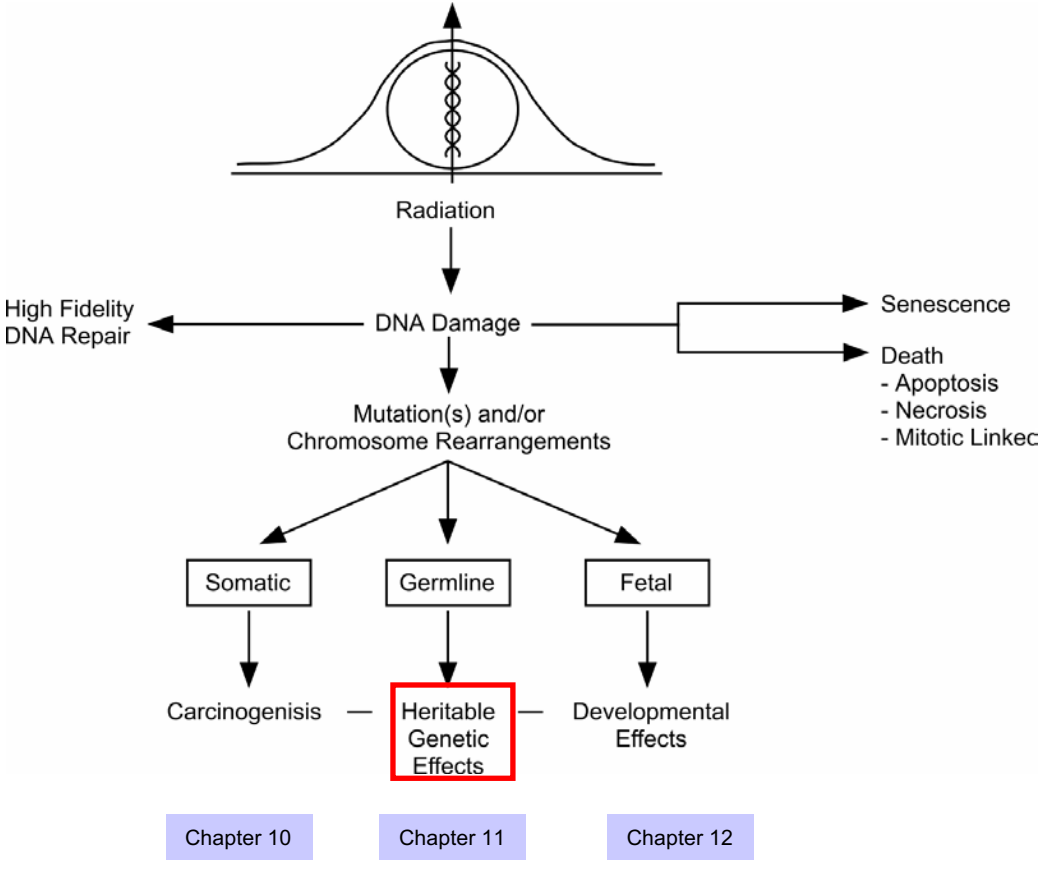
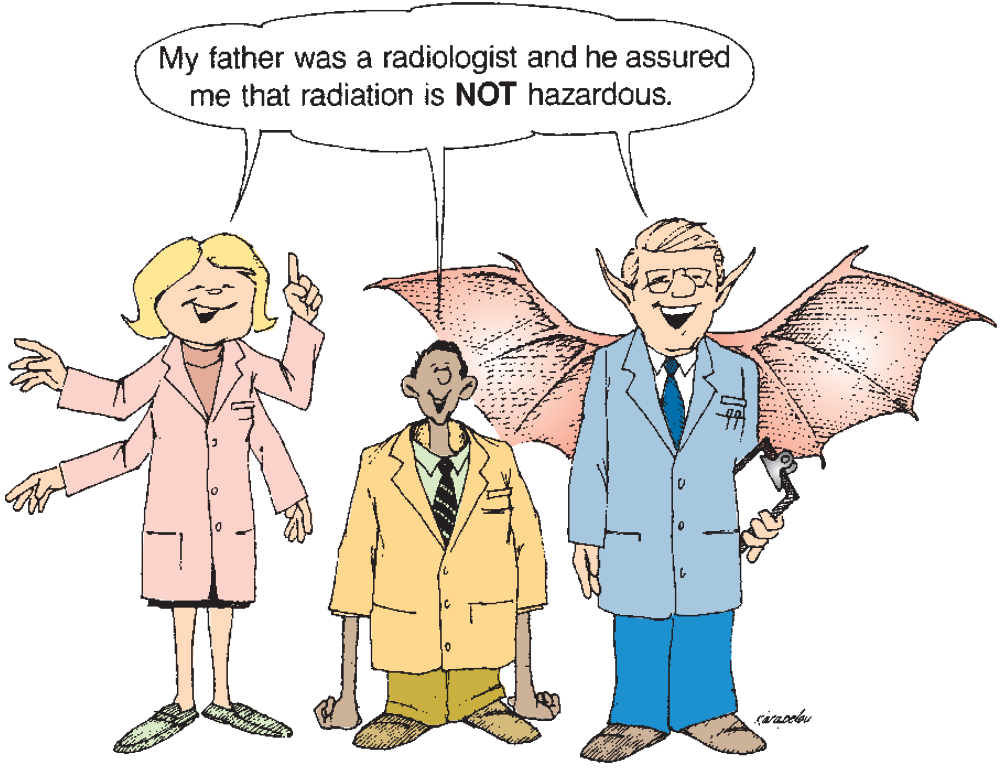
Chapter 11 – Hereditary Effects of Radiation

10/31/2024

DNA as the Target



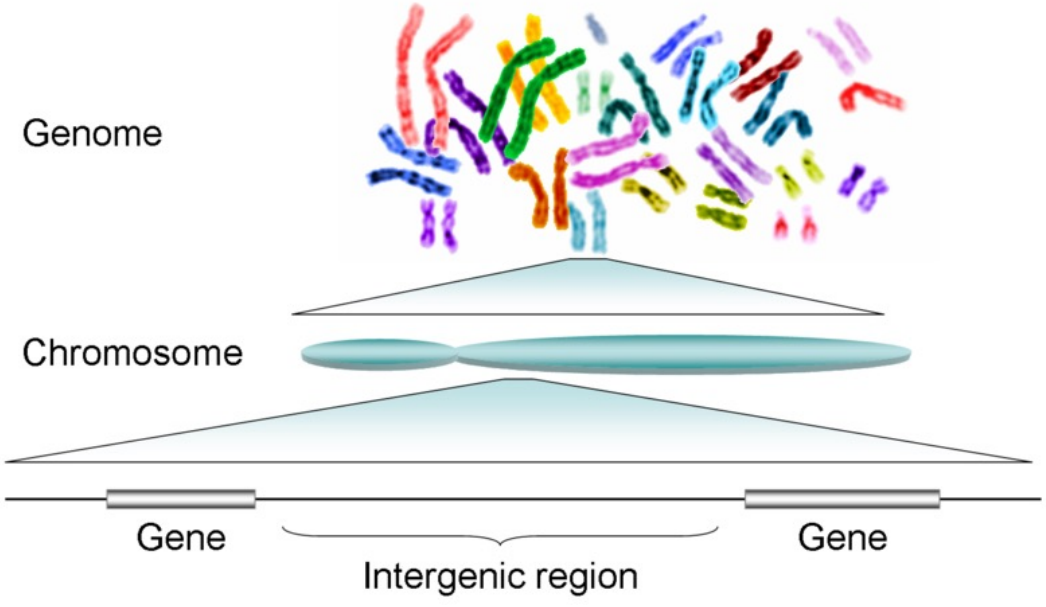
Heritable Effects of Radiation



Outline

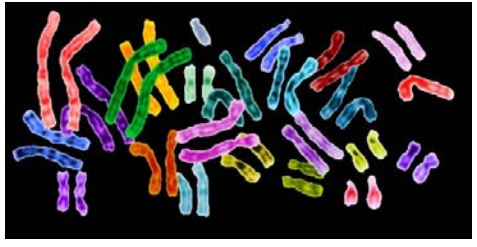
- **Genetics 101**
- Radiation-Induced Hereditary Effects in Fruit flies
- Radiation-Induced Hereditary Effects in Mice
- Radiation-Induced Hereditary Effects in Human
- Radiation Effects on Fertility
- Effect of Radiation on Epigenetics (Medical residents only)

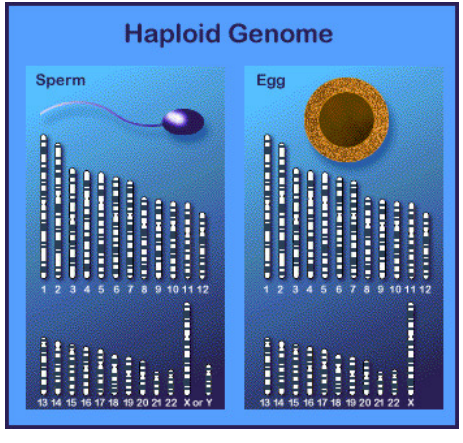
The Human Genome



The human **genome** is composed of **23 pairs** of chromosomes, each of which contain hundreds of ***genes*** separated by ***intergenic regions***. Intergenic regions may contain ***regulatory sequences*** and non-coding DNA.

