Radiation Reactions in Mouse Leg Skin

Arbitrary Score[a]	Observations
0.5	50/50; doubtful if any difference from normal or not
1–	Because 1 covers a wide range of reddening, even before reaching the severity or additional factors requiring 1+, it is necessary to have 1– for "definite reddening (i.e., definitely not normal), but only a very slight degree."
1	Definite abnormality; definite reddening, top or bottom of leg; "clean" appearance means not greater than 1
1+	Severe reddening or reddening with definite white marks in creases under foot; query breakdown; query puffiness
1.5	Some breakdown of skin (usually seen on bottom of foot first); scaly or crusty appearance; definite puffiness, plus (query) breakdown; very marked white marks in creases plus puffiness or severe redness
1.5+	Query possibly moist desquamation in small areas
2	Breakdown of large areas of skin or toes stuck together; possibly moist in places but not all moist
2.5	Breakdown of large areas of skin with definite moist exudate
3	Breakdown of most of the skin with moist exudate
3.5	Complete necrosis of limb (rarely seen so far)

Rodent Skin

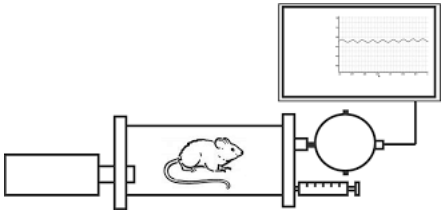
Average daily skin reaction scores for six mice irradiated in 10 fractions of 6 Gy each

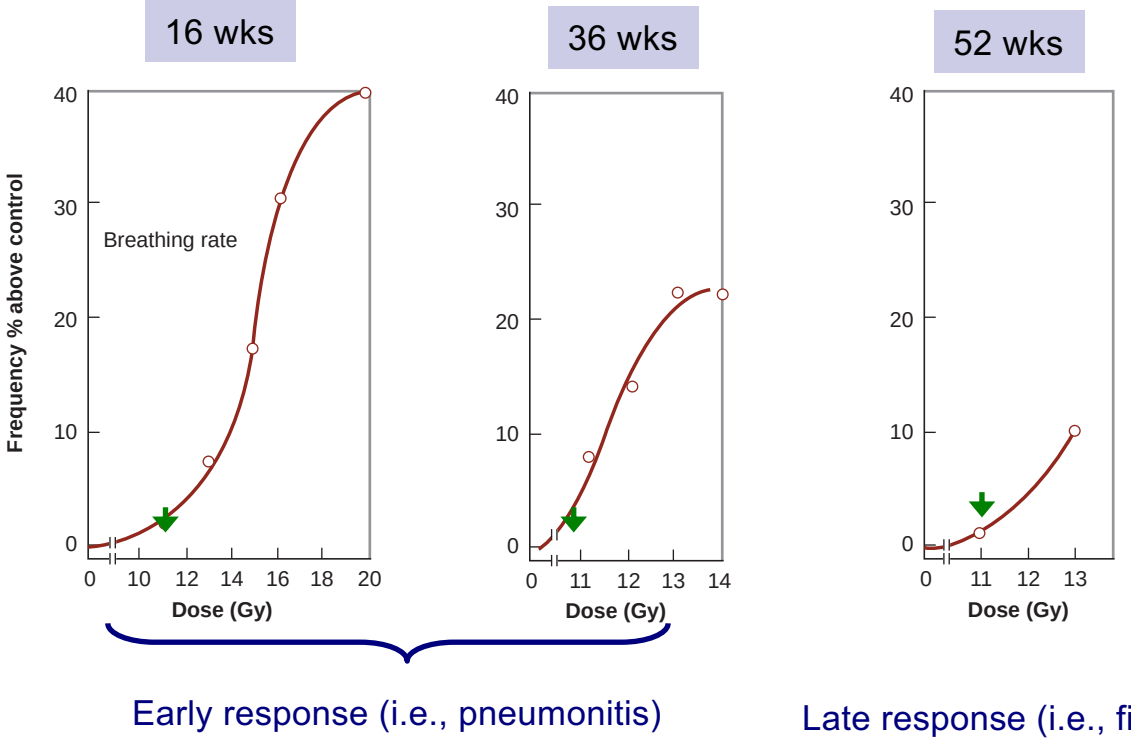


A dose-response curve can be obtained by averaging the skin reaction over a period of time and plotting this average as a function of dose

Early and Late Response of Lung

Breathing frequency was used as a measure of radiation lung damage





Note the sigmoid shape and a threshold dose of ~ 11 Gy

Early response (i.e., pneumonitis)

Late response (i.e., fibrosis)

