Normal Tissue Responses to Radiation

Normal Tissue Responses to Radiation

- In clinical RT the total dose of radiation that safely can be administered is limited not by the sensitivity of the tumor itself, but by the risk of morbidity arising in those normal tissues unavoidably included within the treatment volume

Normal Tissue Responses to Radiation

- In cellular radiobiology, radiation effects measured in terms of the capacity of individual cells to exhibit infinite proliferation, defined as clonogenicity
- Normal tissue responses can be viewed in the same manner; normal tissue injury will reflect the loss of a particular target cell population as these cells divide

Normal Tissue Responses to Radiation

Normal tissue response to radiation classified on the time taken to exhibit clinical injury

- Acute responding tissues: express injury during or within 2-3 weeks of the completion of radiotherapy e.g., skin, oral mucosa

- Late responding tissues: express injury several months to years after irradiation e.g., kidney, lung, CNS

Pathogenesis of Radiation-induced Normal Tissue Injury

Vascular hypothesis: Rubin and Casarett (1968)

States that late radiation effects are caused by damage to blood vessels. This vascular injury, with a long latency reflecting the slow turnover time of the vasculature, leads to vessel occlusion, ischemia, and secondary loss of parenchymal cells

Parenchymal hypothesis: Withers (1979)

Both acute and late effects result directly from cell depletion; the rate of development of injury depends upon the rate at which cells in the tissue normally divide.

Measuring Normal Tissue Responses to Radiation

Clonogenic assays

- Endpoint depends directly on the reproductive integrity of individual cells, based on techniques developed by Withers and colleagues. These systems are directly analogous to cell survival *in vitro*

Measuring Normal Tissue Responses to Radiation

Functional endpoints

- These are reproducible, quantitative endpoints; tend to reflect the **minimum number of functional** cells remaining in a tissue or organ, rather than the number of clonogenic cells

Measuring Normal Tissue Responses to Radiation

Models of dose-response curves

- Infer dose-response curves by assuming the shape of the dose-response curve and performing a series of multifraction experiments. Allows generation of values for α/β for normal tissues in which these parameters cannot be directly measured

Clonogenic Endpoints

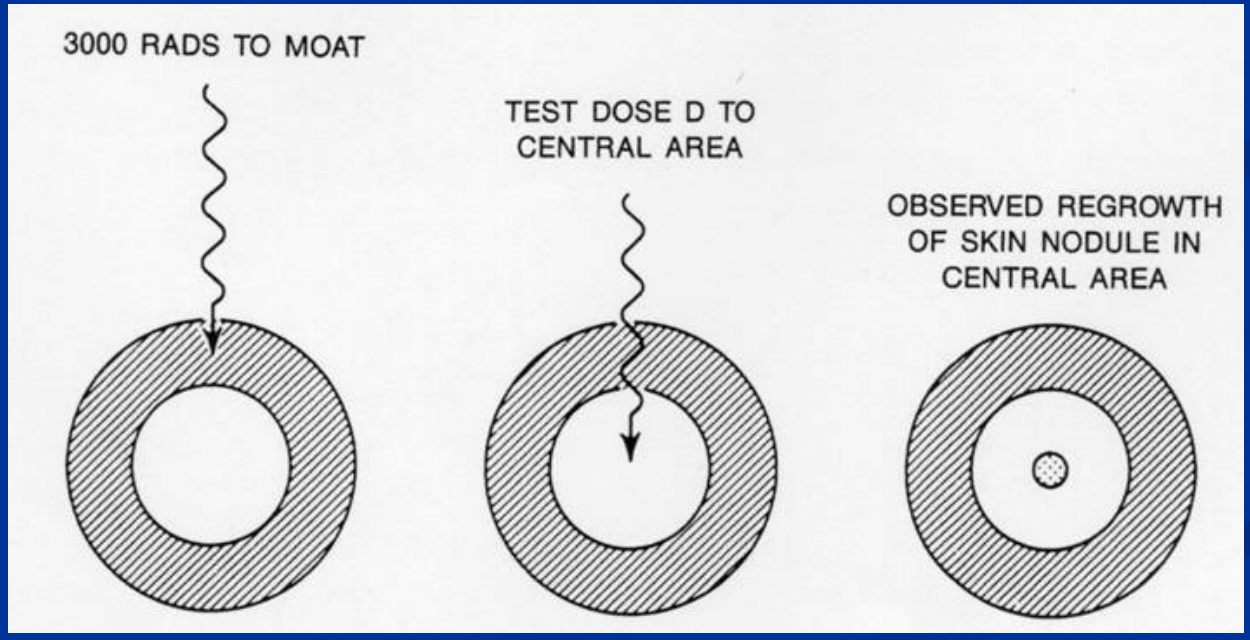
Clones regrowing in situ.

Skin: Withers (1967) determined the survival curves for mouse skin cells *in vivo*

- Hair plucked from an area on the back of a mouse, and a superficial (30 kV) x ray machine used to irradiate an annulus of skin to ~30 Gy
- This produced a “moat” of dead cells, in the center of which was an island of intact skin protected during irradiation by a metal sphere (ball-bearing)
- Moat of dead cells used to prevent migration of viable skin cells into the irradiated area during the time course of the study

Clonogenic Endpoints

- The small area irradiated with a test dose (D Gy) and subsequently observed for skin regrowth
- If ≥1 stem cells survived, a nodule of recovering cells could be seen later.

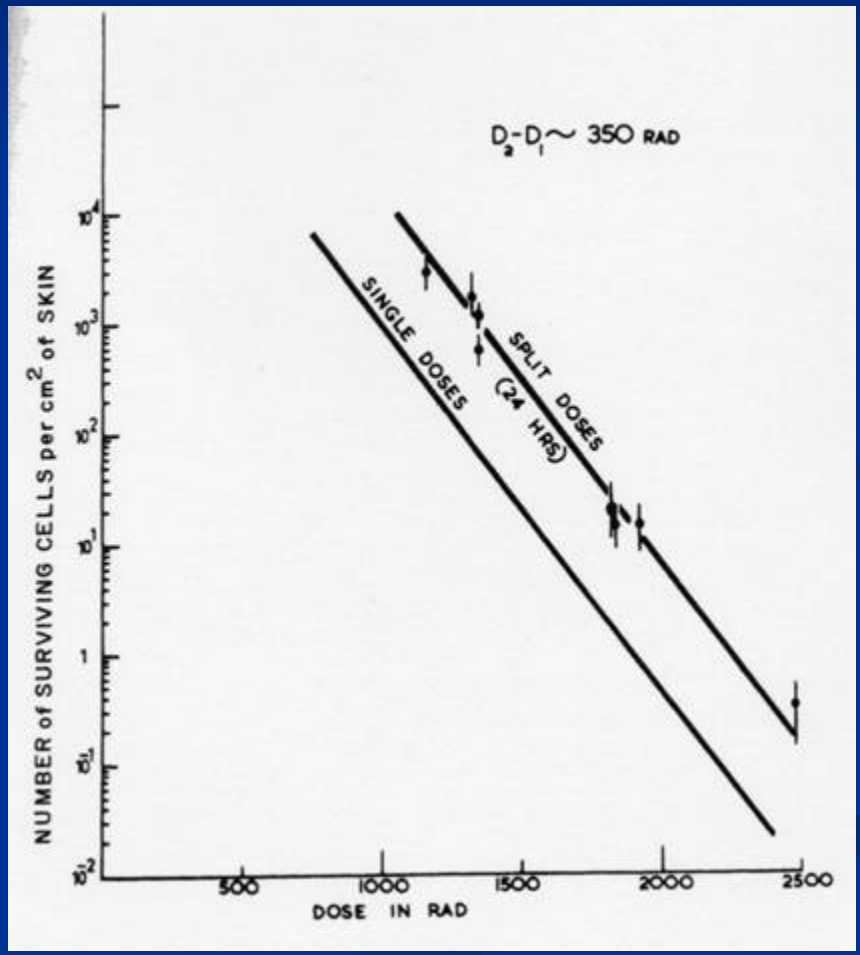


Clonogenic Endpoints

Obtained a straight cell survival curve, D_0 = 1.35 Gy

Some limitations to the technique

- Cannot irradiate too large an area on the back of the mouse to produce the moat
- Even 30 kV x rays scatter, therefore field cannot be too small



Clonogenic Endpoints

Cannot directly obtain values of *n* (extrapolation number), because don't know how many skin stem cells are present/unit area, and thus cannot convert data to SF

However, can calculate *n* indirectly by obtaining a survival curve for doses given in 2 fractions separated by 24 hours; measures repair of SLD

This allows calculation of D_q, where

$$\mathbf{D_q = D_o \log_e (n)}$$

$$\mathbf{and \log_e n = D_q/D_o}$$

Clonogenic Endpoints

Mouse jejunal crypt cells

Withers and Elkind (1969): allows determination of survival characteristics of crypt cells

Mice receive TBI (11-16 Gy), sterilize all dividing cells in the crypt, *has no effect on non-dividing differentiated cells*