The Human Chromosomes





The 46 chromosomes in our somatic cells are two sets of 23 chromosomes – a *maternal set* and a *paternal set*

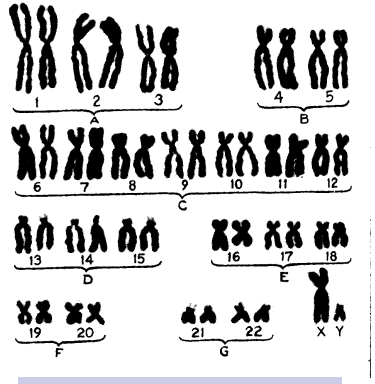
A cell with one set of chromosomes is called a haploid cell

A cell with two of each kind of chromosome is called a diploid cell and is said to contain a diploid, or 2n, number of chromosomes

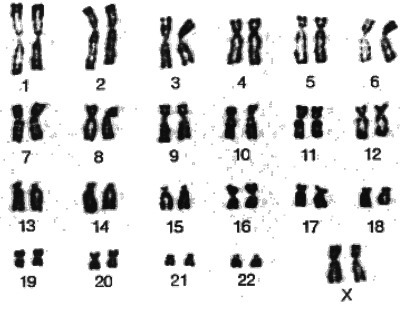
A haploid genome contain 3×10^9 base pairs, and an estimated 20,000 – 25,000 protein-coding genes

In fact, only ~ 1.5% of the genome codes for proteins, while the rest consist of *RNA genes*, *regulatory sequences*, *introns*

The Human Chromosomes



A male karyotype



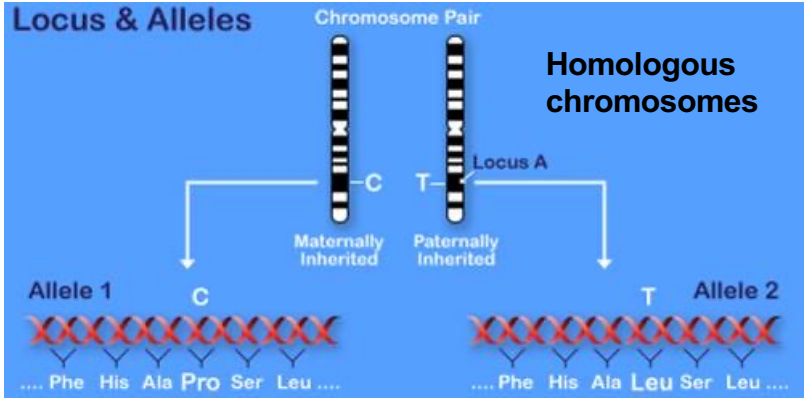
A female karyotype

The two members of a pair of chromosomes carry the same genes in the same sequence, and they are said to be homologous

Chromosomes 1-22 are autosomal chromosomes, or simply autosomes. They are numbered roughly in order of decreasing size

The pair of chromosomes that determine sex are sex chromosomes
Females have two X chromosomes.
Males have an X and a Y chromosome.

Gene, Locus & Alleles



The ***locus*** is the position of a gene on a chromosome

The different forms of a gene are called ***alleles***

- An individual is said to be ***heterozygous*** for a particular gene if the two inherited alleles are different from each other, if even by a single base pair
- An individual is said to be ***homozygous*** for a particular gene if the two inherited alleles are exactly the same
- An individual is said to be ***hemizygous*** for a particular gene if only one allele was inherited. This can happen if one allele is deleted from one of the chromosomes

Inheritance Pattern

A **dominant trait** refers to a genetic feature that hides the recessive trait in the phenotype (visible or detectable characteristic) of an individual. A dominant trait is a phenotype that is seen in both the homozygous **AA** and heterozygous **Aa** genotypes.

The term **"recessive allele"** refers to an allele that causes a phenotype that is only seen in homozygous genotypes (ie. **aa**) and never in heterozygous genotypes (i.e., Aa)

Characteristics that result from recessive genes on the X-chromosomes, so that they are expressed almost exclusively in male children are said to be **sex-linked**

Inheritance Pattern

	DOMINANT TRAITS	RECESSIVE TRAITS
eye coloring	brown eyes	grey, green, hazel, blue eyes
vision	farsightedness	normal vision
	normal vision	nearsightedness
	normal vision	night blindness
	normal vision	color blindness*
hair	dark hair	blonde, light, red hair
	non-red hair	red hair
	curly hair	straight hair
	full head of hair	baldness*
	widow's peak	normal hairline
facial features	dimples	no dimples
	unattached earlobes	attached earlobes
	freckles	no freckles
	broad lips	thin lips

	DOMINANT TRAITS	RECESSIVE TRAITS
appendages	extra digits	normal number
	fused digits	normal digits
	short digits	normal digits
	fingers lack 1 joint	normal joints
	limb dwarfing	normal proportion
	clubbed thumb	normal thumb
	double-jointedness	normal joints
other	immunity to poison ivy	susceptibility to poison ivy
	normal pigmented skin	albinism
	normal blood clotting	hemophilia*
	normal hearing	congenital deafness
	normal hearing and speaking	deaf mutism
	normal- no PKU	phenylketonuria (PKU)

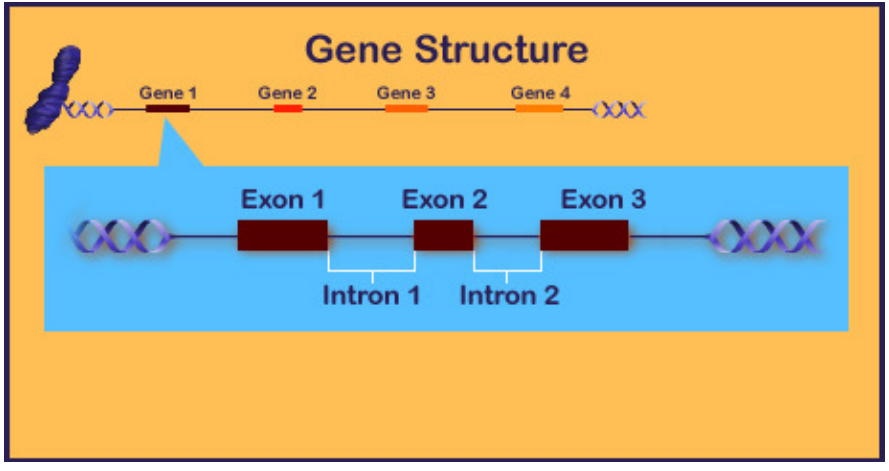
*** sex-linked characteristic**

Inheritance Pattern

Dimple

Curly Hair

Gene Structure

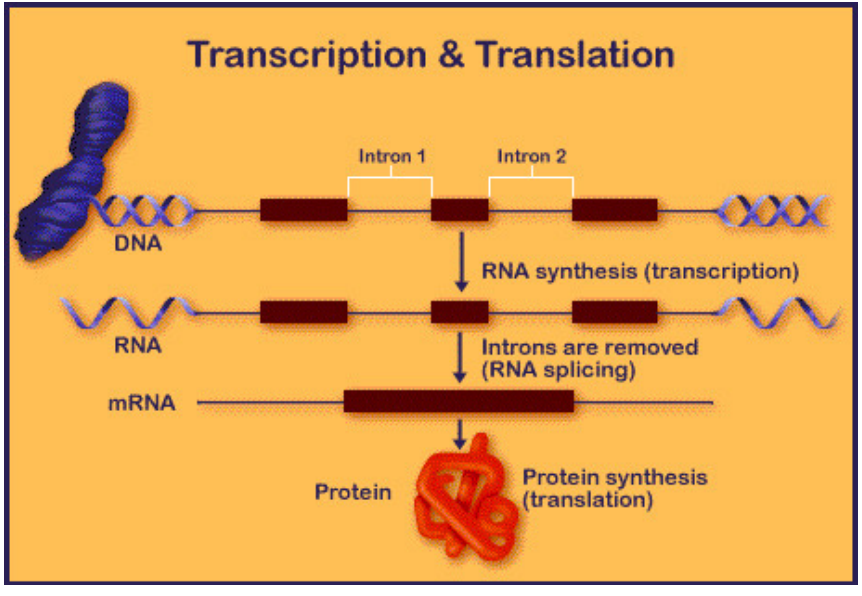


Most genes are discontinuous – the information is split between **exons** (which code for the gene) and **introns** (non-coding sequence).

During gene expression, the introns will be removed via a process called **splicing**

Genetic disease is generally caused by mutations to the exons, since they code for proteins, but mutations in introns can also cause genetic disease

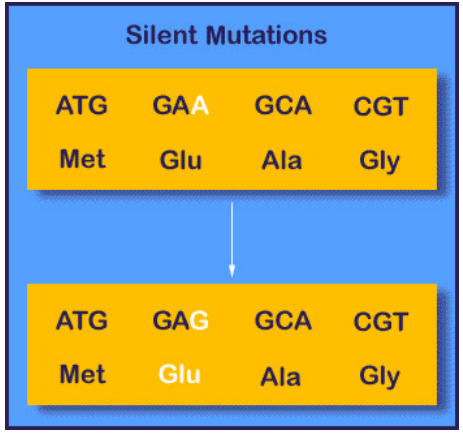
Gene Expression



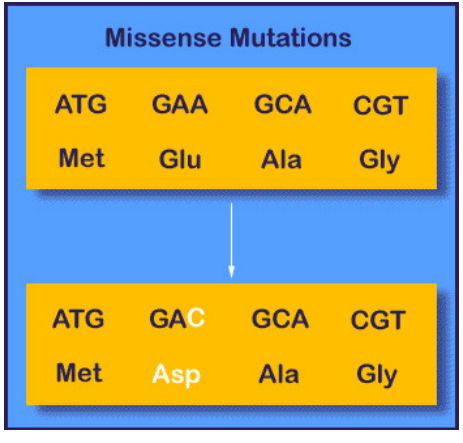
The simple model of gene expression is that DNA is **transcribed** into RNA, which is then **translated** into protein.