Spinal Cord Myelopathy

neurologic deficit related to the spinal cord

Spinal cord is a late responding tissue

Rats
Latency – 4 to 12 months Symptoms – palpable muscle atrophy followed by impaired use of the hind legs

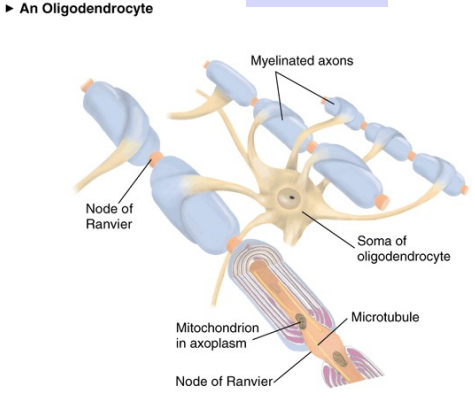
Human
First 6 months – demyelination 1-2 years post-irradiation – glial atrophy and white matter necrosis

Mechanism – thought to be primarily due to killing of glial progenitor cells; vascular injury may accelerate, precipitate, or even initiate the white-matter changes leading to necrosis (***this is an area of some controversy***)

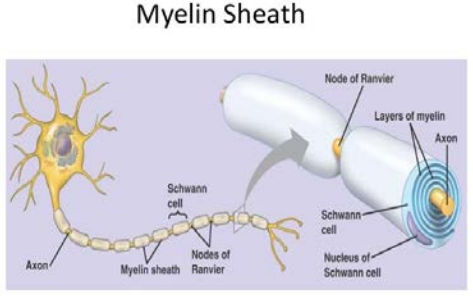
Myelin

- Most long nerve fibers are covered with a whitish, fatty material, called **myelin**, which has a waxy appearance
- Myelin protects and insulates the fibers and increases the transmission rate of nerve impulses
- Axons *outside the CNS* are myelinated by **Schwann cells**
- Within the CNS, it is the **oligodendrocytes** that form myelin sheaths