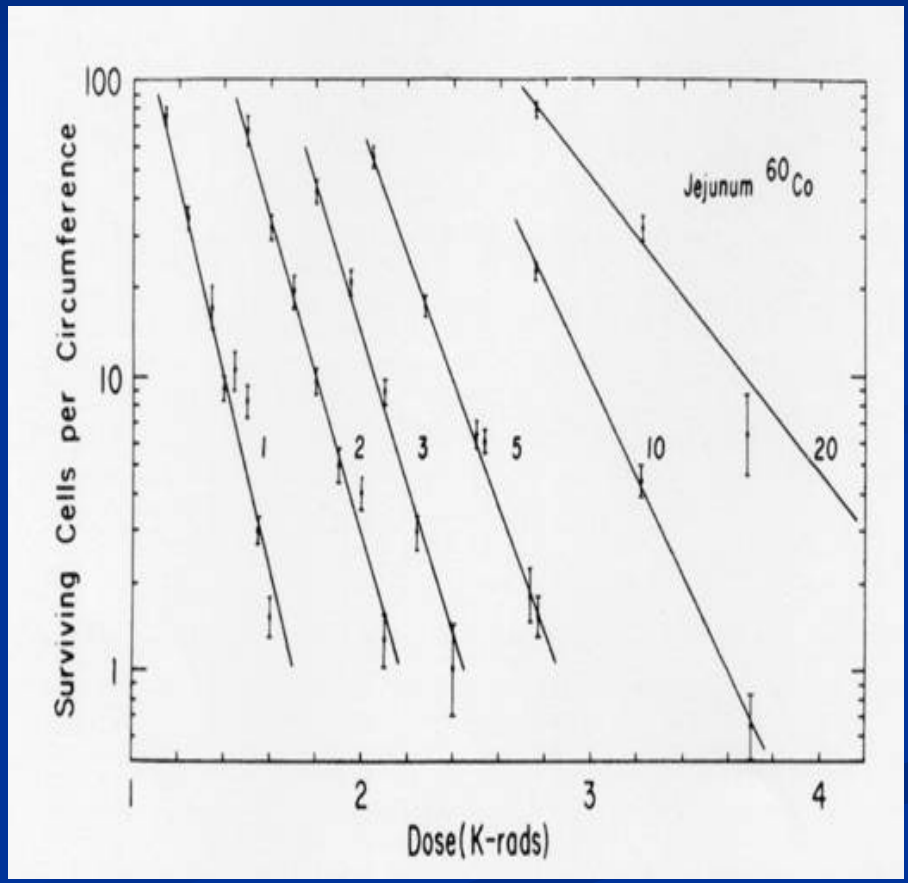
After 3 days, animal sacrificed and sections made of the jejunum. At this time the crypts are just starting to regenerate and it is relatively simple to see them

Radiation damage scored as number of regenerating crypts/circumference. Plot as a function of dose, and yields the survival curve

Clonogenic Endpoints

Limitations:

- Don’t plot the SF, but number of surviving crypts
- Experiments can only be done at doses of ≈ 10 Gy or more, as need sufficient level of biological injury to be able to quantitate.

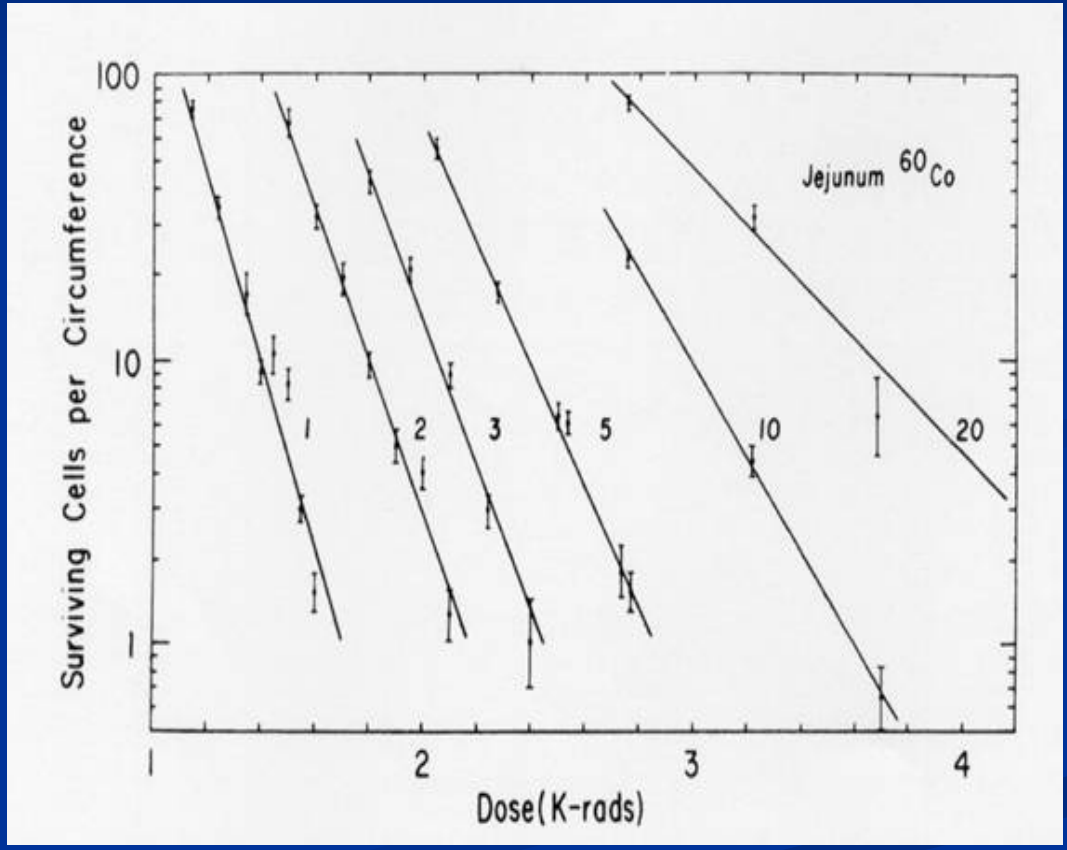


Clonogenic Endpoints

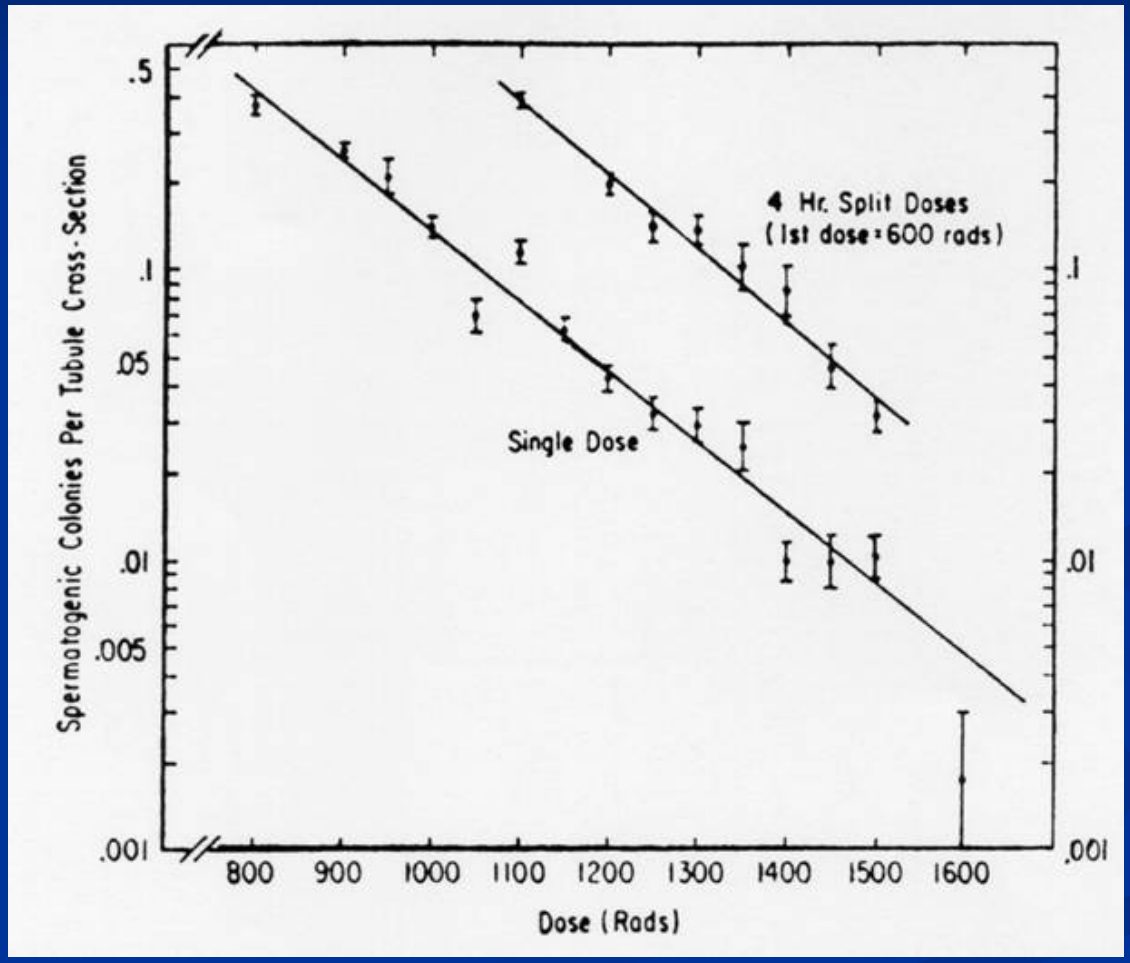
- Can give high doses by using a large number of small fractions, as long as the total dose gives the biological response

Assume

- Each dose/fraction gives same level of cell kill
- Certain number of clonogens/crypt



Clonogenic Endpoints



Clonogenic Endpoints

Cells transplanted to Different Site/Transplantation Assays

- Single-cell suspensions made from irradiated tissue and transplanted to suitable sites in immunologically identical animal.