Mutations

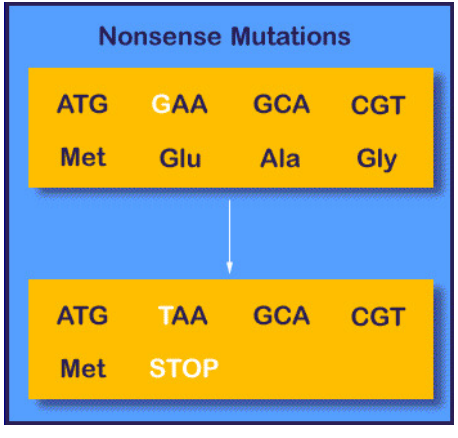
A **mutation** is a small-scale change in the nucleotide sequence of a DNA molecule



A silent mutation has no effect on the functioning of the genome

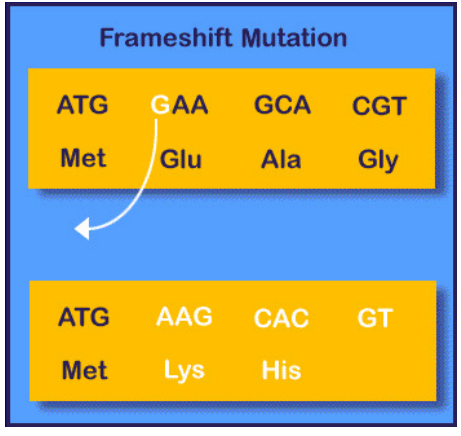


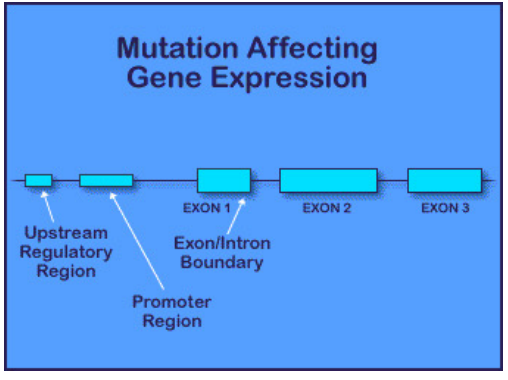
A missense mutation causes a change in a single amino acid



A nonsense mutation results in a shortened protein

Mutation

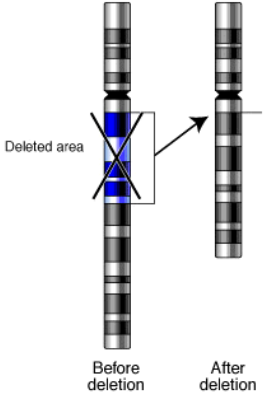


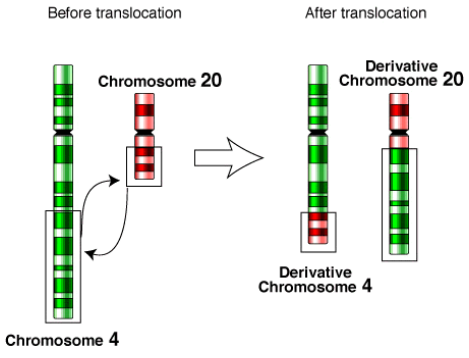


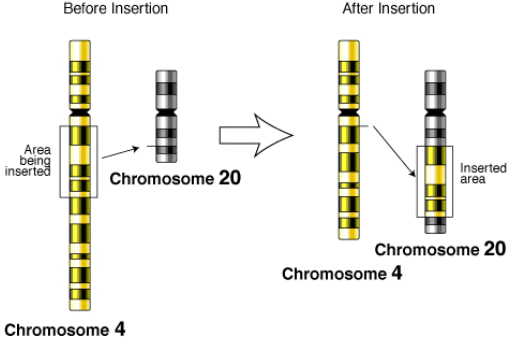
A frameshift mutation changes all of the codons downstream

Mutations in the promoter region or splice sites can affect gene expression

Mutation







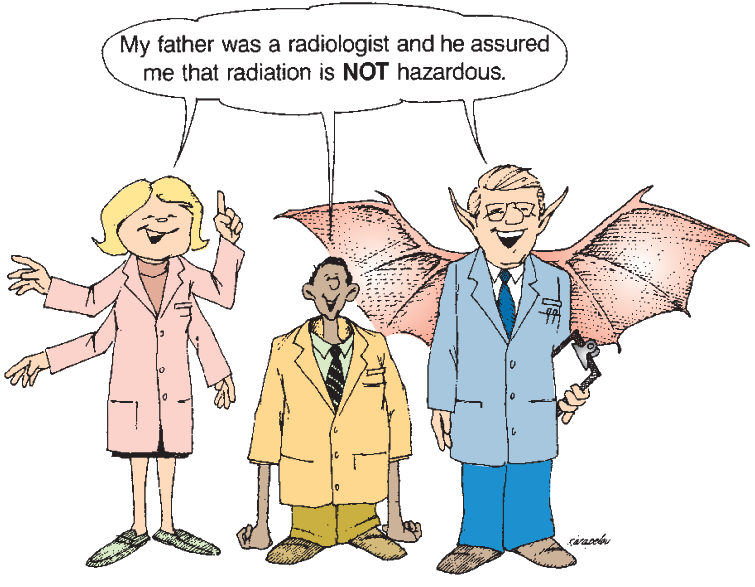
Deletions can be of an entire gene, part of a gene, a single codon, or a single nucleotide

A translocation is when two chromosomes swap pieces of their arms

An insertion is when one portion of a chromosome is inserted into another

Hereditary Disease

Hereditary disease may result when mutations occurring in the ***germ cells*** of parents are transmitted to ***progeny***



Radiation does not result in hereditary effects that are new or unique but rather increases the frequencies of the same mutations that already occur spontaneously or naturally in that species

Hereditary Disease

Hereditary diseases are classified into 3 principal categories – ***Mandelian***, ***chromosomal***, and ***multifactorial***

TABLE 11.1. *Heritable Effects of Radiation*

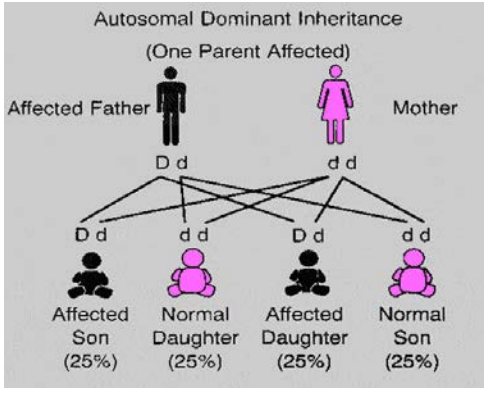
Heritable Effect	Example
Gene mutations[a] **Mandelian**	
Single dominant 736 (753)	Polydactyly, Huntington's chorea
Recessive 521 (596)	Sickle-cell anemia, Tay–Sachs disease, cystic fibrosis, retinoblastoma
Sex-linked 80 (60)	Color blindness, hemophilia
Chromosomal changes	
Too many or too few	Down's syndrome (extra chromosome 21), mostly embryonic death
Chromosome aberrations, physical abnormalities	Embryonic death or mental retardation
Robertsonian translocation	
Multifactorial	
Congenital abnormalities present at birth	Neural tube defects, cleft lip, cleft palate
Chronic diseases of adult onset	Diabetes, essential hypertension, coronary heart disease

[a]The numbers following types of gene mutations refer to the number of human diseases known to be caused by such a mutation. The numbers in parentheses refer to additional possible diseases.

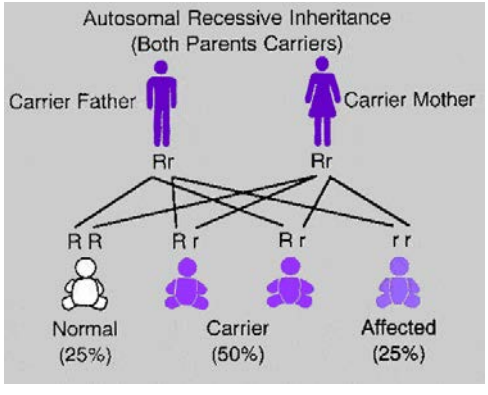
These are the diseases that occur normally that **could be enhanced by exposure to ionizing radiation**

Mandelian

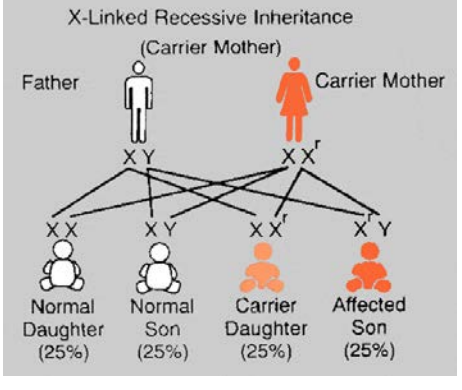
Single gene disorders – diseases or traits where the phenotypes are largely determined by the action, or lack of action, of mutations at ***individual loci***



Autosomal Dominant
(Ex: polydactyly)



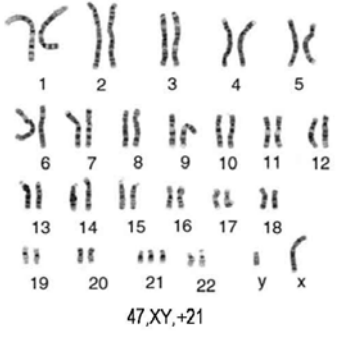
Autosomal Recessive
(Ex: sickle cell anemia)