CNS

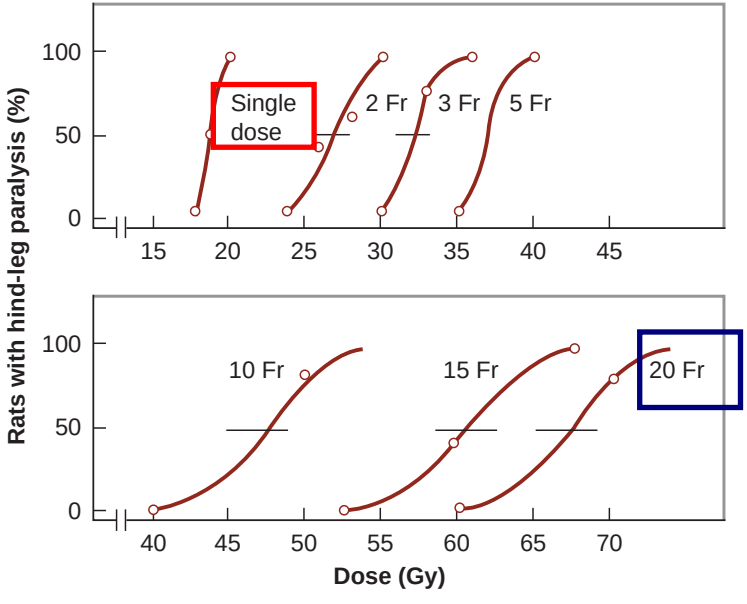


PNS



Spinal Cord Myelopathy

50% paralysis at 19 Gy;
steep dose-response



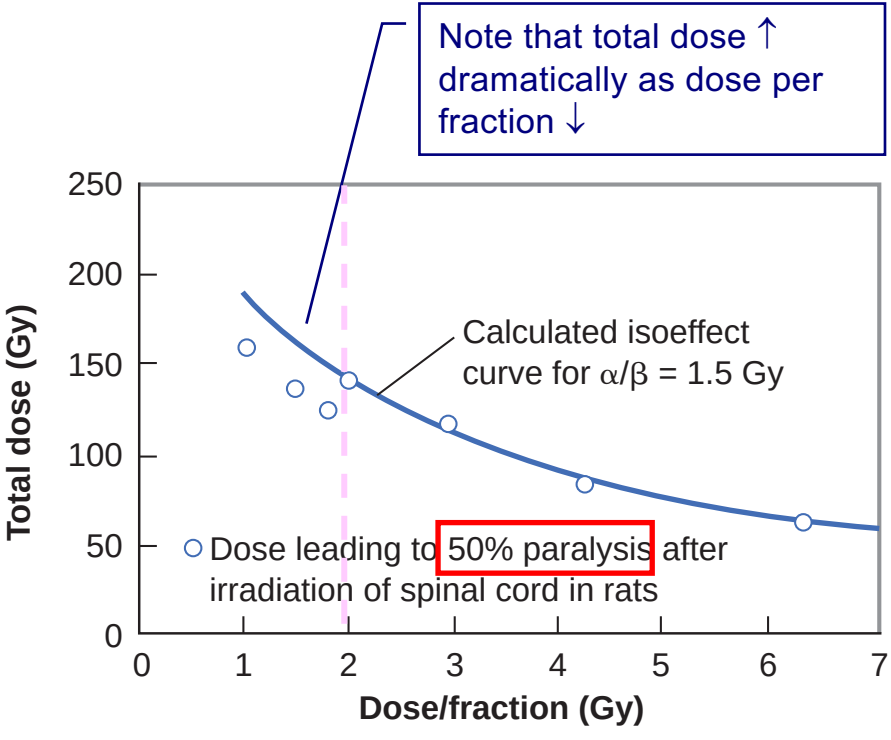
For single doses (25-60 Gy), **latency** decreases as dose increases (~ 2 days/Gy)

50% paralysis requires 65 Gy;
shallower dose-response

Note the dramatic sparing from fractionation

Dose-response curves for the induction of hind-leg paralysis following irradiation of a section of the spinal cord L2-L5

Spinal Cord Myelopathy



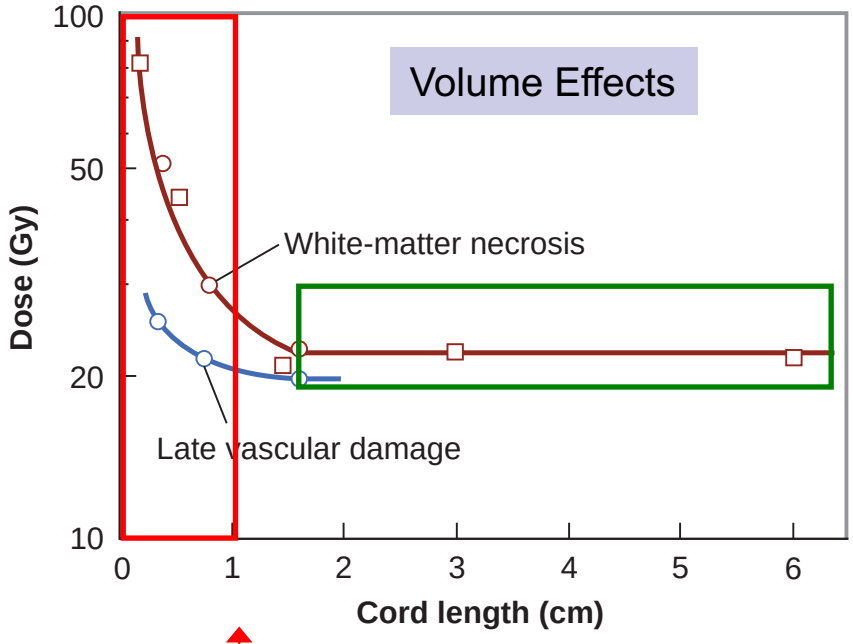
Iso-effect curve

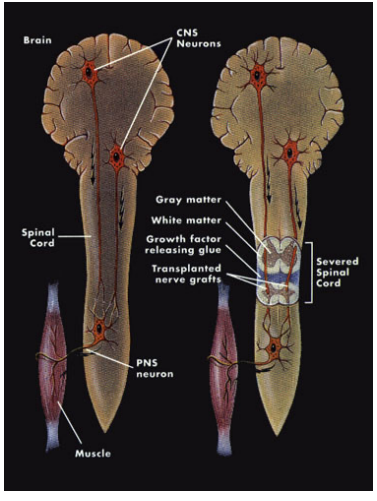
Late responding tissue typically has a **small α/β ratio**, and are sensitive to dose fractionation

Experimental data suggest that the L-Q model overestimates the tolerance for dose per fraction less than 2 Gy – likely due to incomplete repair

Repair of sublethal damage may have "fast" and "slow" components; for this reason, if multiple doses per day area used to the spinal cord, **the interfraction interval should be at least 6 – 8 hours**

Spinal Cord Myelopathy





Below 1 cm, tolerance for white-matter necrosis shows a marked dependence on the length of cord irradiated

Beyond a few cm, the tolerance is virtually independent of the length of the cord irradiated, which is explained by the linear arrangement of the functional subunits