Clonogenic Endpoints

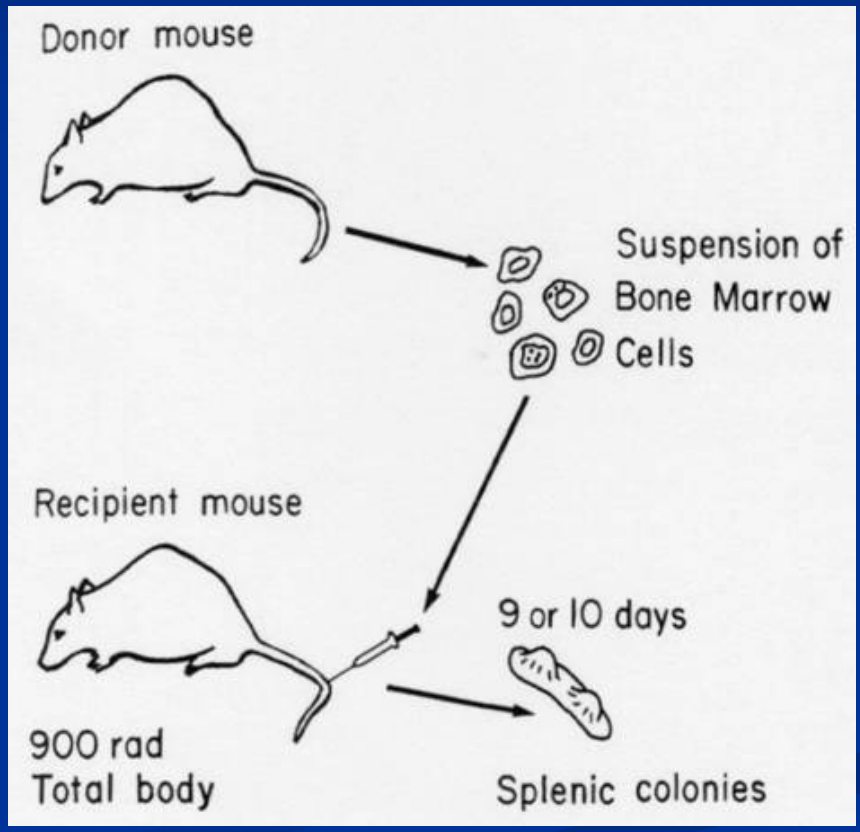
Till and McCulloch (1961)

Developed technique to determine the survival curve for colony-forming bone marrow cells. Requires a *donor* and a *recipient* mouse.

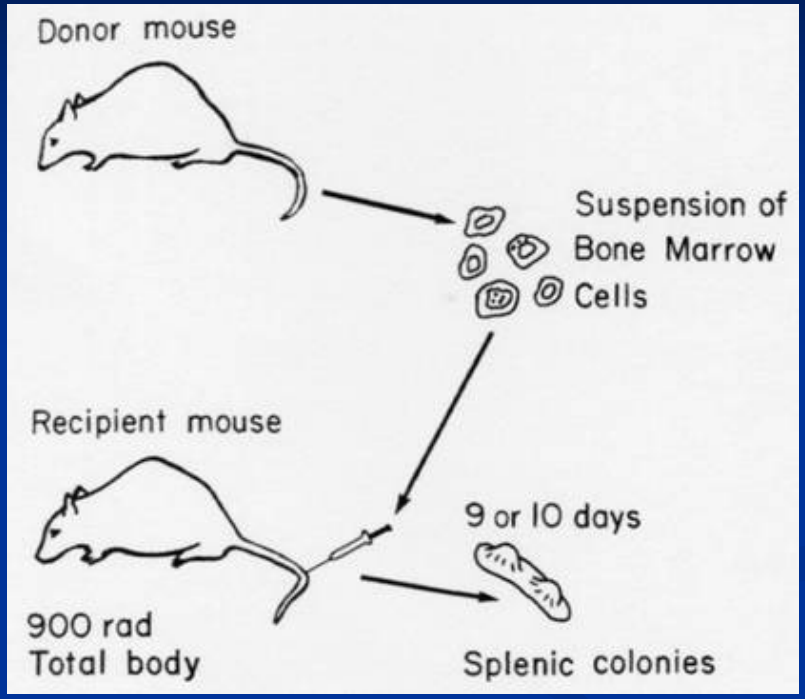
Donor: used to withdraw suspension of nucleated isologous bone marrow cells. A known number of these are injected into the recipient mouse.

Recipient: receives TBI (9 Gy), sterilizes the spleen. Then injected with donor cells, some of which lodge in the spleen and form colonies.

Spleen removed 9-10 days later, and colonies counted.



Clonogenic Endpoints



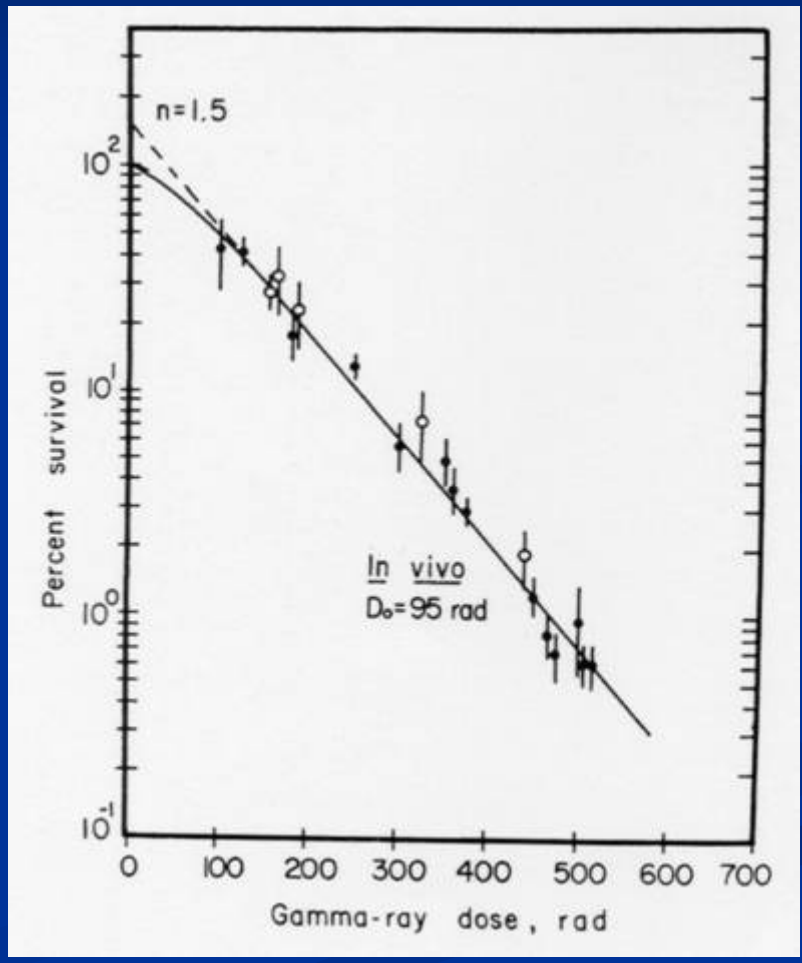
Majority of donor cells are fully differentiated cells and will not form colonies, thus require $\approx 10^4$ cells per colony

SF for a dose D = Colonies counted / Cells innoculated x PE

PE = plating efficiency, # cells required to produce a colony in an unirradiated animal.