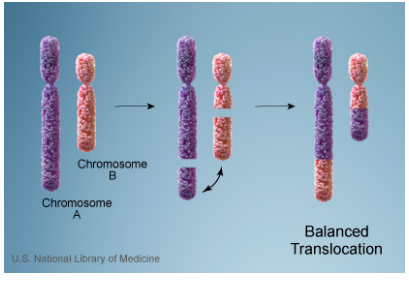
X-linked
(Ex: hemophilia)

Chromosomal Changes

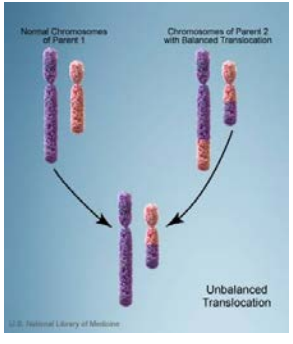
Chromosomal abnormalities – diseases where the phenotypes are largely determined by ***physical changes in chromosomal structure*** - deletion, inversion, translocation, insertion, rings, etc., ***in chromosome number*** - trisomy or monosomy, or ***in chromosome origin*** - uniparental disomy



Trisomy 21



Balanced translocation



Unbalanced translocation

Majority of these abnormalities are incompatible with life, resulting in spontaneous abortion or stillbirth

Multifactorial

Multifactorial traits – diseases or variations where the phenotypes are strongly influenced by the action of ***mutant alleles at several loci*** acting in concert

They are characterized by
- ✓ Known to have a genetic component
- ✓ Transmission pattern not simple Mandelian
- ✓ Interaction with environmental factors

Examples include
Congenital abnormalities – cleft lip with or without cleft palate, neural tube defect
Adult onset – diabetes, essential hypertension, coronary heart disease

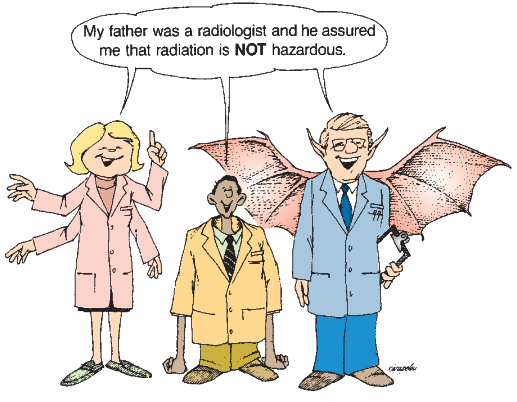
Baseline Frequencies of Genetic Diseases in Human Population

Disease Class	Frequency per Million	
Mandelian Diseases		24,000
Autosomal dominant diseases	15,000	
X-linked diseases	1,500	
Autosomal recessive diseases	7,500	
Chromosomal Diseases		4,000
Multifactorial Diseases		710,000
Chronic diseases	650,000	
Congenital abnormalities	60,000	
Total		738,000

UNSCEAR 2001

Hereditary Disease

Hereditary disease may result when mutations occurring in the ***germ cells*** of parents are transmitted to ***progeny***



Radiation does not result in hereditary effects that are new or unique but rather increases the frequencies of the same mutations that already occur spontaneously or naturally in that species

The hereditary effect of radiation is thus expressed as a **doubling dose**, i.e., the amount of radiation required to produce as many mutations as occur spontaneously in a generation

Outline

- Genetics 101
- **Radiation-Induced Hereditary Effects in Fruit flies**
- Radiation-Induced Hereditary Effects in Mice
- Radiation-Induced Hereditary Effects in Human
- Radiation Effects on Fertility
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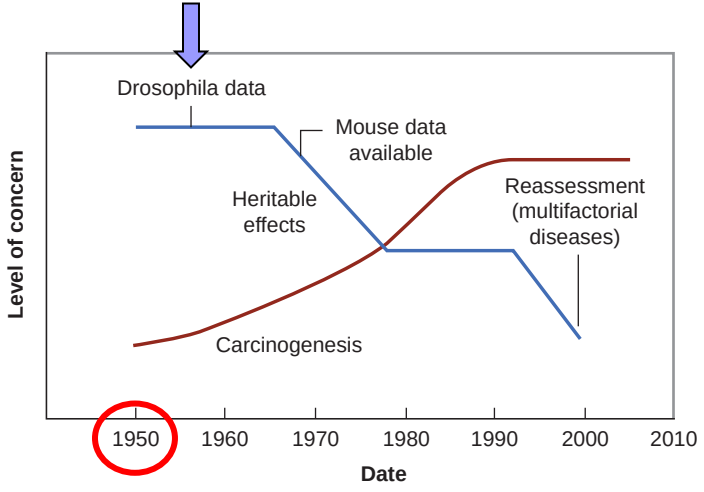
To estimate doubling dose

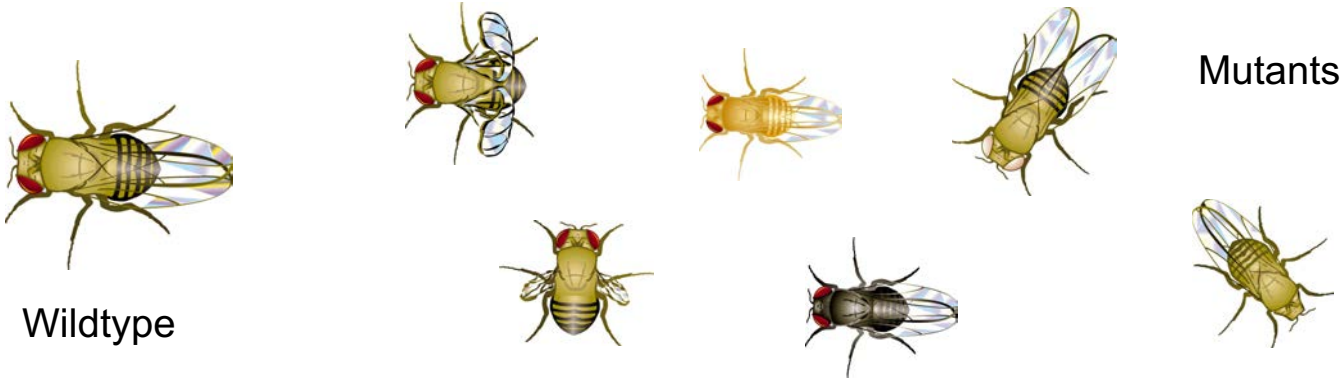
Changing Concerns for Risks

- In the 1950s, heritable changes were considered the principal hazard of exposure to ionizing radiation
- Little was known of the carcinogenic potential of low doses of radiation



Radiation-Induced Hereditary Effects in Fruit Flies

- The earliest mutation experiments were carried out with the fruit fly *Drosophila melanogaster*
- Radiation-induced mutants did not appear different from those that occur spontaneously



The current annual dose limit of 50mSv/year for radiation workers came from fruit fly studies!!!

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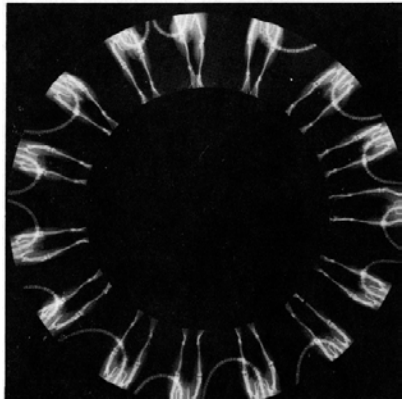
The Megamouse Project

Russell and Russell at Oak Ridge National Laboratory mounted an experiment to determine specific locus mutation rates in the mouse induced by radiation

7 million mice had been used, and the project is referred to as the "megamouse project"

7 specific locus mutations were used to study radiation-induced hereditary effects, shown here were 3 coat color mutations



15 mice in position on an X-ray exposure wheel

These mutations occur spontaneously, and their incidence is increased by irradiation.

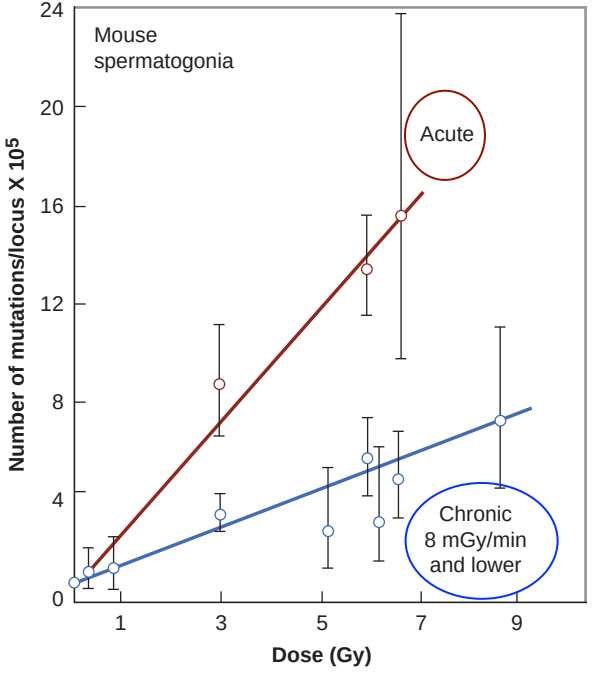
Conclusions of the Megamouse Project

- The radiosensitivity of different mutations varies by a significant factor of ~ 35, so that it is only possible to speak in terms of *average mutation rates*
- We now know that this is due simply to a size difference between the various genes involved

Conclusions of the Megamouse Project

- There is a substantial **dose-rate effect**, so that spreading the radiation dose over a period of time results in fewer mutations for a given dose than in an acute exposure
- This was attributed to a repair process

Mutations in mice as a function of dose



Conclusions of the Megamouse Project

- Essentially all of the radiation-induced hereditary data came from experiments with male mice
- In the mouse, the oocytes are exquisitely radiosensitive, and are readily killed by even low doses of radiation