Spinal Cord Myelopathy

The spinal cord does recover to some extent after long time periods following irradiation

Retreatment after Long Time Interval

Experiments with rats indicate that after an initial treatment to **50%** tolerance, the retreatment tolerance approaches **90%** of the tolerance of untreated control group by about **a year** after the initial irradiation

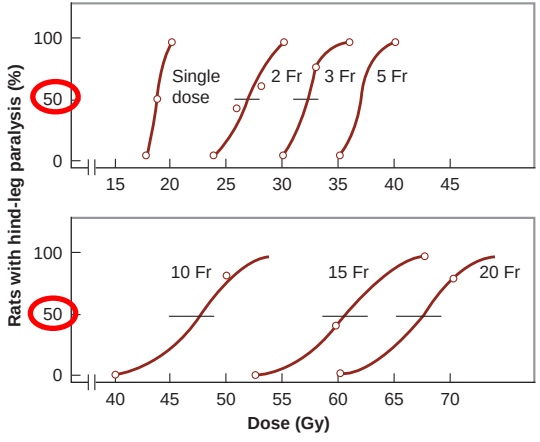
Outline

- Dose-Response Relationship
  - Therapeutic Ratio
- Mechanisms of Cell Death
- Assays for Dose-Response Relationship
  - Clonogenic Endpoints
  - Functional Endpoints
  - **Inferring α/β from Multifractional Experiments**

Inferring α/β Ratio from Multifraction Experiments

- First, you need an experimental system with scorable endpoints (e.g., moist desquamation of > 50% of the area irradiated; 50% paralysis)

- Next, doses that result in the same effect (iso-effect) using various multifraction regimens must be determined experimentally



In this case, take 50% paralysis as endpoint:

n – number of fractions
d – dose per fraction
nd – total dose

is determined for for each multifractionted regimen

Inferring α/β Ratio from Multifraction Experiments

Assumptions

1. The dose response relationship is represented adequately by the LQ equation

$$S = e^{-\alpha D - \beta D^2}$$

2. Each dose in a fractionated regimen produces the same biologic effect

3. Full repair of sublethal damage takes place between dose fractions, but no cell proliferation occurs

Inferring α/β Ratio from Multifraction Experiments

Suppose total dose **D** is divided into **n** equal fractions of dose **d**, i.e., **D = nd**

Substitute D with nd,

$S = e^{-\alpha D - \beta D^2}$ can be rewritten as $S = (e^{-\alpha d - \beta d^2})^n$

$$\ln S = n(-\alpha d - \beta d^2)$$

Rearrange,

$$-\ln S / nd = \alpha + \beta d$$

Inferring α/β Ratio from Multifraction Experiments

$$-\ln S / nd = \alpha + \beta d \quad \longrightarrow \quad 1/nd = -\beta/\ln S(d) - \alpha/\ln S$$

If we plot 1/nd (i.e, reciprocal of total dose) against d (i.e, dose per fraction), we would obtain a straight line

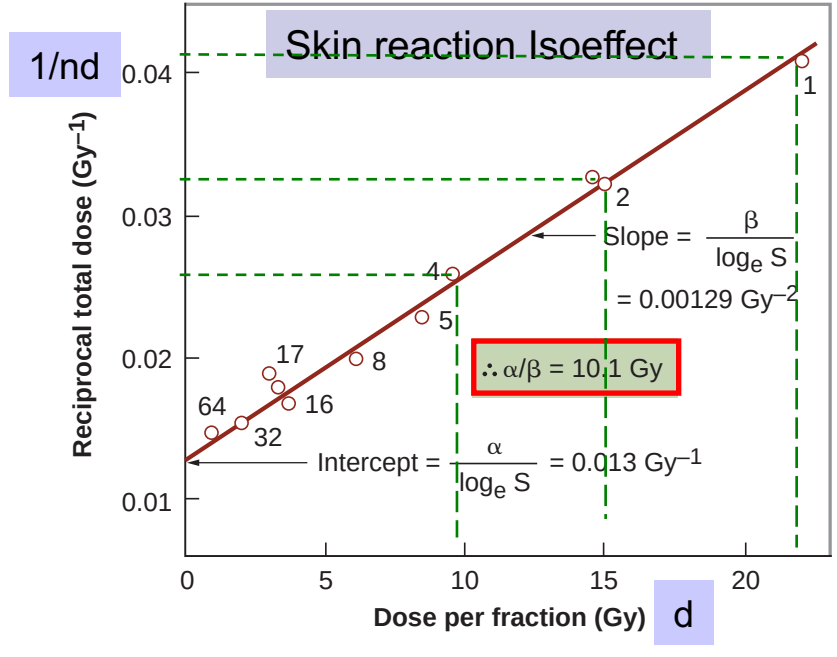
Intercept = $-\alpha/\ln S$

Slope = $-\beta/\ln S$

$$\frac{\text{intercept}}{\text{slope}} = \alpha/\beta$$

This gives an estimate of α/β ratio

Inferring α/β Ratio from Multifraction Experiments



Intercept = 0.013 **Gy^{-1}**
Slope = 0.00129 **Gy^{-2}**

⇨ α/β = 0.013/0.00129 = 10.1 Gy

Determine the total dose and dose per fraction for the **same biologic endpoint** (e.g., 50% moist desquamation) with single dose and various multifractionation regimen