Clonogenic Endpoints

- Repeat the process for a range of doses, and obtain a survival curve
- These bone marrow cells are the most sensitive mammalian cells to die a mitotic death
- D_0 is ~ 0.95 Gy, with little or no shoulder



Dose Response Relationships for Functional End-Points

- Can use essentially any functional end-point to determine tissue's response to radiation, providing the endpoint can be reproducibly quantified/assessed
- One of the most widely used systems in experimental radiobiology has been the skin
- Advantages: relatively simple system, easy to assess injury

Dose Response Relationships: Pig Skin

- The use of pig skin was pioneered by Fowler and colleagues (1963)
- Fields were tattooed onto the flank of pigs and irradiated with graded doses of X rays
- Reactions were scored daily using an arbitrary scale

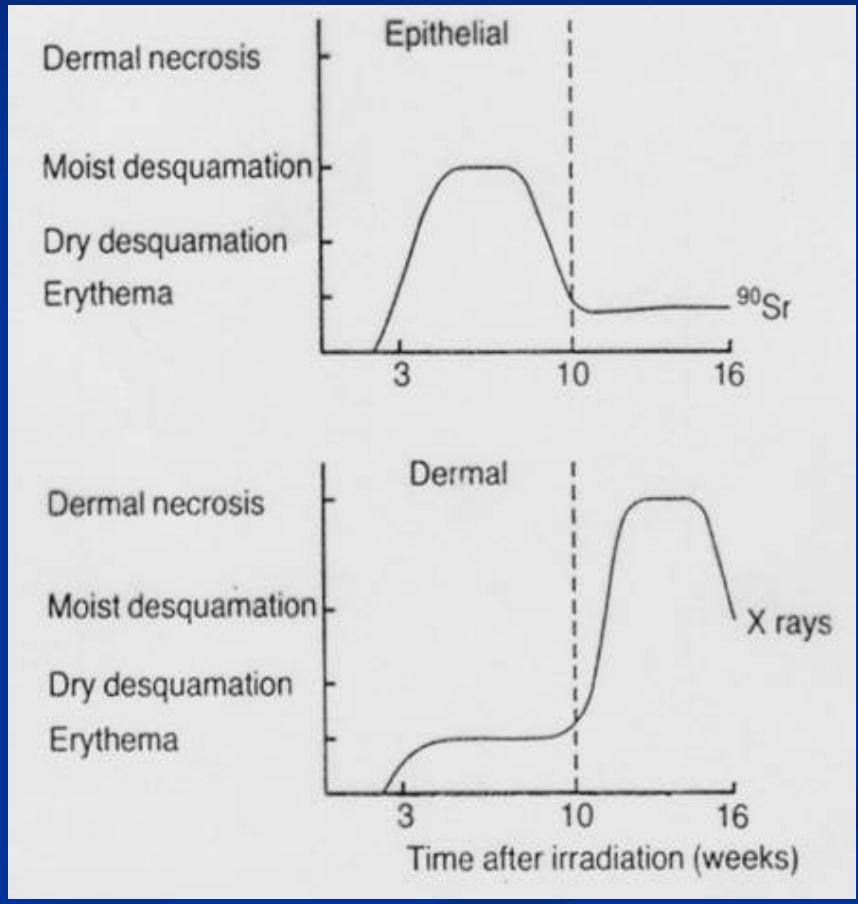
Dose Response Relationships: Pig Skin

ARBITRARY SCORE	REACTION
0	No visible reaction
1	Faint erythema
2	Erythema
3	Marked erythema
4	Moist desquamation of < 50% of irradiated area
5	Moist desquamation of > 50% of irradiated area

Dose Response Relationships: Pig Skin

- Due to statistical difficulties in analyzing such non-parametric ordinal data, a scoring system based purely on the incidence of a particular reaction has been developed in Oxford by Hopewell *et al*

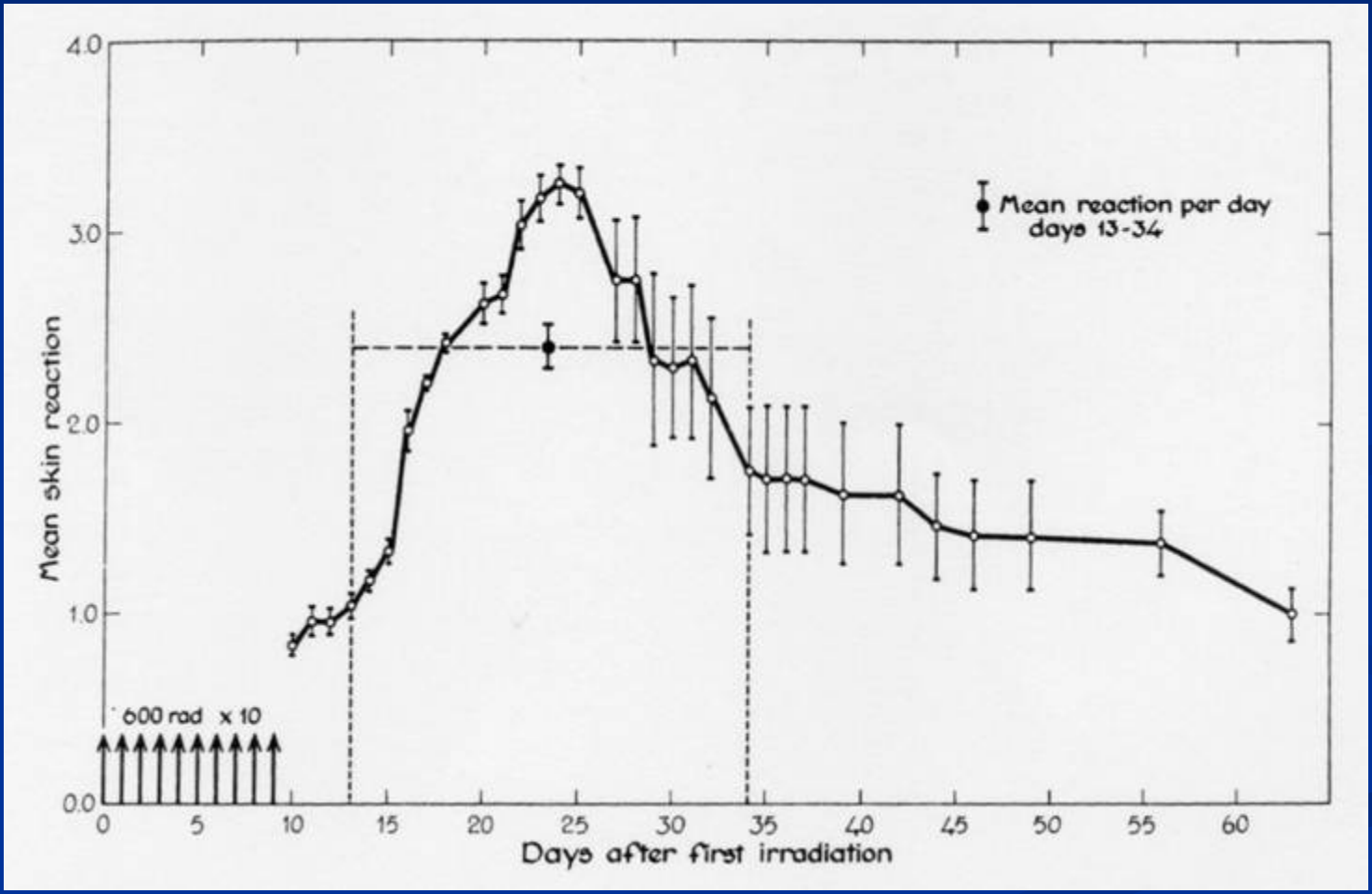
- Response of pig skin is biphasic.



Dose Response Relationships: Pig Skin

- Initial response erythema, can be followed by moist desquamation. Always seen approx. 5 weeks PI
- Second wave: Dusky-mauve erythema, and dermal necrosis, seen approx. 10-16 weeks PI
- Major disadvantage: large size of animal, expensive to keep
- Alternative is to use rodents, e.g. use mouse leg/foot
- Usually irradiate one hind-limb, other serves as control. Score daily using arbitrary numerical scale.

Dose Response Relationships: Mouse Skin

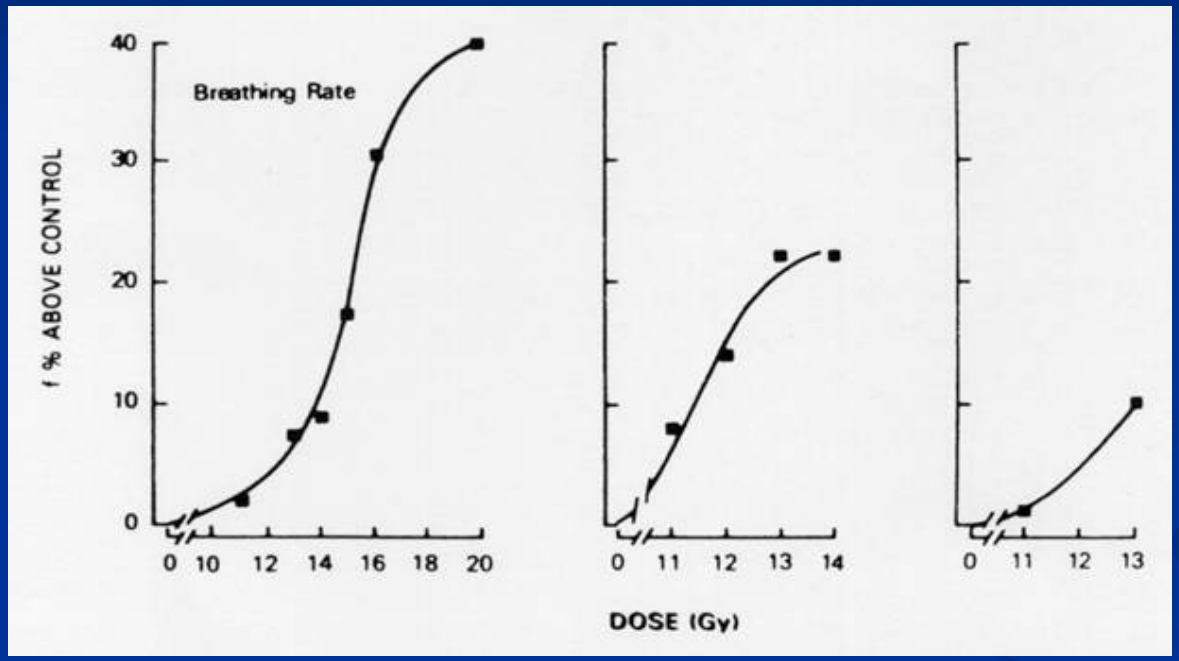


Dose Response Relationships: Mouse Lung

- Travis *et al* (1980) developed a non-invasive assay of breathing frequency to assess early and late damage to mouse lung

