MEDICAL UNIVERSITY – PLEVEN
FACULTY OF PUBLIC HEALTH
CENTER FOR DISTANCE LEARNING

INFLUENCE OF RADIATION ON MOLECULAR, CELLULAR AND TISSUE LEVELS

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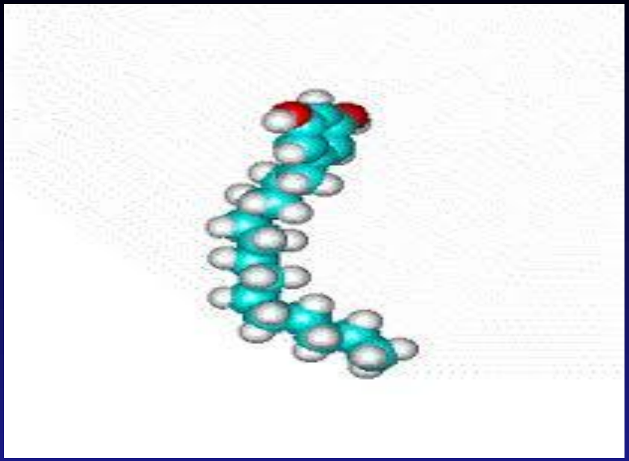


Proteins

Lipids

RADIATION EFFECTS ON THE MOLECULAR LEVEL

DNA

I. Effects on DNA

- **DNA** is **key molecule** in the living organism.
  - Many experimental data indicate that DNA is the most likely **target** in the cell for the radiation.
  - The effect of radiation on DNA is dependent on **dose, time** and **the phase of the cell cycle**.
  - Many of the damages in DNA can be and are repaired by the cell.

- Radiation damage to DNA can be divided into **four categories**:

1. **Base damage** - change or loss of a base.
2. **Single-strand breaks** **(SSB)** - break in the backbone of one chain of DNA molecule.
3. **Double-strand breaks** **(DSB)** - break in both chains of the DNA molecules.
4. **Crosslinks** - either within the DNA molecule (**intrastrand**) or from one molecule to another (**DNA - interstrand** or **DNA - protein**).

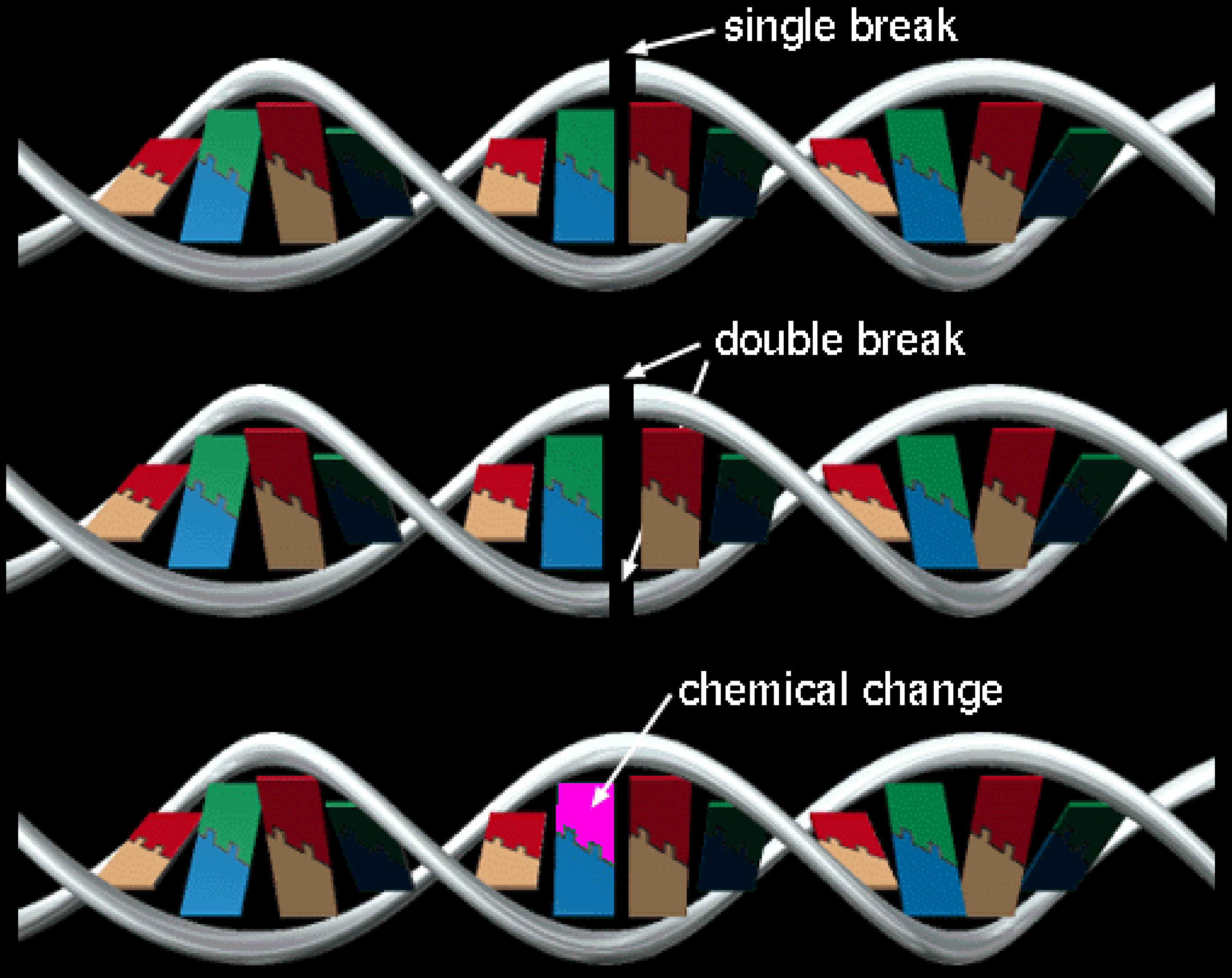
- **Base damage**
  - The loss or change of a base on the DNA chain results in an **alteration of the base sequence** since it is the sequence of these bases that stores and transmits **genetic information**.
  - This damage can be of major consequence of the cell.
  - Loss or change of a base is considered a **type of mutation**.
- **Single-strand breaks**
  - Breaks in just one of strands of DNA molecules are most likely **efficiently repaired**, with little, if any, long-term consequences to the cell.