↓

Plot 1/nd vs. d, and fit for a straight line

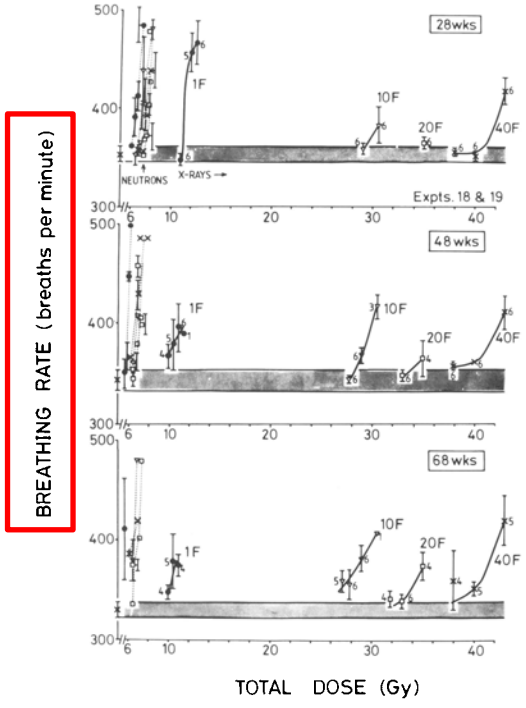
↓

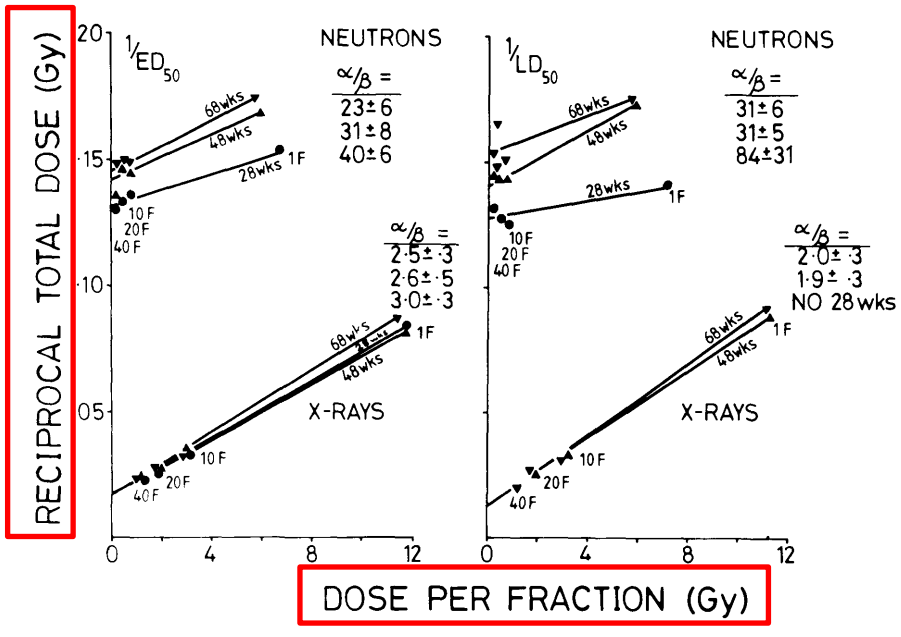
Determine intercept and slope

↓

Calculate α/β

Inferring α/β Ratio from Multifraction Experiments – Breathing Rate

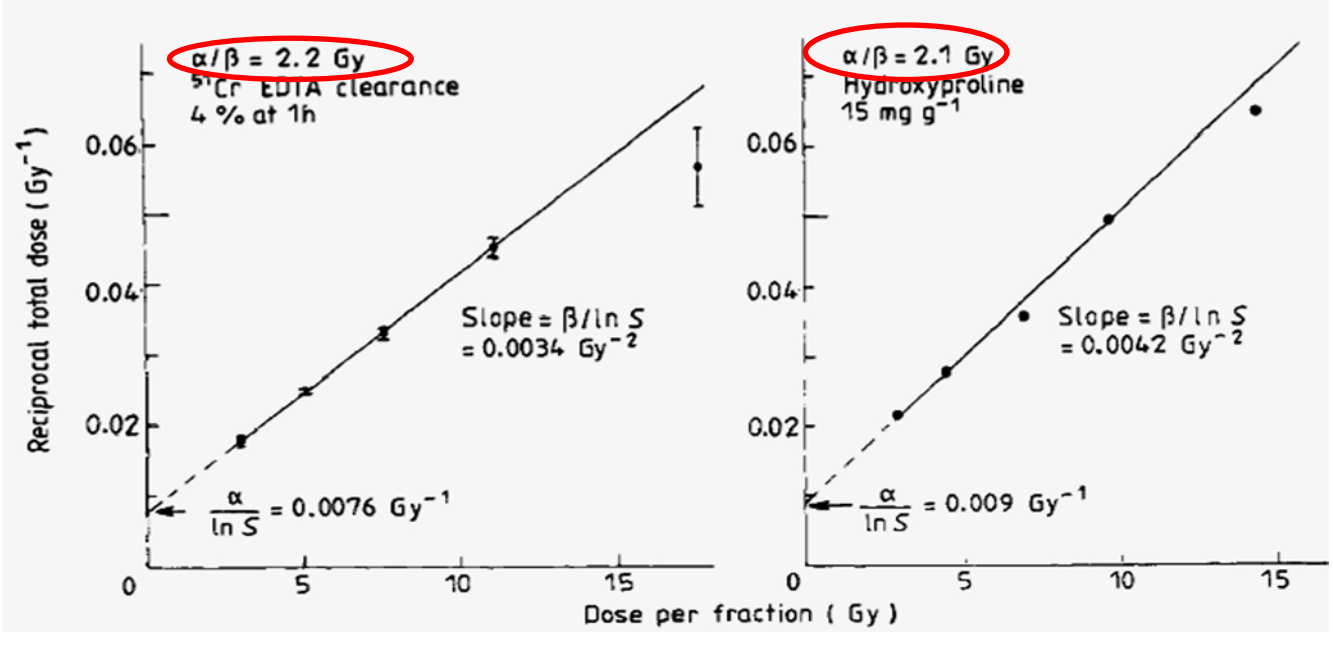




Parkins & Fowler 1985; British J of Radiology 58: 1097-1103

Inferring α/β Ratio from Multifraction Experiments

Mouse kidney function at 6-9 months



Inferring α/β Ratio from Multifraction Experiments

Table 12.1 *Values for the α/β ratio for a variety of early- and late-responding normal tissues in experimental animals*

	α/β	References
Early reactions		
Skin		
—Desquamation	9.1–12.5	Douglas and Fowler (1976)
	8.6–10.6	Joiner *et al.* (1983)
	9–12	Moulder and Fischer (1976)
Jejunum		
—Clones	6.0–8.3	Withers *et al.* (1976)
	6.6–10.7	Thames *et al.* (1981)
Colon		
—Clones	8–9	Tucker *et al.* (1983)
—Weight loss	9–13	Terry and Denekamp (1984)
Testis		
—Clones	12–13	Thames and Withers (1980)
Mouse lethality		
—30d	7–10	Kaplan and Brown (1952)
—30d	13–17	Mole (1957)