Conclusions of the Megamouse Project

- The hereditary consequences of a given dose can be reduced greatly if a time interval is allowed between irradiation and conception
- This again was thought to be a consequence of some repair process

Although there is no data for humans, it is recommended that a period of **6 months** be allowed between exposure to radiation and planned conception in radiotherapy patients or others whose gonads receive doses in excess of about **0.1 Gy**

Conclusions of the Megamouse Project

- The genetic effects of radiation are frequently represented by the "doubling dose"

Doubling dose – the amount of radiation required to produce as many mutations as occur spontaneously in a generation

- The estimate of the doubling dose favored by the BEIR V and the USCEAR 88 is **1 Gy** based on the ***low dose rate exposure***

No more than **1- 6%** of spontaneous mutations in humans may be ascribed to background radiation

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Risk Estimation in Human

Disease Class	Frequency per Million	
Mandelian Diseases		24,000
Autosomal dominant diseases	15,000	
X-linked diseases	1,500	
Autosomal recessive diseases	7,500	
Chromosomal Diseases		4,000
Multifactorial Diseases		710,000
Chronic diseases	650,000	
Congenital abnormalities	60,000	
Total		738,000

- To estimate the risk of radiation-induced hereditary diseases in human, two quantities are required

1. The baseline mutation rate for humans, which is estimated to be 738,000 per million

2. The doubling dose, from the mouse data, which is about 1 Gy

Risk Estimation in Human

- Two correction factors are needed

1. To allow that not all mutations lead to a disease – this is the **mutation component** (MC), which varies for different classes of hereditary diseases

2. To allow for the fact that the 7 specific locus mutations used in the mouse project are not representative of inducible hereditary diseases in the human because they are all nonessential for the survival of the animal or cell

UNSCEAR Estimates of Hereditary Risks

TABLE 11.4 **Current Estimates of Genetic Risks from Continuing Exposure to Low-LET, Low-Dose, or Chronic Irradiation (from UNSCEAR 2001) (Assumed Doubling Dose: 1 Gy)**

Disease Class	Based Frequency per 10^6 Live Births	Risk per Gy per 10^6 Progeny: First Generation	Risk per Gy per 10^6 Progeny: Up to Second Generation
Mendelian			
Autosomal dominant and X-linked	16,500	750–1,500	1,300–2,500
Autosomal recessive	7,500	0	0
Chromosomal	4,000	[a]	[a]
Multifactorial			
Chronic	650,000	~250–1,200	~250–1,200
Congenital abnormalities	60,000	2,000	2,400–3,000
Total	738,000	~3,000–4,700	3,950–6,700
Total risk per Gy expressed as percent of baseline	—	~0.41–0.64	~0.53–0.91

[a]Assumed to be subsumed in part under the risk of autosomal dominant and X-linked diseases and in part under congenital abnormalities.

From United Nations Scientific Committee on the Effects of Atomic Radiation: *Hereditary Effects of Radiation: The UNSCEAR 2001 Report to the General Assembly with Scientific Annex*. New York, United Nations, 2001.

Note that the total risk per Gy is only about **0.41 – 0.64% of the baseline risk** of 738,000 per million live births, which is a relatively small proportion

Data pertain to a **"reproductive"** population

UNSCEAR 2001 Estimation of Genetic Risks

Assumed doubling Dose of 1 Gy

- Not all mutations lead to a disease (mutation component)
- Not all mutations are compatible with life, i.e., recoverable in live births

Baseline risk – 738,000 per 10^6 live births

What is the excess risk per 10^6 progeny per Gy?

Corrected risk – ~ 3000-4,700 per 10^6 progeny per Gy

Corrected risk per Gy expressed as percent of baseline = ~ 3000-4,700/738,000 = ~ 0.41-0.64% = ~ 0.5%

ICRP Estimates of Hereditary Risks

- Based on the data calculated by UNSCEAR 2001 and corrected for "reproductive age"
- In other words, only **genetically significant dose (GSD)** was considered

Genetically significant dose (GSD) – The dose to the gonads weighted for the age and sex distribution in those members of the population expected to have offspring

Table 11.5 Heritable Effects – ICRP (2003)

- Total population 0.2%/Sv
- Working population 0.1%/Sv
- Based on:
  - Heritable risks for first two generations
  - Life expectancy 75 years; reproductive age 30 years
  - Total population $\frac{30}{75}$ of reproductive population
  - Working population $\frac{30-18}{75}$ of reproductive population

ICRP Estimates of Hereditary Risks

Total Population

Assumption – average life expectancy of 75 years; mean reproduction age stopping at 30 years
The risk coefficients = 30/75 = 40% for reproductive population

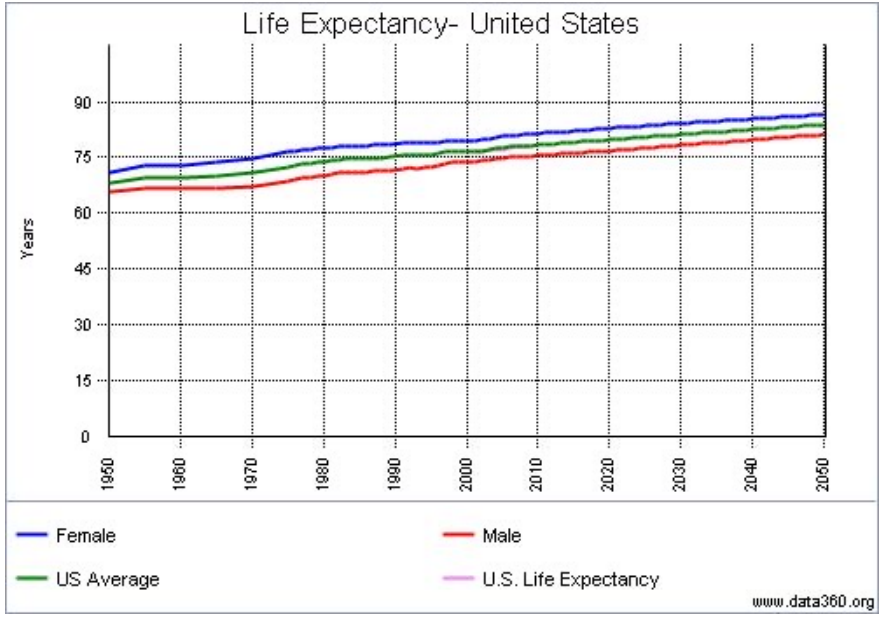
Risk ≅ 0.5% (UNSCEAR data) x 40% = **0.2%/Sv**

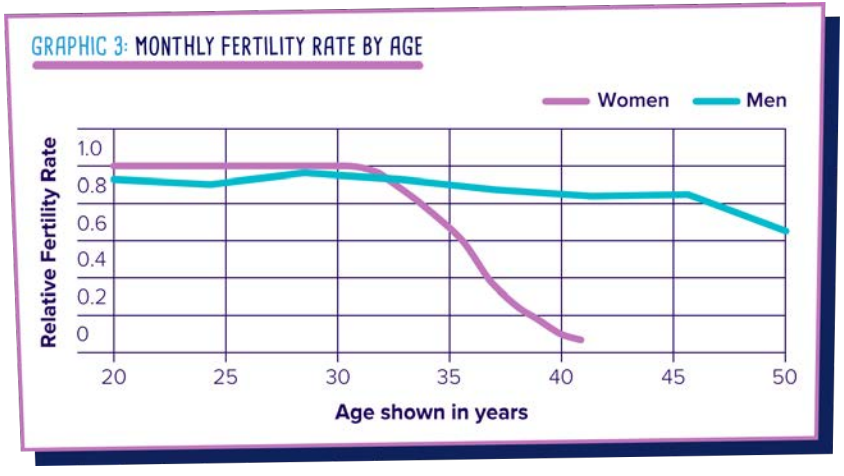
Radiation Workers

Radiation workers start working at age 18, so the relevant reproductive years = 30-18 = 12 years
The risk coefficients = 12/75 = 16%

Risk ≅ 0.5% (UNSCEAR data) x 16% = **0.1%/Sv**

Life Expectancy and Fertility Rates in US





Data from A-Bomb Survivors

- Children of the A-bomb survivors have been studied for a number of indicators, and compared to a control cohort
- None of the differences reached statistical significance
- 3 indicators were used to estimate the doubling dose

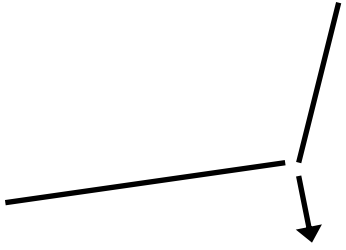
Human Data

TABLE 11.6 **Doubling Dose (Gametic) in the Offspring of Survivors of the Atomic Bomb Attacks on Hiroshima and Nagasaki**

Genetic Indicator	Doubling Dose, Sv
Untoward pregnancy outcome	0.69
Childhood mortality	1.47
Sex chromosome aneuploidy	2.52
Simple average	1.56

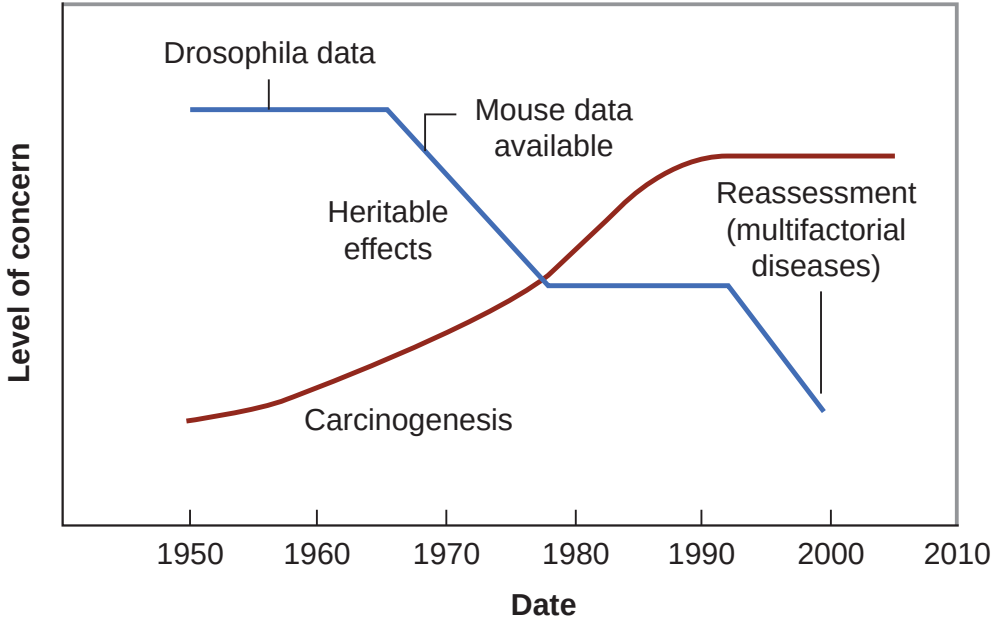
Adapted from Schull WJ, Otake M, Neal JV. Genetic effects of the atomic bomb: A reappraisal. *Science*. 1981;213:1220–1227, with permission.