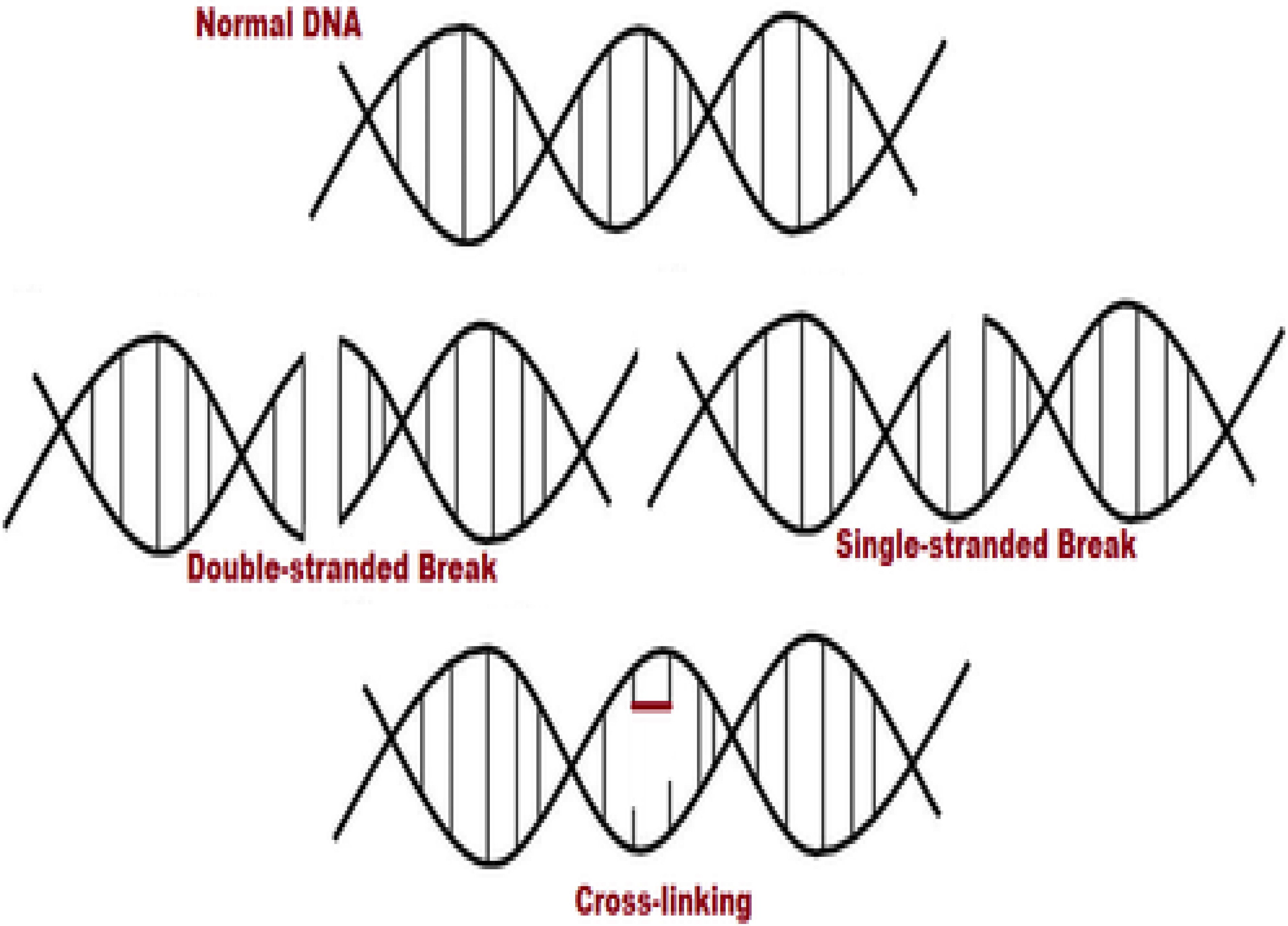
Double-strand breaks

- Breaks in either chains or backbones of DNA if they are in proximity can have a **significant impact** on the cell.
- This type of damage is more difficult for the cell to repair accurately.
- If repair does not take place, the **DNA chains can separate**, with greater consequence to the life of the cell.

Crosslinking

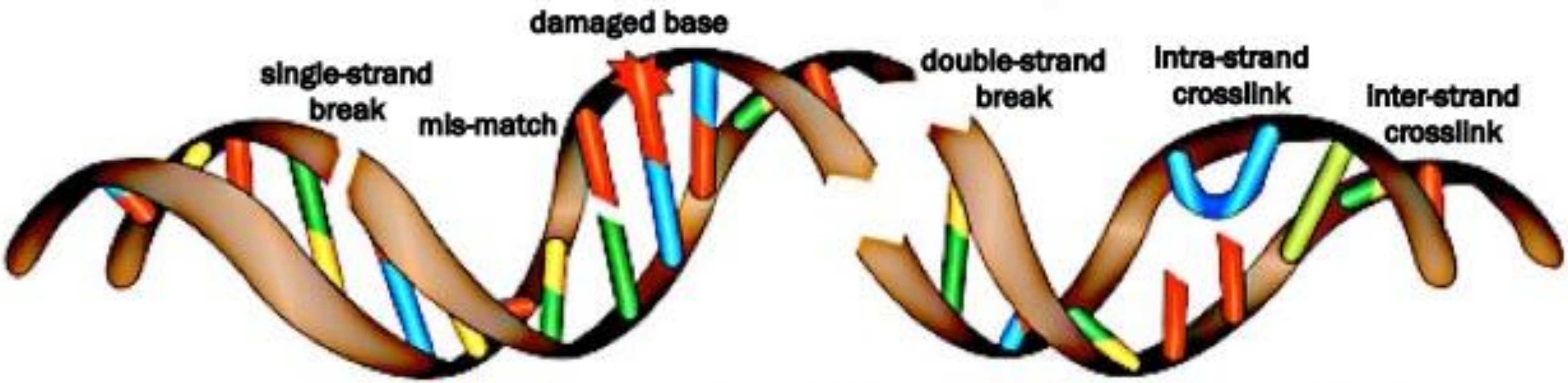
- Radiation can produce **covalent crosslinks** involving DNA.
  - An **intrastrand** **crosslink** can be formed between two regions of the same DNA strand.
  - Alternatively **interstrand** **crosslink** can be produced either between the two complementary DNA strands or between completely different DNA molecules.
  - DNA molecules can become **covalently linked to a** **protein molecule** (DNA - protein crosslink).