- Observed that breathing rate ↑ progressively with dose following a threshold of ~11 Gy

Dose Response Relationships: Mouse Lung

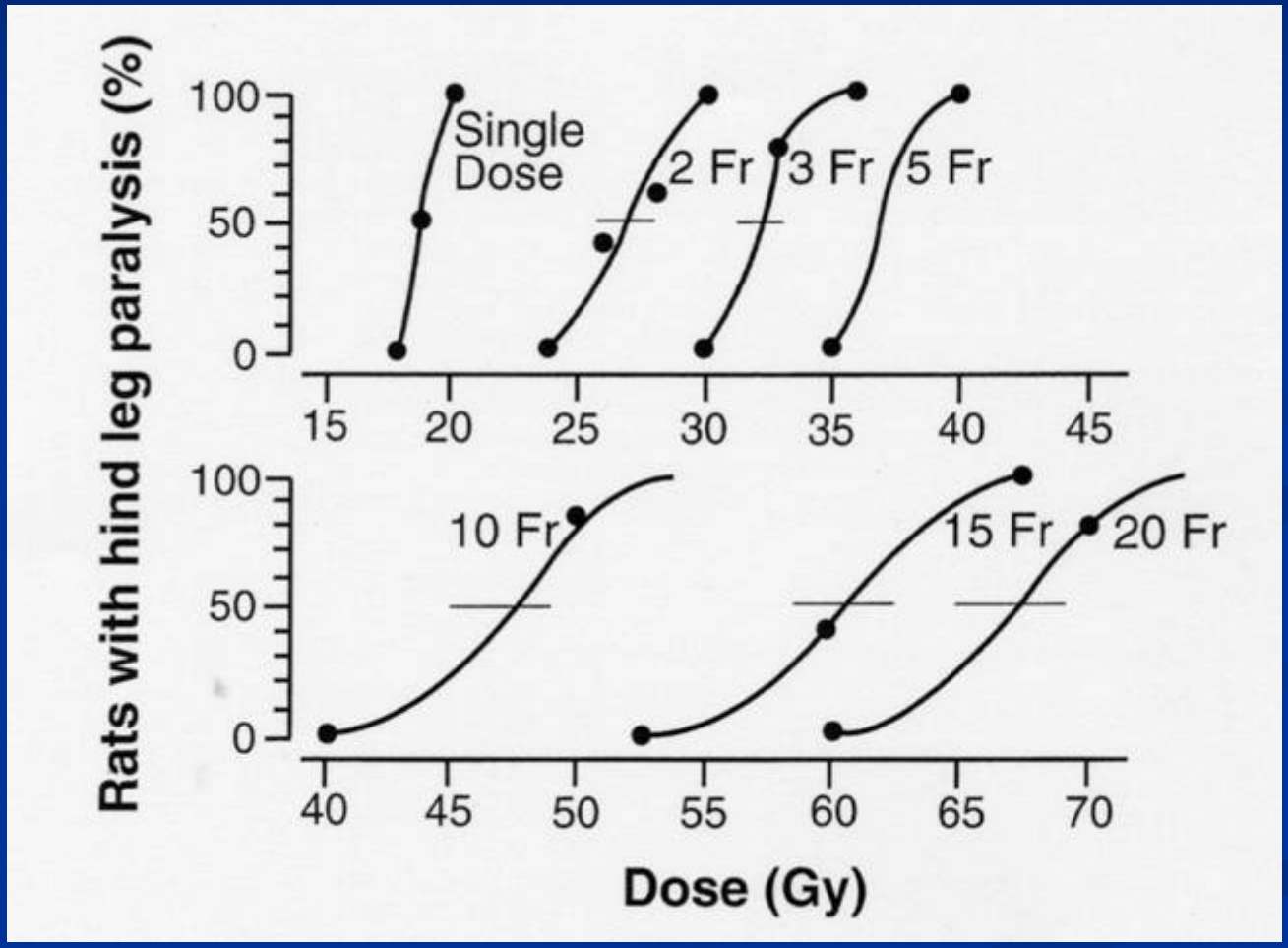


- Between 14-24 weeks PI increased frequency reflects early pneumonitic phase
- By 52 weeks the increased frequency is associated with the late fibrotic phase

Dose Response Relationships: Rat Spinal Cord

- Dose-response curves can be generated following local irradiation of the rat spinal cord (van der Kogel 1980)
- After latent periods of ~ 4-12 months animals start to exhibit signs of radiation myelopathy e.g. hind limb paralysis
- Can construct dose-response curves by plotting the incidence or probability against dose

Dose Response Relationships: Rat Spinal Cord



Determining α/β from Multifraction Experiments

- Can determine parameters of dose response curve for any normal tissue system in which a functional end point can be observed by performing a multifraction experiment
- Determine total dose required to give a defined biological response following multifraction irradiation
- Assume: ***Dose-response relationship is adequately represented by the L-Q formula***

Determining α/β from Multifraction Experiments

$$S = e(-\alpha D - \beta D2)$$

where S = SF cells following Dose D, α and β are constants

Experimental studies have shown that each successive fraction in a fractionated regimen is equally effective, so that the effect (E) of *n* fractions can be expressed as

$$E = \alpha D + \beta D2$$

if D is delivered in *n* fractions of dose *d* then this becomes

$$E = n(\alpha d + \beta d2)$$

Determining α/β from Multifraction Experiments

Can be rewritten as the following terms

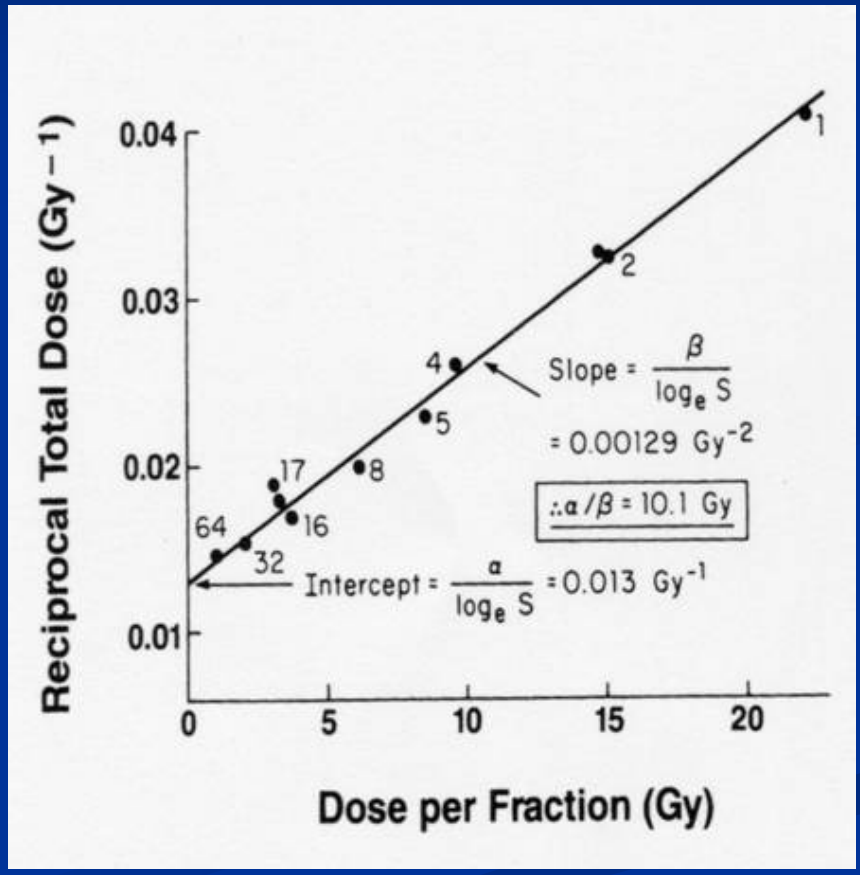
Equation 1: $1/D = (\alpha/E) + (\beta E)\, d$

Equation 2: $1/n = (\alpha/E)d + (\beta E)d^2$

Fe-Plot (Douglas & Fowler 1976)