Recent review by Neel estimated the doubling dose to be about **2 Sv**, with a lower limit of 1 Sv, and an upper limit that is indeterminate



Both refer to acute radiation exposure

Changing Concerns for Risks

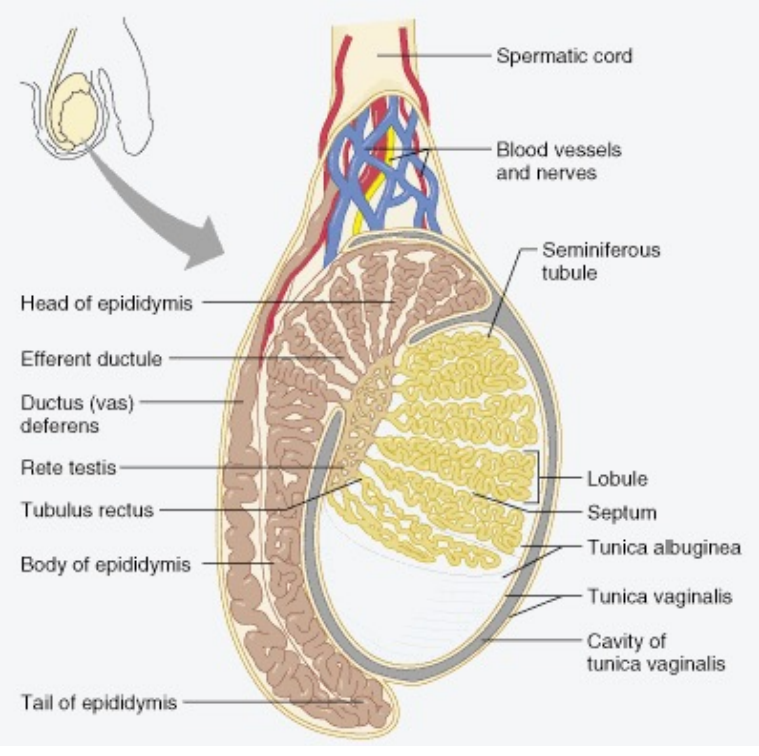


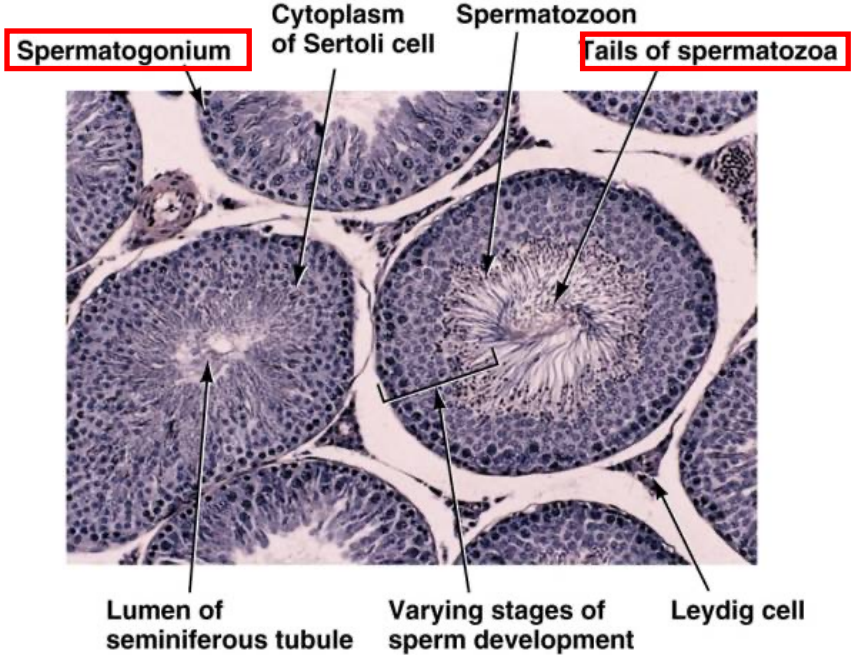
Over the years, concern has switched from heritable effects to radiation carcinogenesis

Outline

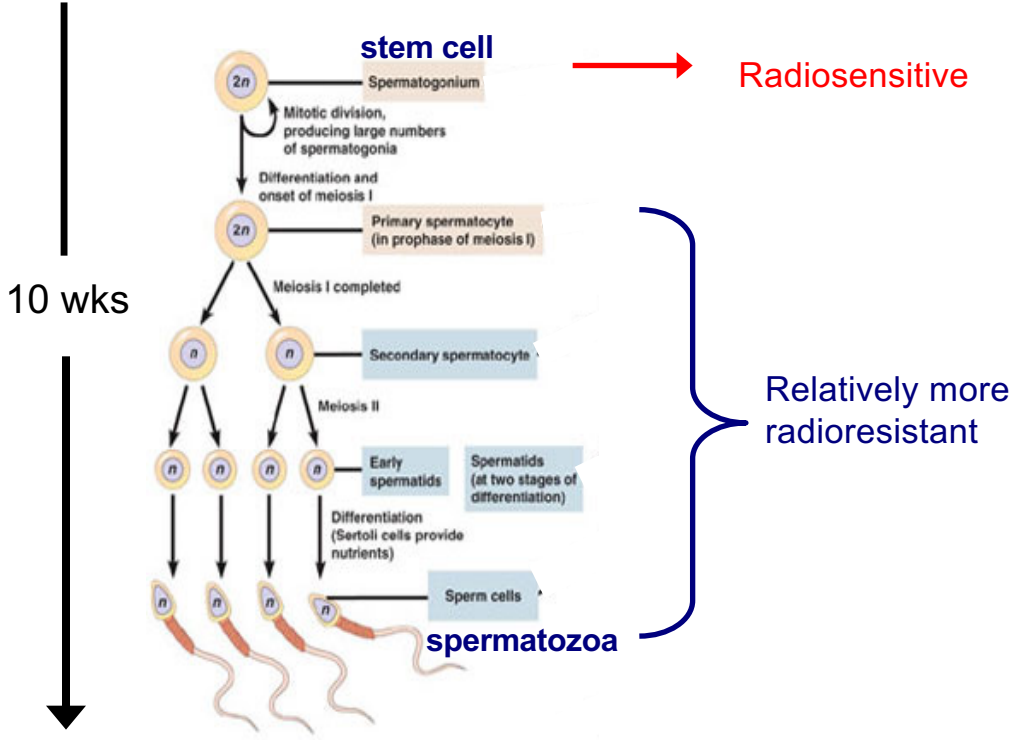
- Genetics 101
- Radiation-Induced Hereditary Effects in Fruit flies
- Radiation-Induced Hereditary Effects in Mice
- Radiation-Induced Hereditary Effects in Human
- **Radiation Effects on Fertility**
- Effect of Radiation on Epigenetics (Medical residents only)

The Male Reproductive System





Spermatogenesis



Following a moderate dose of radiation, individual will remain fertile as long as mature sperm cells are available (= **latent period**)

However, if sperms are used up, temporary sterility will result until spermatogonia repopulate