single break
double break
chemical change

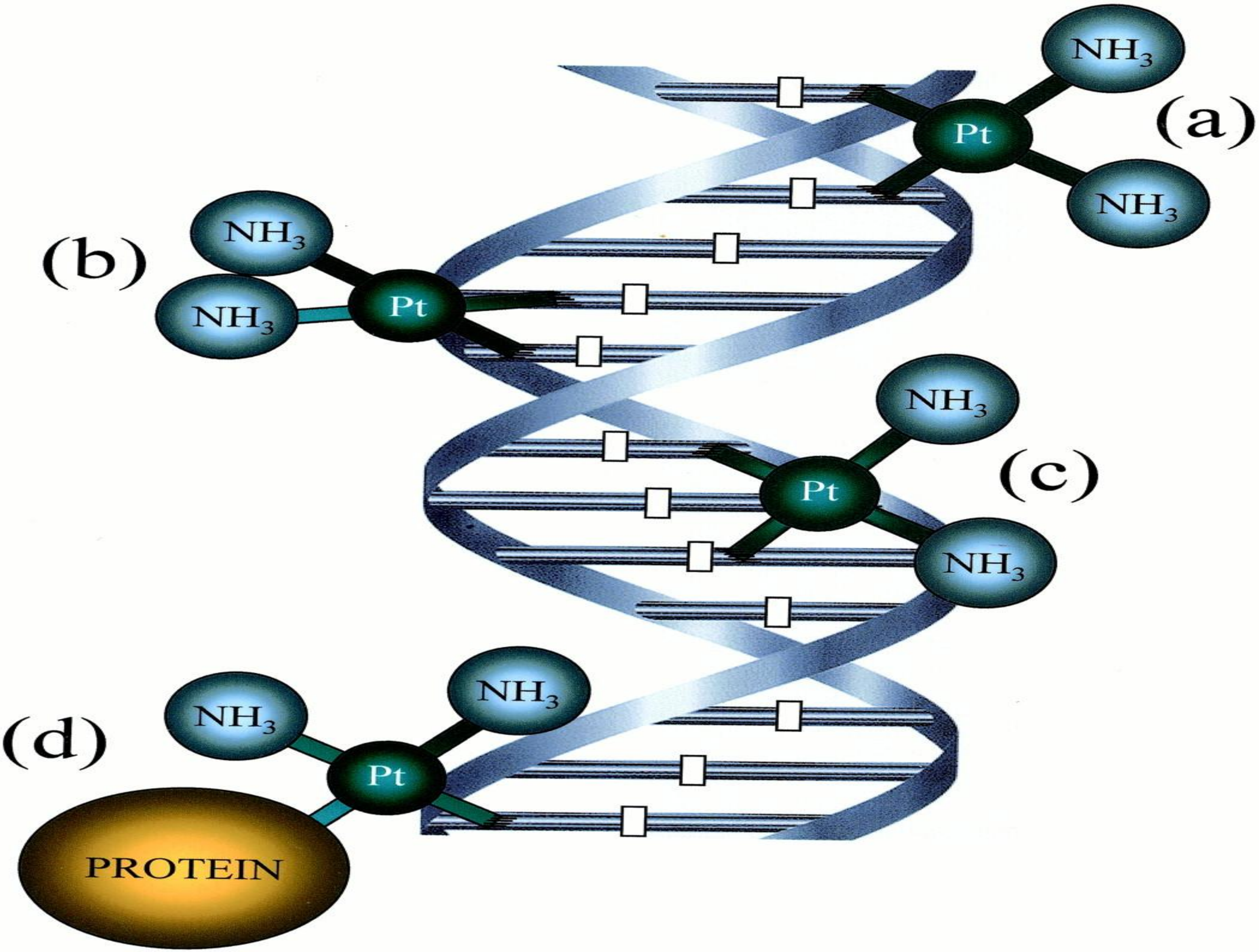

Normal DNA
Double-stranded Break
Single-stranded Break
Cross-linking

Defects in Sperm DNA Structure

Single-strand DNA break (ss-DB)
Double-strand DNA break (ds-DB)
Base deletion or modification
Inter or intra-strand cross linkage



Esteves et al 2013; Alvarez and Gosálbez 2011; Ward 2011



NH3
Pt
NH3
(a)
NH3
(b)
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(c)
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NH3
(d)
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PROTEIN

Damage of DNA synthesis

- Experimental studies show that the ionizing radiation **can depress** DNA synthesis.
- The exact mechanism is unknown, but **some hypotheses** have been suggested as follow:
  - **Decrease of DNA polymerize activity**
  - The pool size of **DNA precursors may be altered** either by a change in membrane permeability or an alteration of other biochemical factors necessary for the synthesis of nucleosides and nucleotides.
- In general the total **RNA synthesis** is **less radiosensitive** than DNA synthesis.

II. Effects of radiation on the enzymes

1. **Thimidine kinase** and **DNA polymerase** are important enzymes for the synthesis of DNA. After whole body irradiation with 1500 R (1.5 Gy) the activities of these two enzymes are reduced strongly.
2. **Monoamine oxidase** (**MAO**) is a mitochondrial enzyme and it plays an important role in deamination of the biogenic amines.
   - **A decrease** (71%) in the MAO activity in mouse **liver** is observed 3 day after 2 000 rads; however, in the kidney, heart and brain, no changes in the enzyme activities is seen.

3. The radiation releases **lytic enzymes** within the cells and thereby permits them to **attack vital structures**. The specific or total enzymatic activities of most **lysosomal enzymes (acid phosphatase, β-glucuronidase** etc.) **increase**.

- These changes are not found immediately after exposure, but develop progressively as a function of time.

Biochemical indicators of radiation injuries

- Many biochemical parameters are changed after irradiation:
  - **deoxycytidine** level in human urine is markedly **increased**;
  - **β-aminoisobutyric acid**, a metabolite of **thymidine**, markedly **increases** also in the urine of irradiated individuals;
  - the urine levels of several **amino acids - cysteic acid, valine, leucine, hydroxyproline, arginine**, etc. are markedly **increased** after irradiation;
  - **creatine**, a metabolite of **glycine** in urine is linear **increased** within exposure range 50 - 650 R of x-rays.

II. EFFECTS OF RADIATION ON CELLULAR LEVEL

- **Subcellular lesions** and particularly **chromosome damages** are very important effects of the **ionizing radiation on the cells**.
- The overall result of radiation interacting with a chromosome is **breakage of the chromosome**, thereby producing **two or more chromosomal fragments**, each having a broken end.
  - - **These broken ends** have the important capability to joint with other broken ends, possibly forming **new chromosomes**.
  - - The new chromosomes may or may not appear structurally different from the chromosome prior to irradiation.