—30d	11–26	Paterson *et al.* (1952)
Tumour bed		
—45d	5.6–6.8	Begg and Terry (1984)
Late reactions		
Spinal cord		
—Cervical	1.8–2.7	van der Kogel (1979)
—Cervical	1.6–1.9	White and Hornsey (1978)
—Cervical	1.5–2.0	Ang *et al.* (1983)
—Cervical	2.2–3.0	Thames *et al.* (1988)
—Lumbar	3.7–4.5	van der Kogel (1979)
—Lumbar	4.1–4.9	White and Hornsey (1978)
	3.8–4.1	Leith *et al.* (1981)
	2.3–2.9	Amols, Yuhas (quoted by Leith *et al.*, 1981)
Colon		
—Weight loss	3.1–5.0	Terry and Denekamp (1984)
Kidney		
—Rabbit	1.7–2.0	Caldwell (1975)
—Pig	1.7–2.0	Hopewell and Wiernik (1977)
—Rats	0.5–3.8	van Rongen *et al.* (1988)
—Mouse	1.0–3.5	Williams and Denekamp (1984a, 1984b)
—Mouse	0.9–1.8	Stewart *et al.* (1984a)
—Mouse	1.4–4.3	Thames *et al.* (1988)
Lung		
—LD_{50}	4.4–6.3	Wara *et al.* (1973)
—LD_{50}	2.8–4.8	Field *et al.* (1976)
—LD_{50}	2.0–4.2	Travis *et al.* (1983)
—Breathing rate	1.9–3.1	Parkins and Fowler (1985)
Bladder		
—Frequency, capacity	5–10	Stewart *et al.* (1984b)

α/β values are in Gy.
From Fowler (1989); for references, see the original.