Spermatogenesis continue from puberty to death

Radiation Effects on Male Sterility

Self-renewal system: spermatogonia → spermatocytes → spermatids → spermatozoa

Latent period b/w irradiation and sterility

Oligospermia and reduced fertility: **0.15 Gy**

Azoospermia and temporary sterility: **0.5 Gy**

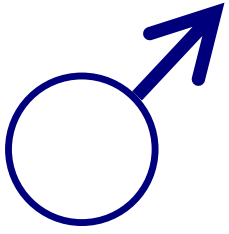
Recovery is dose dependent

Permanent sterility

6 Gy – *single dose*

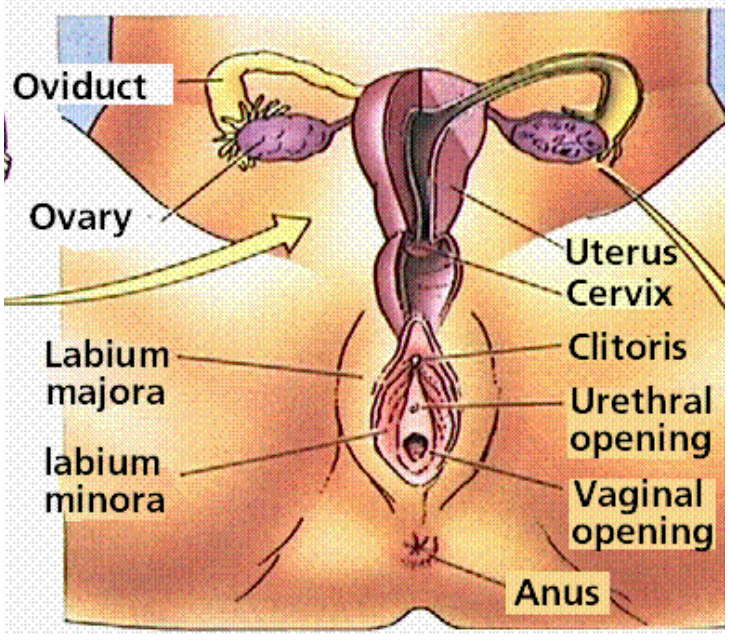
2.5-3 Gy, *fractionated*, 2-4 wks

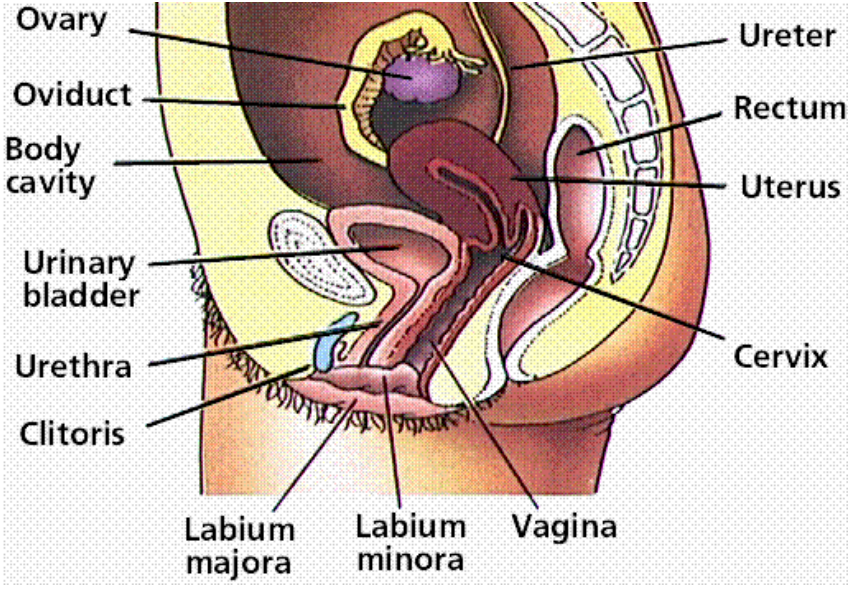
Induction of sterility does not affect hormone balance, libido, or physical capability



In male, fractionated doses cause more gonadal damage than a single dose due to reassortment

The Female Reproductive System



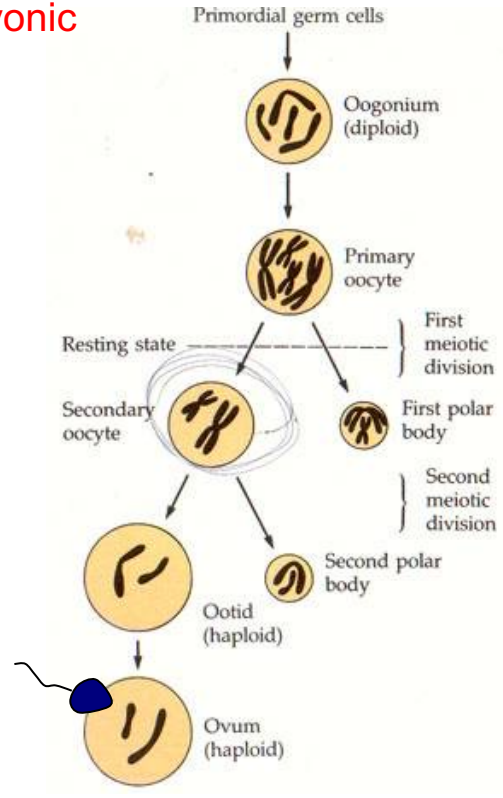


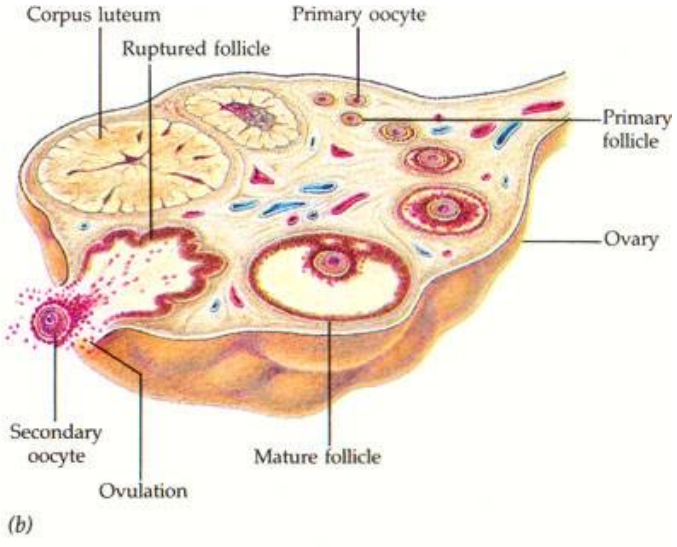
Oogenesis

Embryonic

At birth

Puberty





There are no stem cells (oogonium) in adults
There are ~ 1 million oocytes at birth, 300,000 at puberty

Radiation Effect on Female Fertility

By 3 days after birth, all cells progressed to primary oocyte stage; **no further cell division**

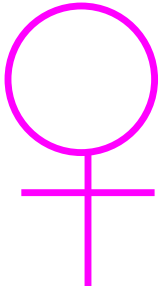
Neither latent period nor temporary sterility in females

Radiation can induce permanent ovarian failure; marked **age dependence**

Permanent sterility

- **12 Gy** – *prepuberty*
- **2 Gy**, *premenopausal*

Radiation sterility produces hormonal changes like those seen in natural menopause



Medical Residents Only

Outline

- Genetics 101
- Radiation-Induced Hereditary Effects in Fruit flies
- Radiation-Induced Hereditary Effects in Mice
- Radiation-Induced Hereditary Effects in Human
- Radiation Effects on Fertility
- **Effect of Radiation on Epigenetics**

Epigenetics

- So far, it is assumed that heritable effect must involve a change in DNA sequence

- However, heritable changes in gene expression or cellular phenotype may also occur via epigenetic mechanisms

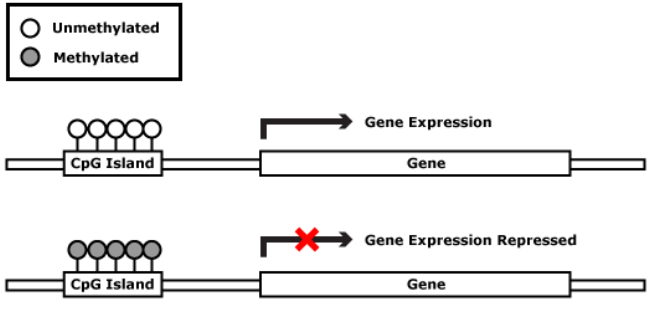
Epigenetics

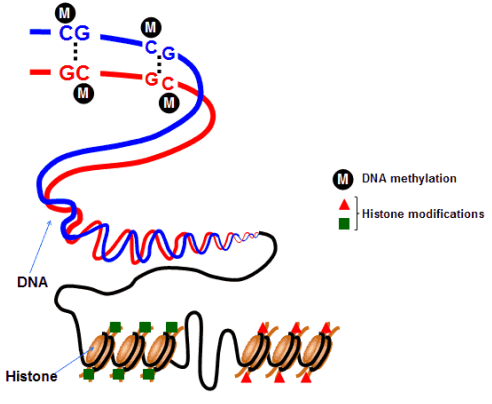
Definition

Epigenetics refers to changes in **gene expression** that do NOT involve a change in the nucleotide sequence