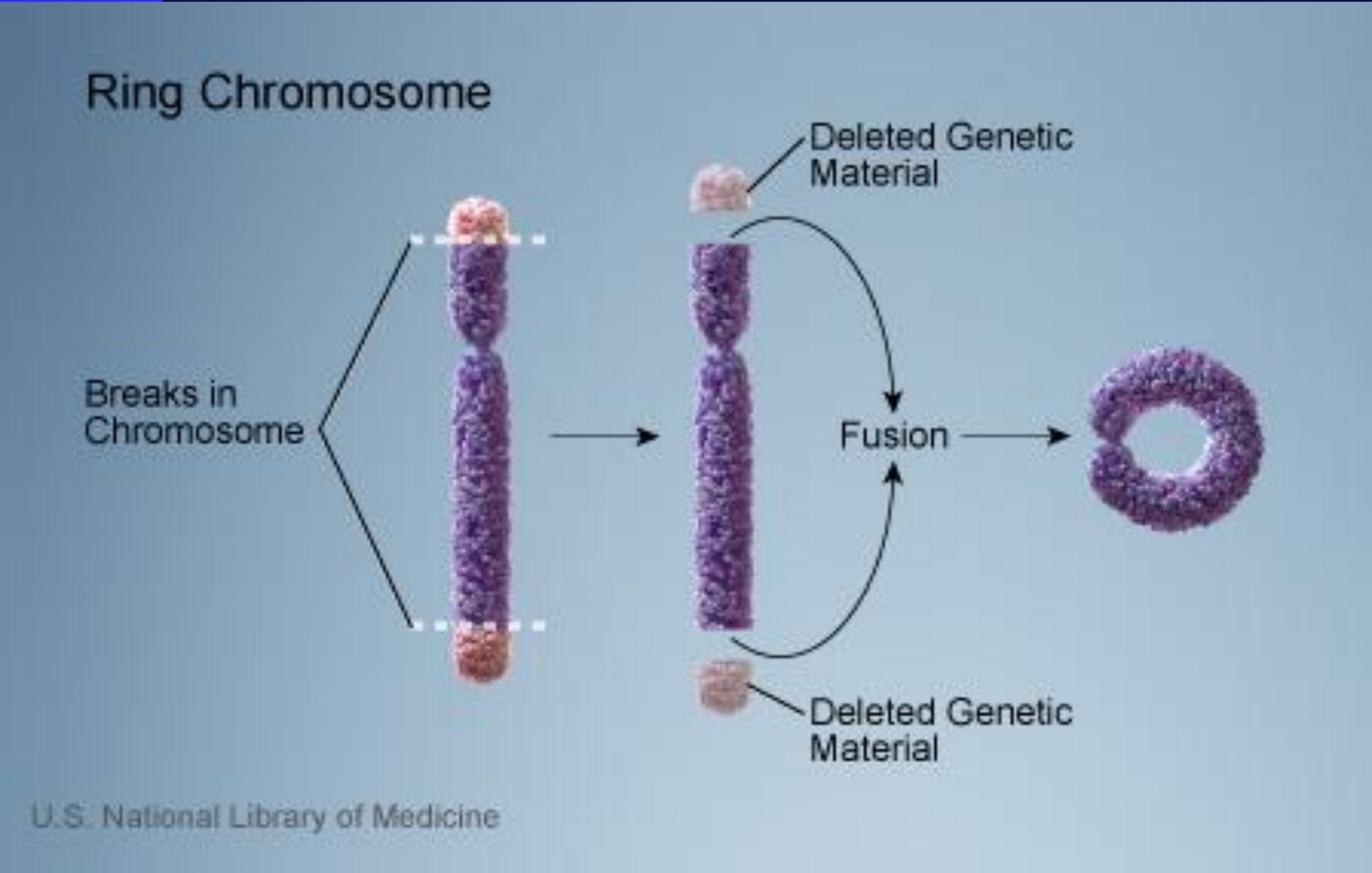
- The radiation may produce a **variety of structural changes**, some of which are:

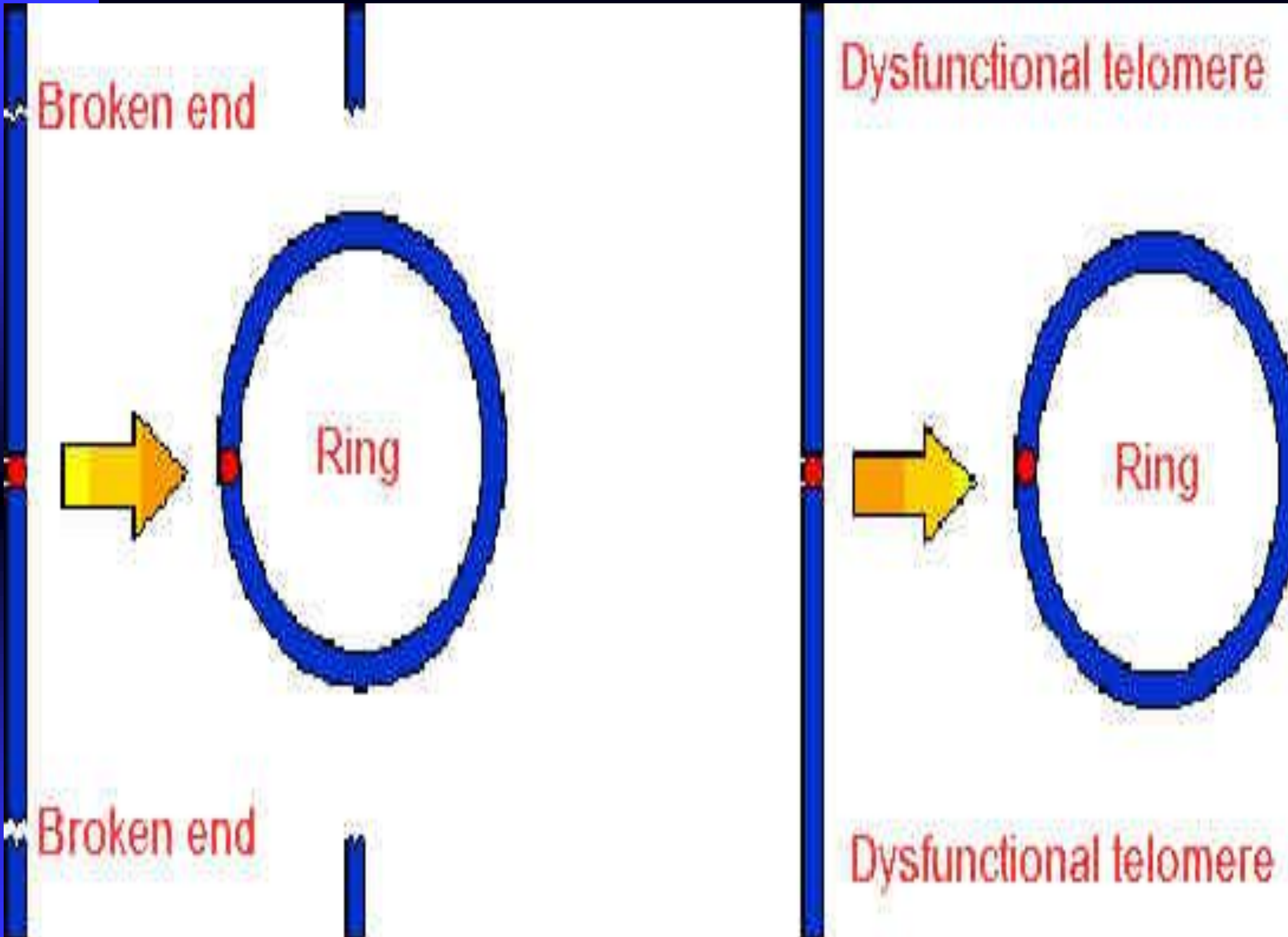
1. **A single break in one chromosome or chromatid**.
2. **A single break in separate chromosomes** or chromatids.
3. **Two or more breaks in the same chromosome or chromatid**.
4. **"Stickiness" or clumping of the chromosomes**.