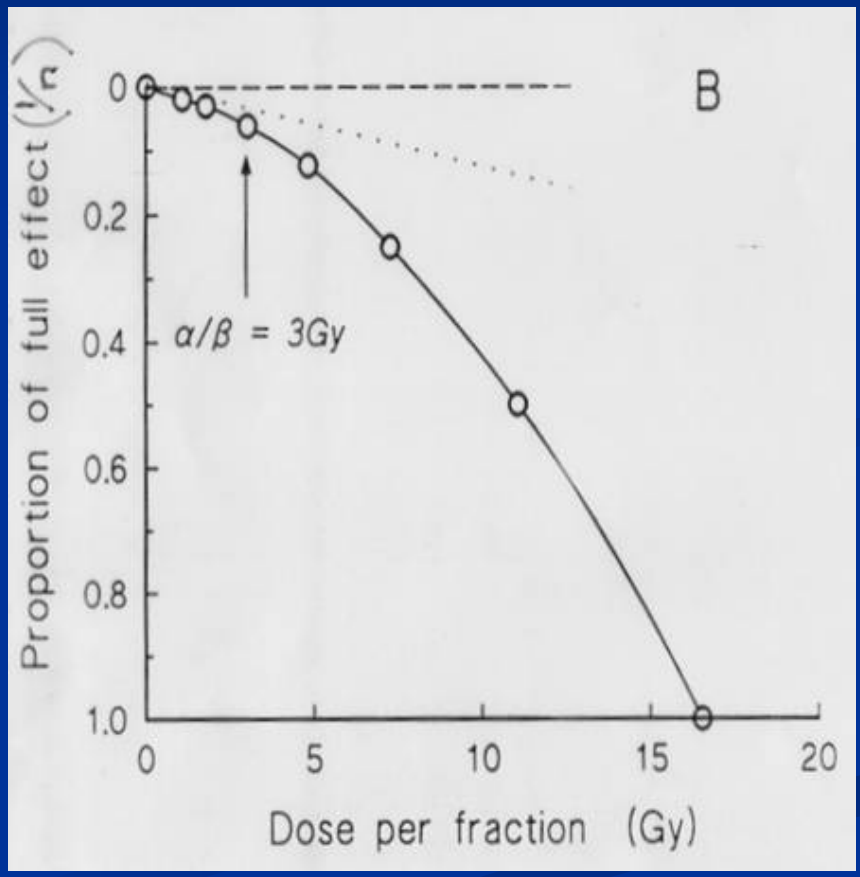
Analyze dose-response curves by

1. Plotting effect vs. total dose
2. Determine iso-effect doses for each fractionation regimen
3. Plot 1/D against corresponding *d*/F
4. Should give a straight line with slope of β/E and intercept on the *y* axis of α/E
5. Where line cuts the *x* axis = -α/β



Alternatively:

1. Plot 1/*n* vs. *d*/F
2. Gives shape of continuously bending cell survival curve
3. Preferred statistically since 1/*n* and *d*/F axes are independent



Volume Effect

- In clinical RT, volume of tissue irradiated is an important factor determining the clinical tolerance of an organ
- This can be very different from the tissue radiosensitivity
- Kidney and lung are among the most radiosensitive organs when the total volume irradiated; $TD_5 \approx 20$ Gy in 2 Gy fractions
- However, if treat small volumes can use much higher doses, reflects the marked reserve capacity of these organs

Volume Effect

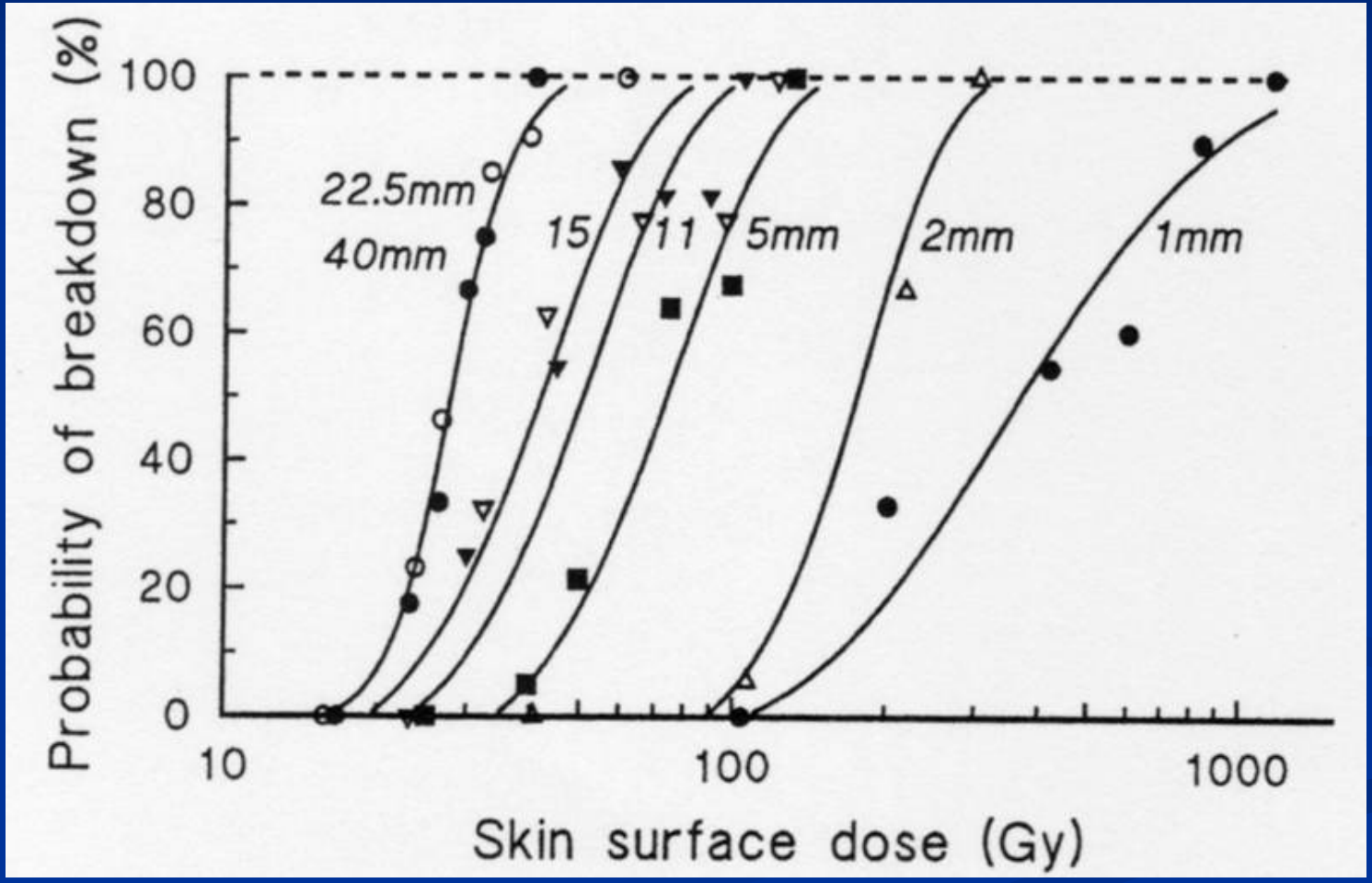
- Organ radiosensitivity depends largely on its arrangement of FSUs, and ability of these FSUs to migrate
- Tissues with a high migratory capacity include skin, mucosa, and GI tract. Small volumes can be treated to relatively high doses, as repopulation will readily occur from outside the irradiated volume
- However, once critical distance reached (migration no longer possible) will see cell loss without adequate replacement
- Above a certain dose will see damage to stroma and vasculature, which may prevent regeneration

Volume Effect

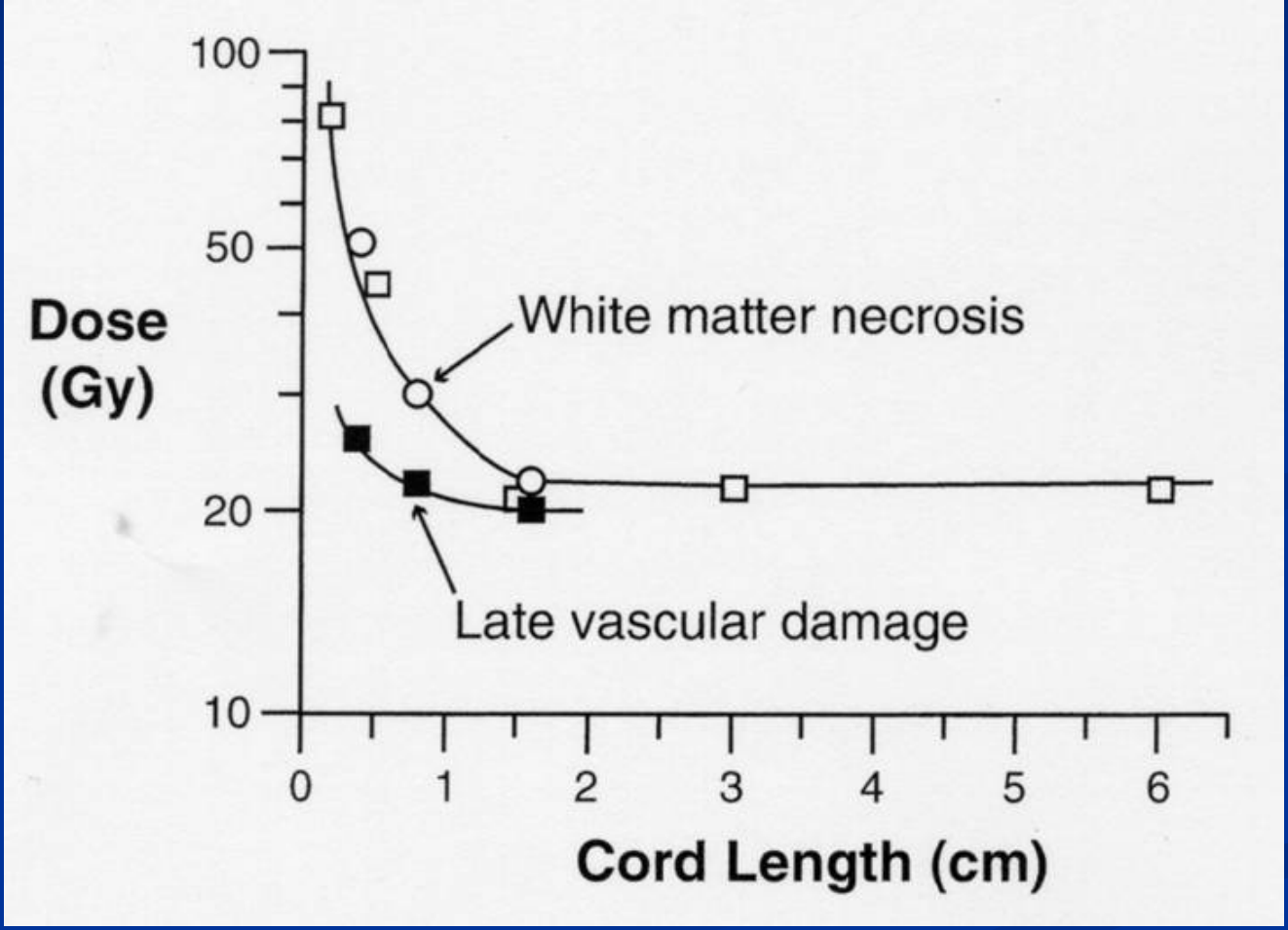
Serial arrangement:

- Integrity of each FSU critical to organ function, elimination of only one resulting in a measurable probability of a complication
- A good example is the spinal cord. Specific functions are controlled by specific segments arranged linearly. Since impulses need to pass along cord, loss of critical cells in any one segment will result in complete failure of the cord
- Thus, as field size increases, the probability of complications increases steeply; the probability of complications is only related to the total irradiated volume
- Not the case for small volumes; here cell migration plays a dominant role

Volume Effect in Pig Skin



Volume Effect in Rat Spinal Cord

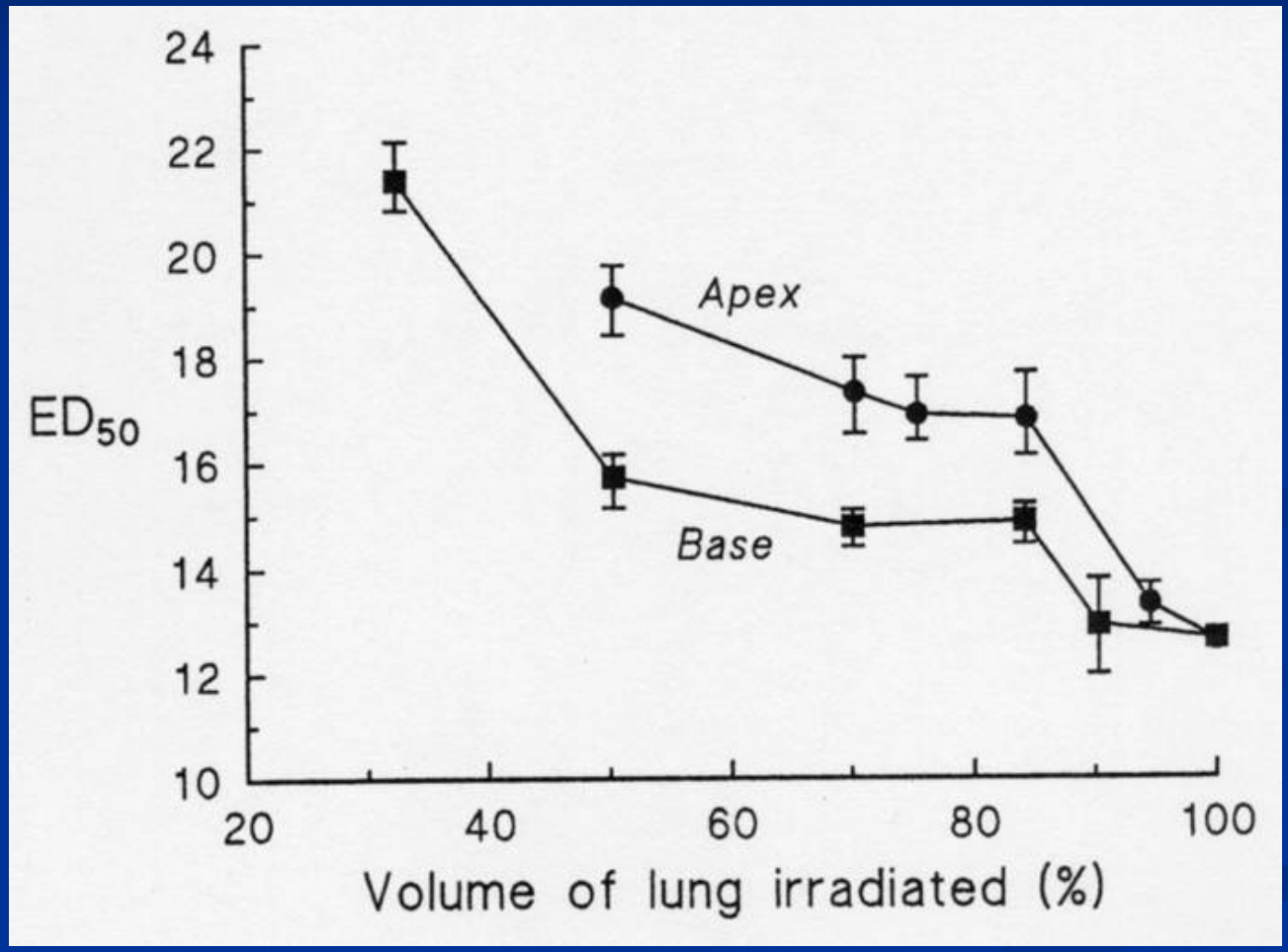


Volume Effect

Parallel arrangement:

- Organs such as kidney and lung, can lose large numbers of FSUs before see marked loss of function

Volume Effect in Mouse Lung



Proliferative Organization of Normal Tissues

- Cell proliferation in normal tissues is highly organized, with cell production under tight homeostatic control
- In adult tissues under non-pathologic conditions cell production is exactly balanced by loss of differentiated mature cells
- Number maintained by proliferative activity of precursor cells, i.e. cells which serve to replace those cells lost due to normal "wear and tear"

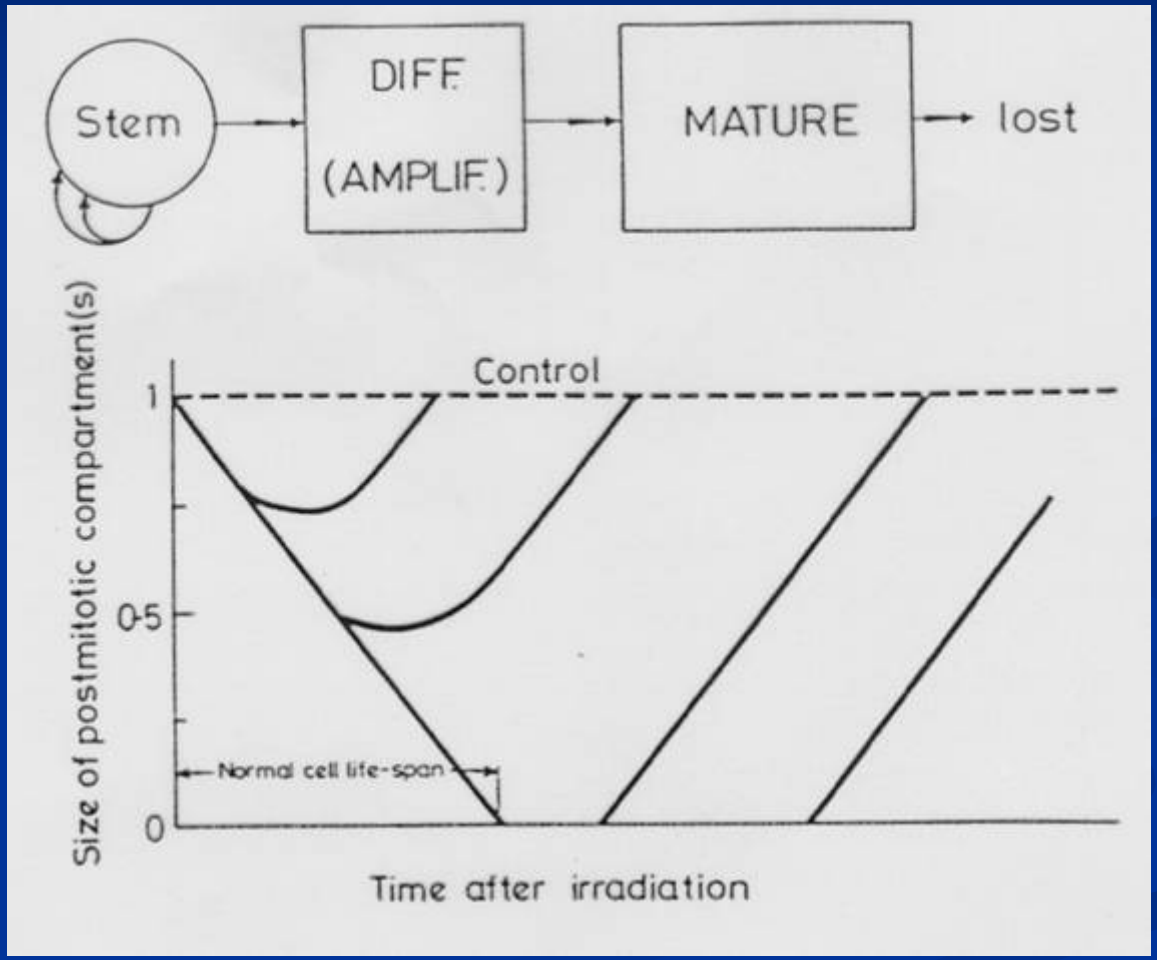
Proliferative Organization of Normal Tissues