Inferring α/β Ratio from Multifraction Experiments

Table 5. Ratio of linear to quadratic terms from multifraction experiments.

	α/β (Gy)
Early reactions	
Skin	9–12
Jejunum	6–10
Colon	10–11
Testis	12–13
Callus	9–10
Late reactions	
Spinal cord	1.0–4.9
Kidney	1.5–2.4
Lung	2.4–6.3
Bladder	3.1–7

α/β tends to be larger for early responding tissues, about 10 Gy, than for late responding tissues, about 2 Gy

We will discuss the clinical implication in Chapter 23

Summary

- A number of assays have been developed that have all pointed to the existence of standard survival curve-like dose-responses *in vivo*
- These assays have permitted the assessment of normal tissue toxicity effects and extrapolation to human exposures
- Isoeffect curves can be used to establish a dose vs. dose/fraction relationship that is different for early- and late-responding tissues

Review Questions

Question 1

The shape of a tumor control probability curve for a series of identical tumors, as a function of total dose above a particular threshold, would best be described as:

A. parabolic

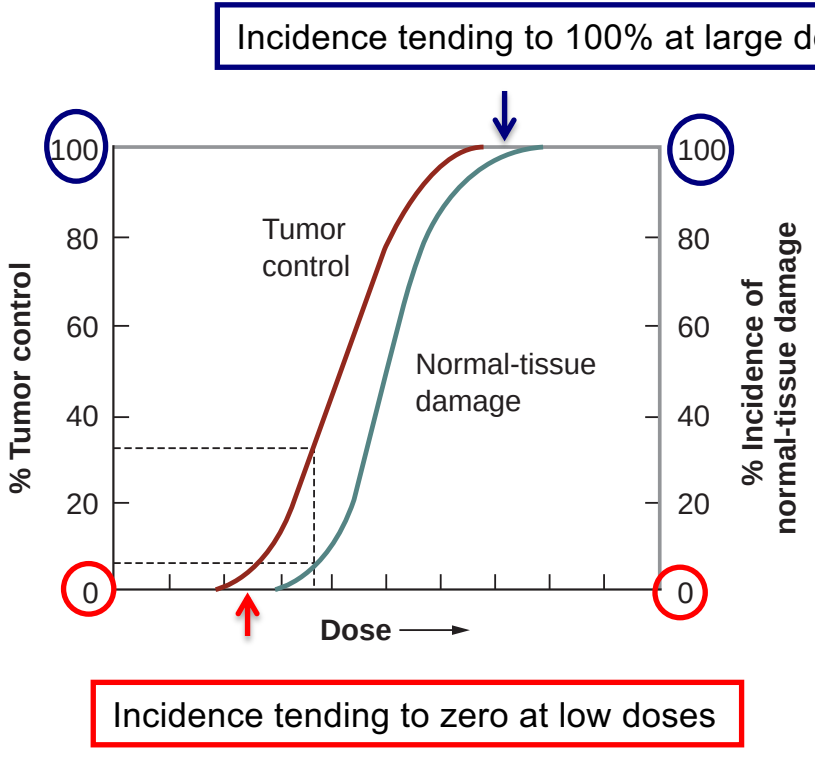
B. sigmoidal

C. linear

D. bell-shaped

E. linear-quadratic

Dose-Response Relationship



The dose-response curves typically have a **sigmoidal (S) shape** for both tumor control and normal-tissue complications

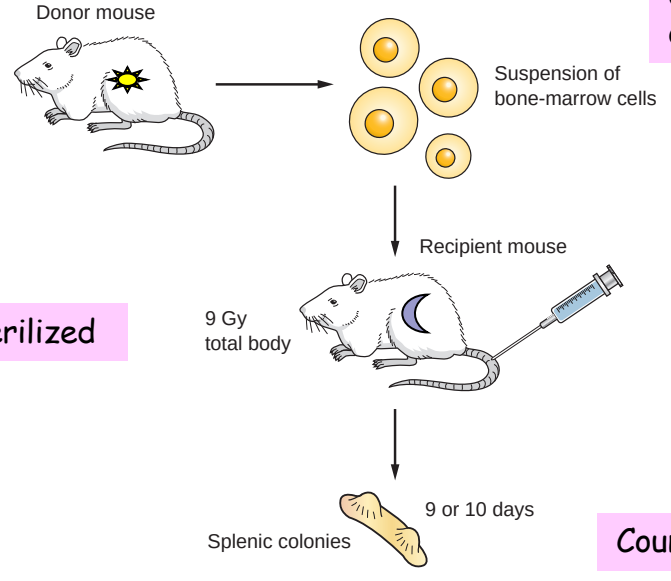
Question 2

The spleen-colony assay:

A. is used to measure hepatocyte radiosensitivity
B. requires roughly three weeks to permit spleen cells to form colonies in recipient animals
C. was used to demonstrate that the radiation survival curve for intestinal crypt cells was linear-quadratic in shape
D. has been used to determine the radiation survival curve for bone marrow stem cells
E. requires that the recipient animal be given a sublethal dose of radiation

Bone Marrow Stem Cells

Irradiated with to some test dose



Contain a small proportion of stem cells

Spleen cells sterilized

Count the # of colonies

Till & McCulloch's Spleen Colony Assay

Question 3

Which of the following *in vivo* assays of radiation response does NOT depend on a functional endpoint?

A. LD50

B. skin nodule formation

C. myelopathy

D. breathing rate

E. cognitive impairment

Clonogenic End Points

- Clones Regrowing *in Situ*
  - Skin Colonies
  - Crypt Cells of the Mouse Jejunum
  - Testes Stem Cells
  - Kidney Tubule

  } Withers et al.

- **Cells Transplanted to Another Site**
  - Bone Marrow Stem Cell → Till & McCulloch
  - Mammary Cells
  - Thyroid Cells

  } Clifton & Gould

Functional Assays

- Not a direct measure of cell survival, but direct relevance to clinical side effects
- Examples include
  - Pig Skin
  - Rodent Skin

  } Fowler
  - Early and Late Response of the Lung → Travis
  - Spinal Cord Myelopathy → van der Kogel

Question 4

A major limitation of in-situ colony formation assays for normal tissues, such as the testes clonogenic assay developed by Withers and his collaborators, is that they:

A. are not useful for doses less than roughly 5 Gy

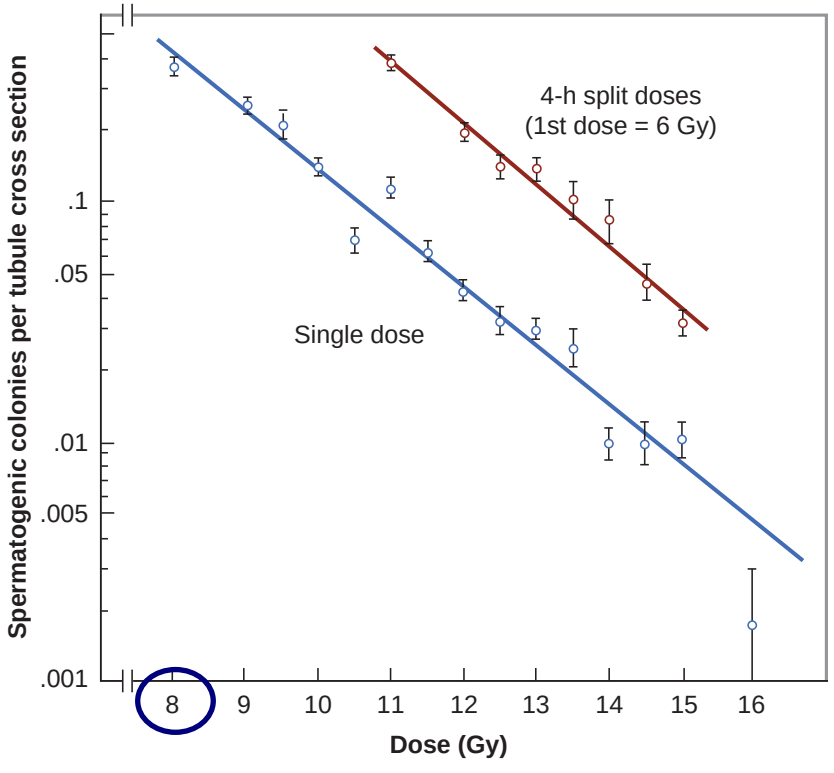
B. are not able to provide estimates of the D_0

C. require explanting cells from the irradiated tissue

D. measure functional endpoints, not cell survival

E. primarily reflect radiation response of vascular endothelial cells

Testes Stem Cells



D_0 = 1.68 Gy

D_q = 2.7 Gy

⇦ Note that a relatively high single dose of 8-16 Gy are necessary to score individual surviving colonies