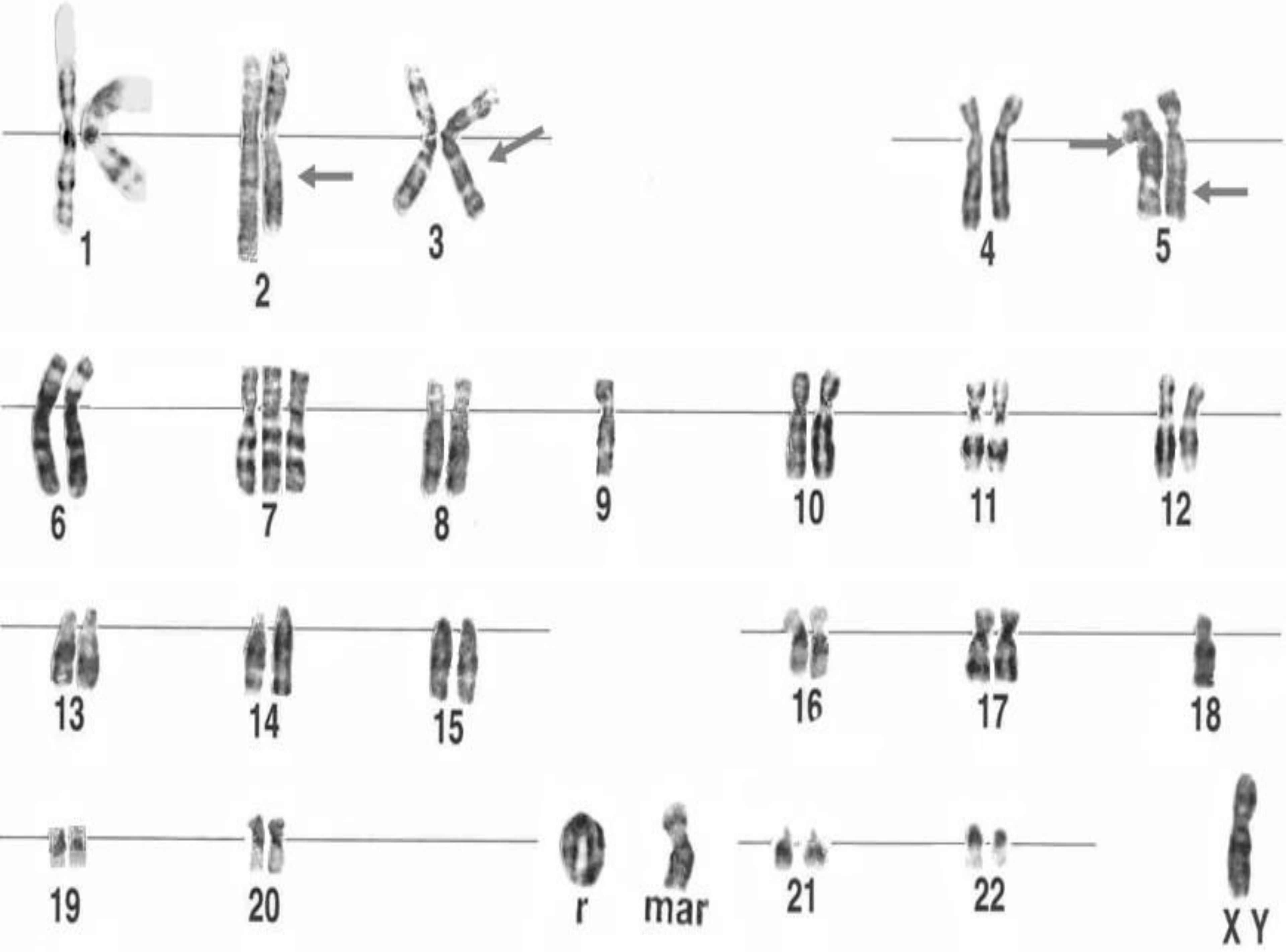
The **general consequence** to the cell of these structural changes may be one of the following:

- a) The broken ends may **rejoin with no visible damage**. This process termed **restitution** results in restoration to its pre-irradiated condition.
- b) **Loss of part of the chromosome or chromatid** at the next mitosis, giving rise of **an aberration**. This process is termed **deletion**. The resultant aberration is an **acentric fragment**.
- c) **Rearrangement of the broken ends**, which can produce a grossly distorted chromosome. Some examples are **ring chromosomes**, **dicentric chromosomes** and **anaphase bridges**.