- The degree of organization of cells within proliferative and functional compartments has been used to distinguish between two categories of tissues, *hierarchical* and *flexible* (Michalowski 1981)

Hierarchical or H-type Tissues

- Clearly recognizable separation between the stem cell compartment, an amplification compartment (usually proliferating rapidly), and a post-mitotic non-dividing compartment of mature functional cells
- Mostly rapidly renewing cell systems: include hematopoietic tissues, skin epidermis, GI tract mucosa and testicular epithelium

Hierarchical or H-type Tissues



Hierarchical or H-type Tissues

- Capacity for clonogenic self-replication restricted to a small subset of cells called stem cells; not functioning cells
- Mature functioning cells develop from stem cells by a process of differentiation during which intermediate transit cells progressively lose their capacity for proliferation while their functional capacity increases

Hierarchical or H-type Tissues

- Mature cells are lost from the tissue at a characteristic rate as a result of wear and tear
- Numbers maintained by a continuous influx of stem cells into the differentiation pathway
- Stem cell number is maintained by self-replication; when not proliferating, the stem cells reside in a quiescent G_0 state

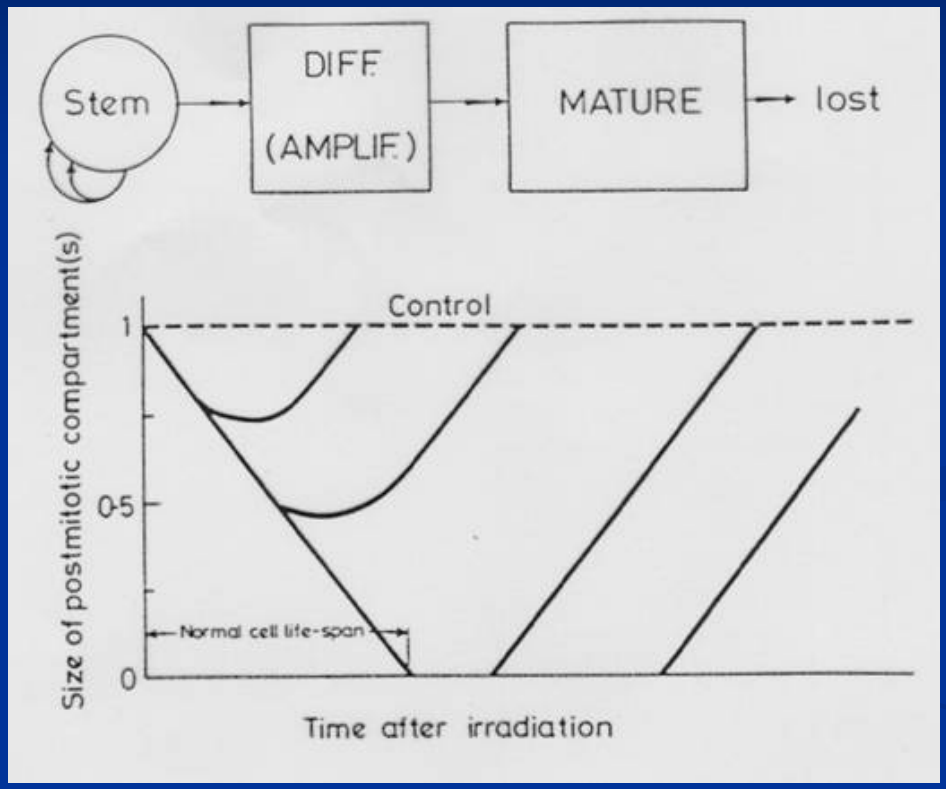
Radiation Response of H-type Tissues

- After irradiation stem cells and cells in transit compartment will die at mitosis; mature functional cells will not be damaged by irradiation
- Causes reduced flow of cells from the stem-transit compartment leading to a reduction in the number of mature cells
- Since the life-span of the post-mitotic functional cell is limited, depletion becomes evident shortly after irradiation, clinically expressed as an early or acute response

Radiation Response of H-type Tissues

- Since under steady-state conditions the rate of flow is constant, depopulation of the post-mitotic pool starts immediately; will proceed linearly, and be **dose-independent**
- Time to reach compete depopulation also **dose-independent**, dependent on the length of mature cell longevity

Radiation Response of H-type Tissues



- At low doses, some stem cells will survive and initiate regeneration; the time required to reach a certain level of depletion will therefore be **dose-dependent**
- With higher doses, see increased stem cell kill

Regeneration Response of H-type Tissues

- Unless all stem cells are lost, regeneration response occurs through activation of regulatory homeostasis
- Subclonogenic proliferation will tend to slow down the rate of cell depletion and delay this homeostatic response with a resultant prolongation of radiation damage
- Regeneration occurs through…

- Recruitment of proliferating stem cells into the proliferation pool. If stem cell intact it will contribute to regeneration

- However, if stem cell dies at division will speed up depletion of the stem cell compartment and stimulate activation of homeostatic control via more intensive proliferation. Therefore, ***recruitment of cells in H-type tissue beneficial***

- Shortening of cell cycle time of proliferating stem cells, usually decrease in G_1

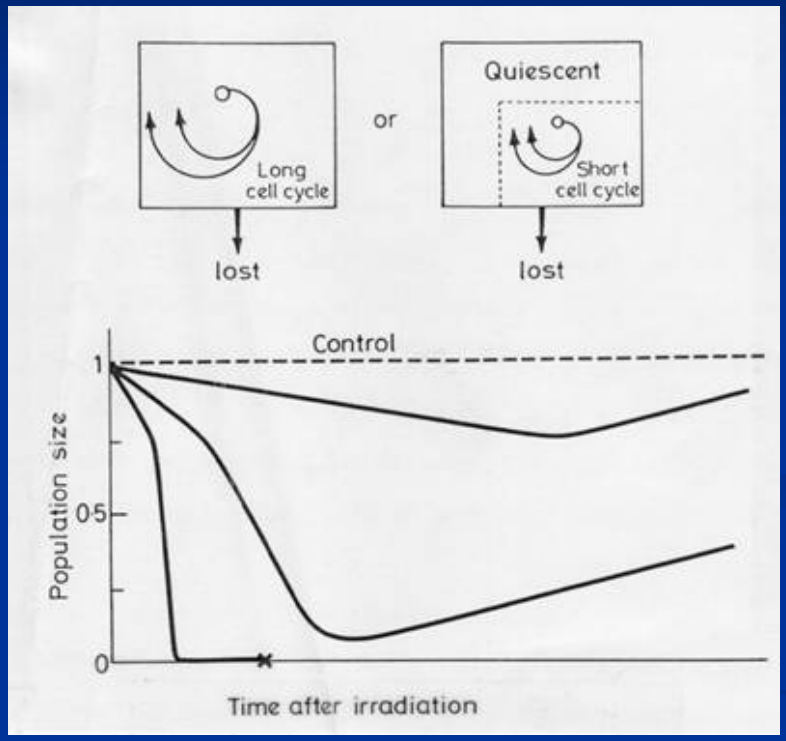
Radiation Response of F-type Tissues

- Irradiation will kill actively dividing cells in a ***dose-dependent*** manner
- Accompanied by normal and constant normal cell loss. Therefore steepness of initial slope of depopulation will be ***dose-dependent***
- These tissues typically have long turnover times, thus an apparent delay in the expression of damage may be seen, and its duration will be inversely related to dose

Radiation Response of F-type Tissues

- Once cell loss detected, proliferation stimulated; could involve either a reduction in cell cycle time and/or an increased growth fraction
- Can cause an acceleration in the rate of cell loss due to mitotic death, resulting in a temporary vicious cycle of cell loss
- This second or **avalanche** phase will follow the initial decline in cell number

Radiation Response of F-type Tissues



- Since during this avalanche phase the probability of mitotic failure will be dose-dependent, the rate of depopulation will also increase with dose
- Therefore, more severe lesions seen earlier than mild injury, in contrast to the H-type tissue reactions. Takes less time for failure to occur with increasing dose.