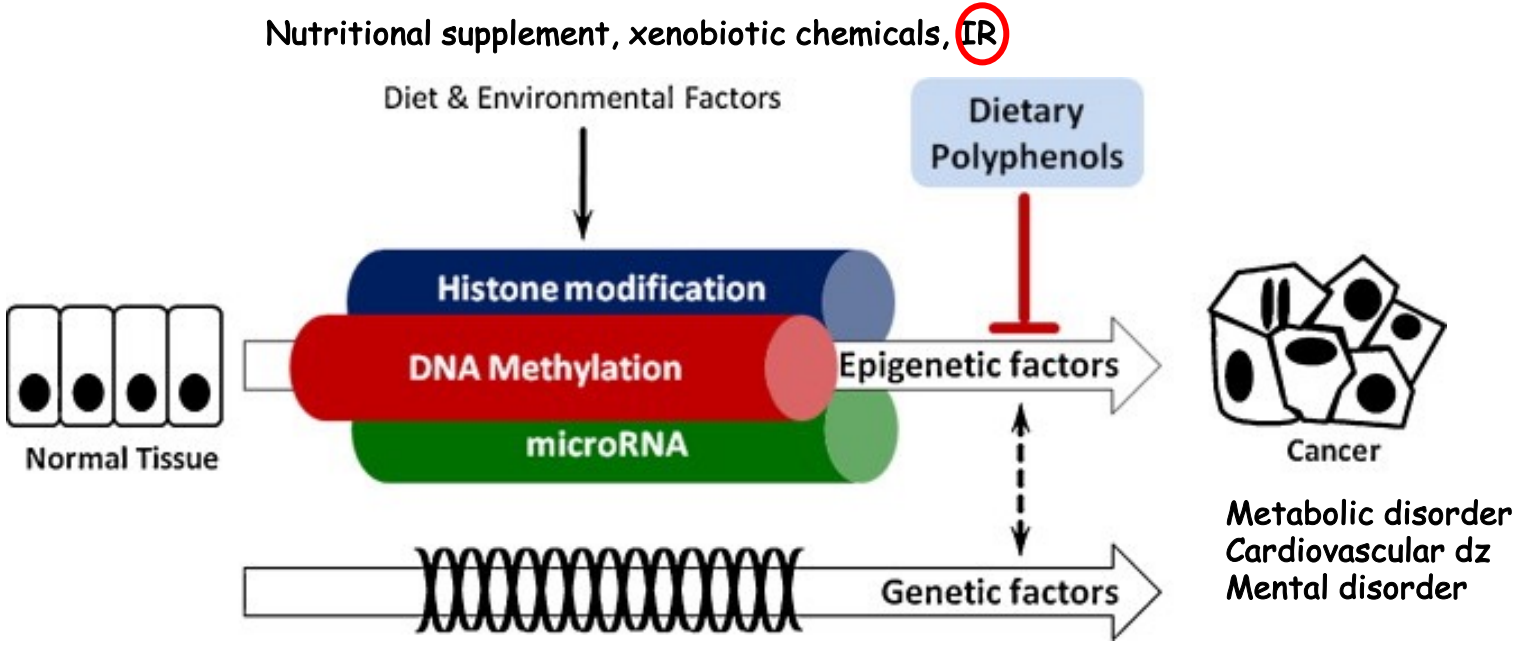
Mechanisms

- **DNA methylation** – occurs at the C-5 position of cytosine in CpG
- Changes to chromatin packaging of DNA by posttranslational **histone modifications**





Epigenetics



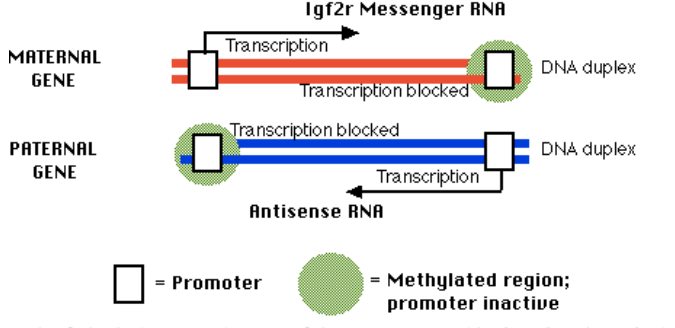
Alteration during prenatal and early postnatal stages	⇒	Risk later in adult
Alteration in parents		Risk in off-springs

Imprinted Genes

Most autosomal genes are expressed from the alleles of both parents, however, ~1% of autosomal genes in humans are “imprinted”

Imprinted genes are genes whose expression is determined by the parent that contributed them

- e.g, the maternal allele is expressed exclusively because the paternal allele is imprinted or vice versa



In the father's (**paternal**) copy of the *IGF2r* gene (the imprinted version)

Several human syndromes, and even some cancers, result from genetic and epigenetic modifications at imprinted loci

Imprinted Genes

Expression of an imprinted gene in the present generation depends on the environment that it experienced in the previous generation

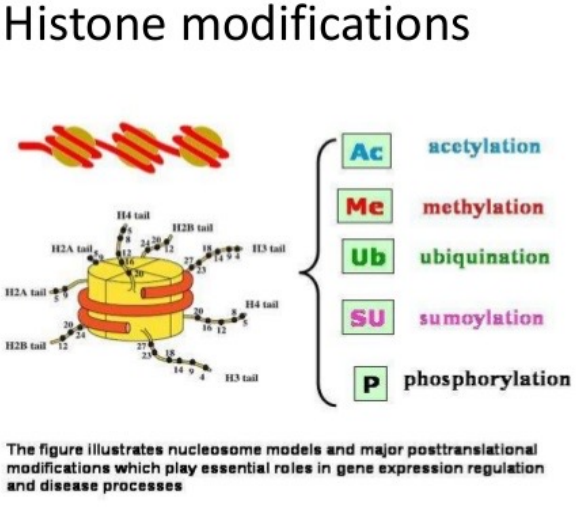
The study of radiation on epigenetics is in its infancy, but it is a factor that may influence the perception of radiation-induced heritable effects in the future

Mutations in DNA are no longer the whole story!

Question

Epigenetic modification of DNA-associated histones can occur through all of the following mechanisms, EXCEPT:

A. Phosphorylation
B. Acetylation
C. Glycosylation
D. Methylation
E. Ubiquitination



Sumoylation involves addition of SUMOs (small ubiquitin-like modifiers)

Review Questions

Question 1

The probability of a hereditary disorder in the first generation born to parents exposed to radiation is estimated to be approximately:

A. 0.02/mSv
B. 0.2/mSv
C. 0.002/Sv
D. 0.02/Sv
E. 0.2/Sv

Question 1

Table 11.5 Heritable Effects – ICRP (2003)

- Total population 0.2%/Sv
- Working population 0.1%/Sv
- Based on:
  - Heritable risks for first two generations
  - Life expectancy 75 years; reproductive age 30 years
  - Total population $\frac{30}{75}$ of reproductive population
  - Working population $\frac{30 - 18}{75}$ of reproductive population

Question 2

A 22-year-old man completed a course of radiation therapy for Hodgkin's lymphoma one year ago. For the previous 6 months, he and his wife tried unsuccessfully to conceive a child. He expressed concern to his radiation oncologist that the radiation exposure (gonadal dose of 0.83 Gy) may have left him sterile. How should the radiation oncologist respond?

A. The radiation dose likely caused permanent sterility

B. The dose of radiation should have had no effect on the patient's sperm count and probably isn't the cause of the couple's fertility problems

C. The patient should not even be attempting to conceive a child due to a significantly increased risk for radiation-induced mutations in the offspring of irradiated individuals

D. Hormonal dysfunction caused by the radiation, not the lowered sperm count per se, probably accounted for the couple's fertility problems

E. This dose should interfere with fertility for no more than about a year, so the patient should keep trying to conceive a child.

Radiation Effects on Male Sterility

Self-renewal system: spermatogonia → spermatocytes → spermatids → spermatozoa

Latent period b/w irradiation and sterility

Oligospermia and reduced fertility: **0.15 Gy**

Azoospermia and temporary sterility: **0.5 Gy**

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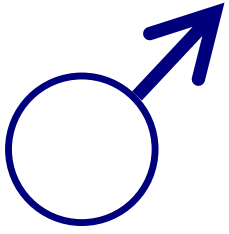


In male, fractionated doses cause more gonadal damage than a single dose due to reassortment

Question 3

Which of the following statements concerning the landmark “mega-mouse” study of radiation mutagenesis, is INCORRECT?

A. The dose response curve for radiation-induced mutations was linear with no threshold.

B. Radiation dose-rate was not found to affect the induction of mutations.

C. Males were more susceptible to radiation-induced mutations than females.

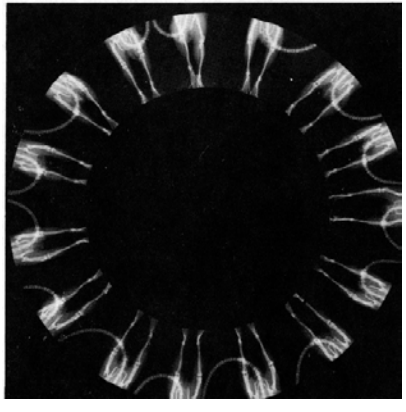
D. Mutation rates at the different loci studied varied widely.

E. The estimated doubling dose for mutations was approximately 1 Gy.

The Megamouse Project

Russell and Russell at Oak Ridge National Laboratory mounted an experiment to determine specific locus mutation rates in the mouse induced by radiation

7 million mice had been used, and the project is referred to as the "megamouse project"



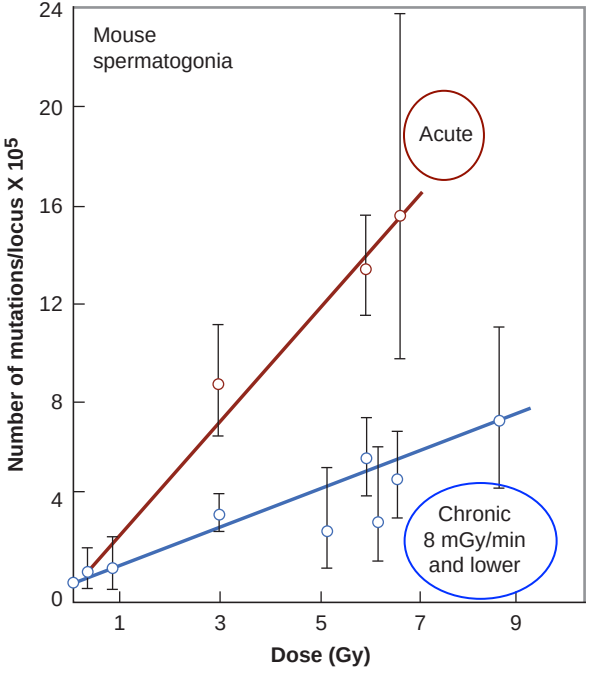
7 specific locus mutations were used to study radiation-induced hereditary effects, shown here were 3 coat color mutations

15 mice in position on an X-ray exposure wheel

These mutations occur spontaneously, and their incidence is increased by irradiation.

Conclusions of the Megamouse Project Summarized

- There is a considerable dose-rate effect



Mutations in mice as a function of dose

Question 4

- Radiation produces unique mutations not otherwise seen spontaneously. T or F