Ring chromosome




Broken end
Ring
Broken end
Dysfunctional telomere
Ring
Dysfunctional telomere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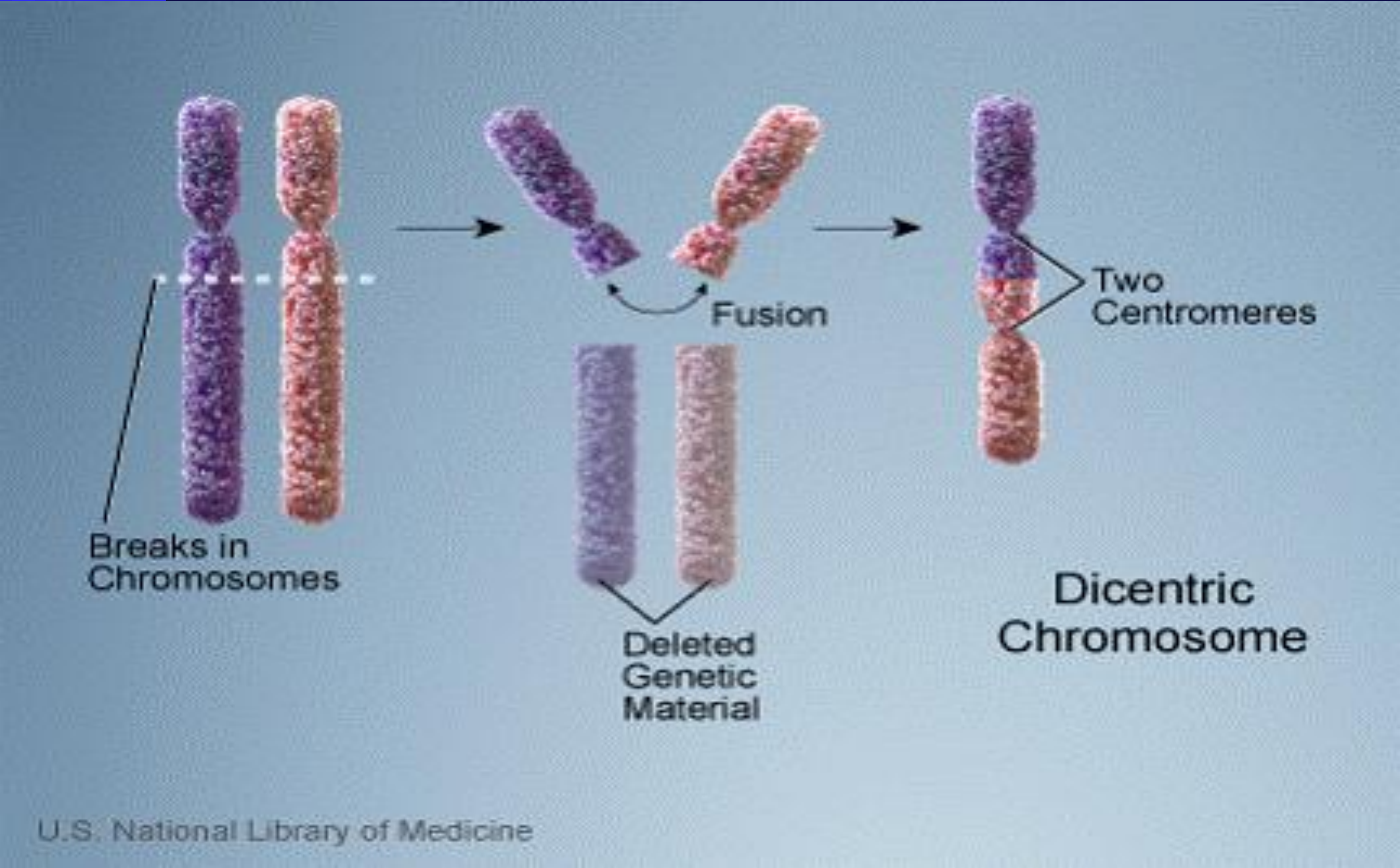
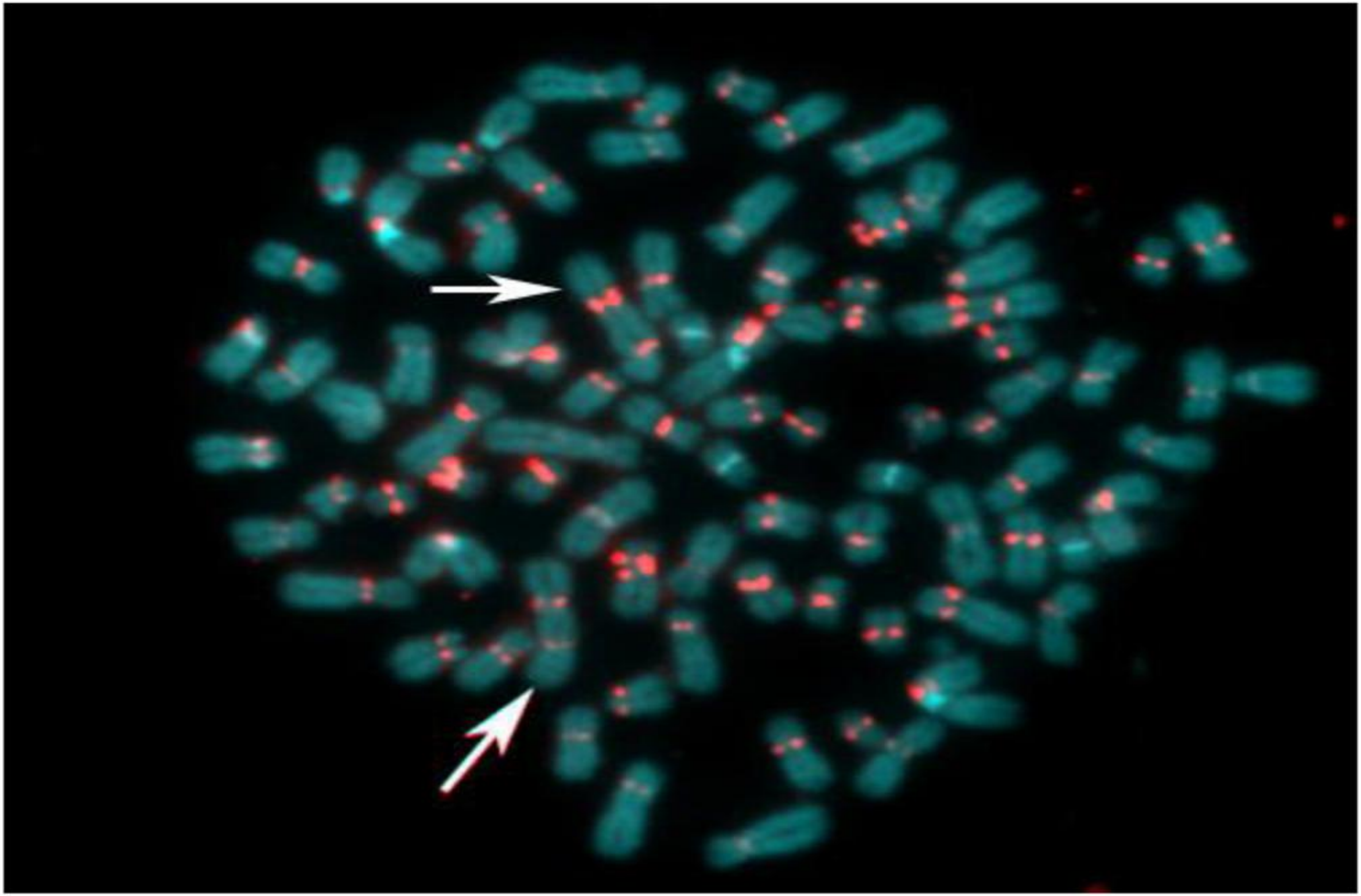
X Y

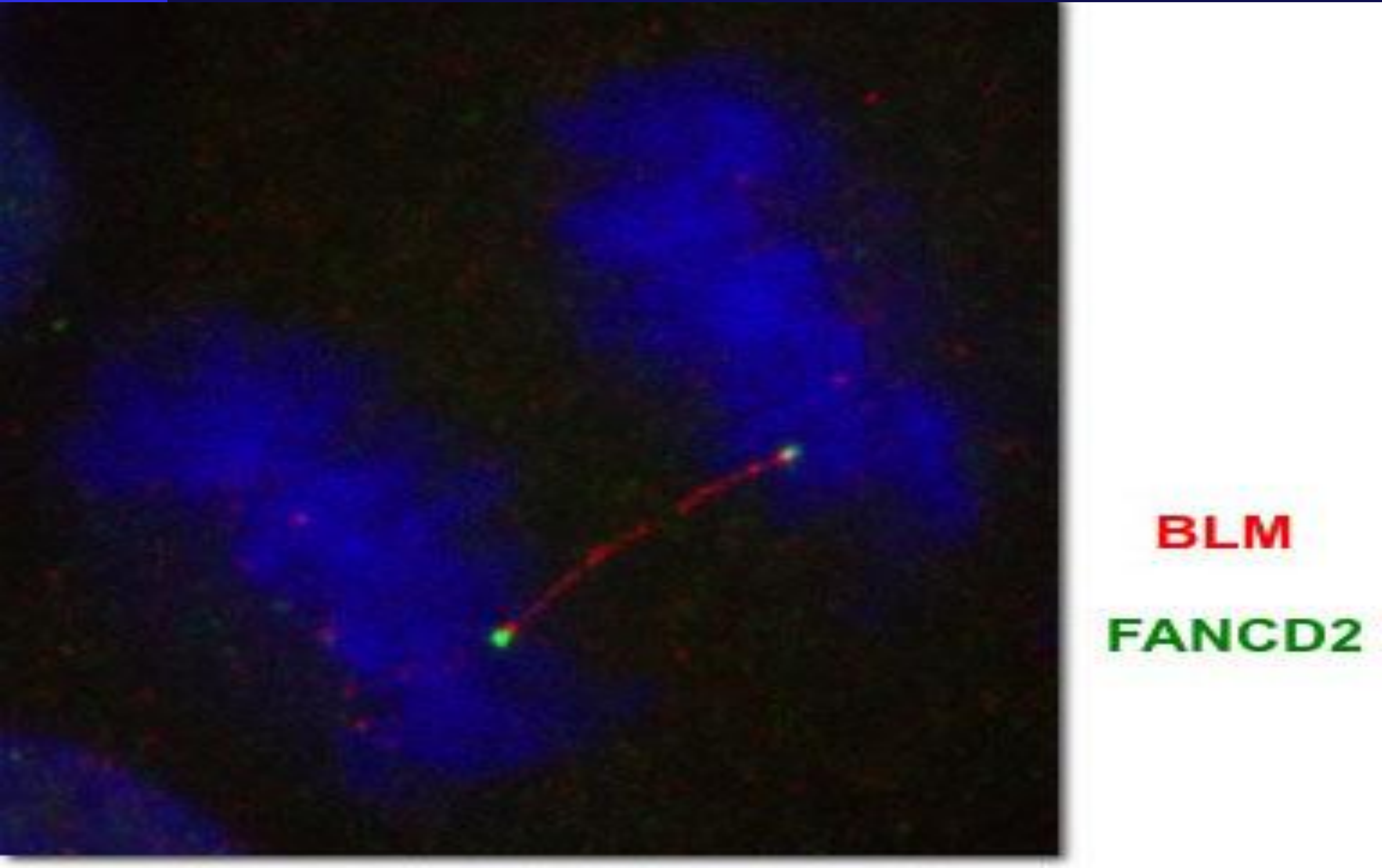
Dicentric chromosomes



Dicentric chromosomes

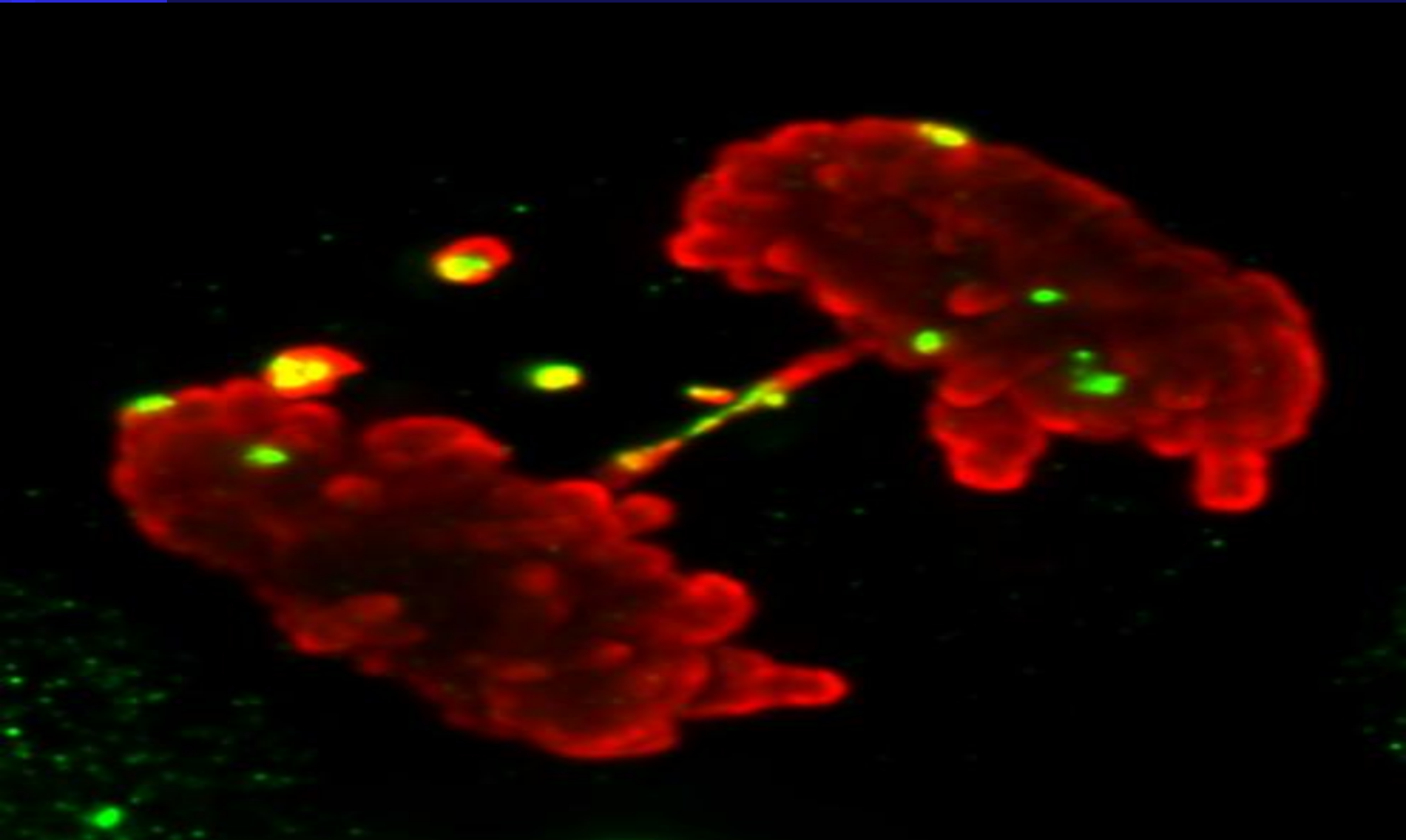


Anaphase bridges

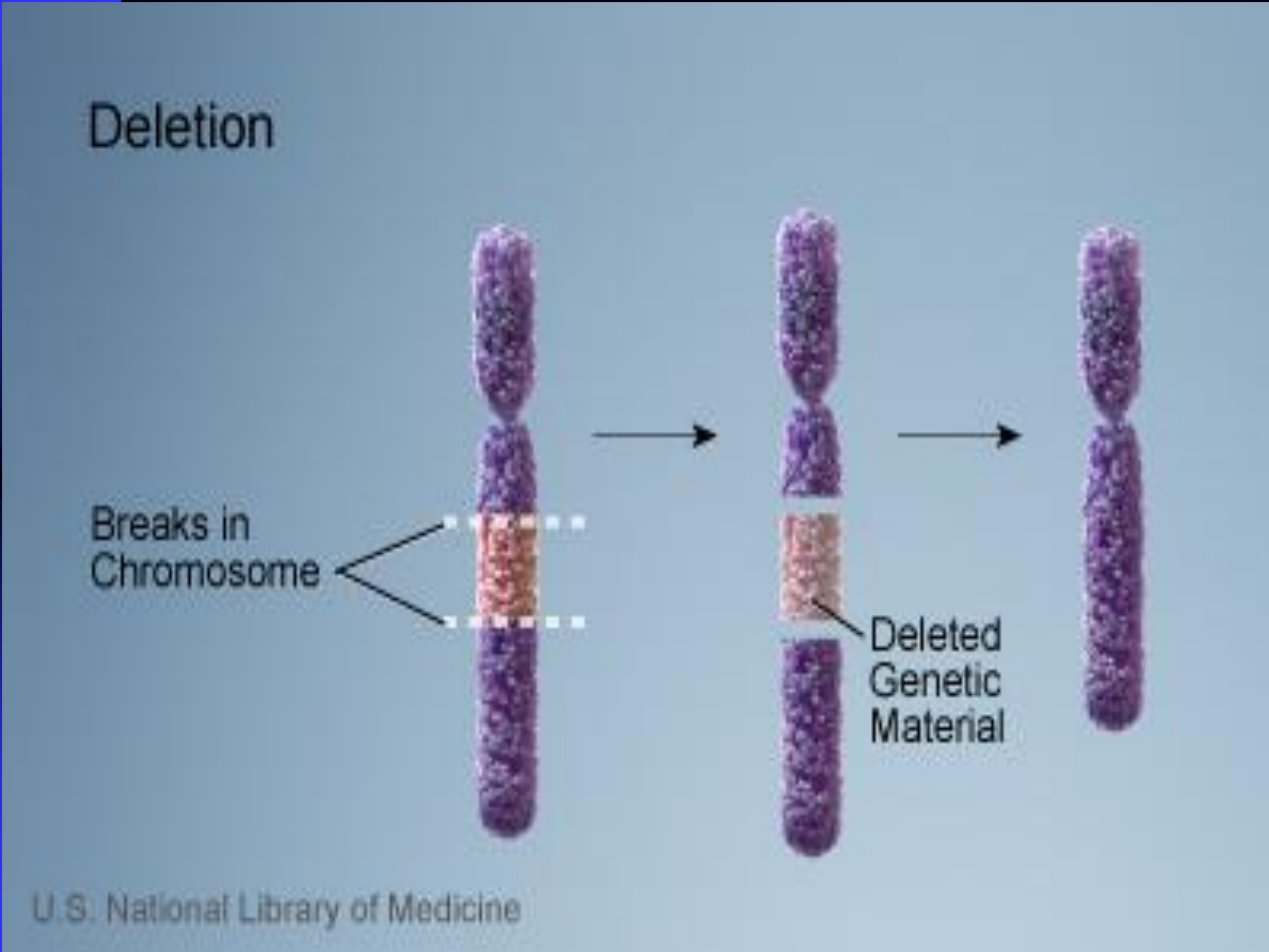


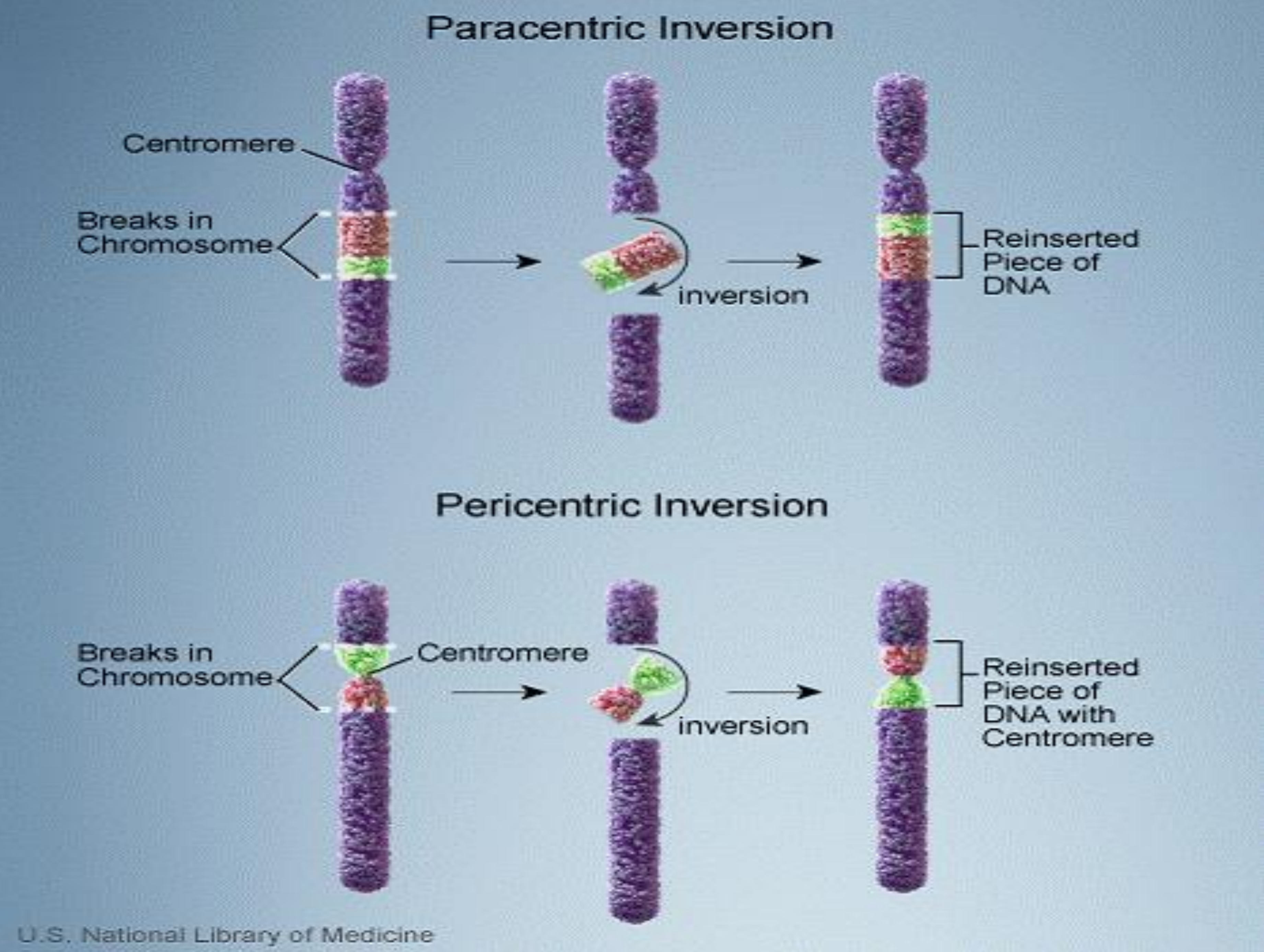
Anaphase bridges

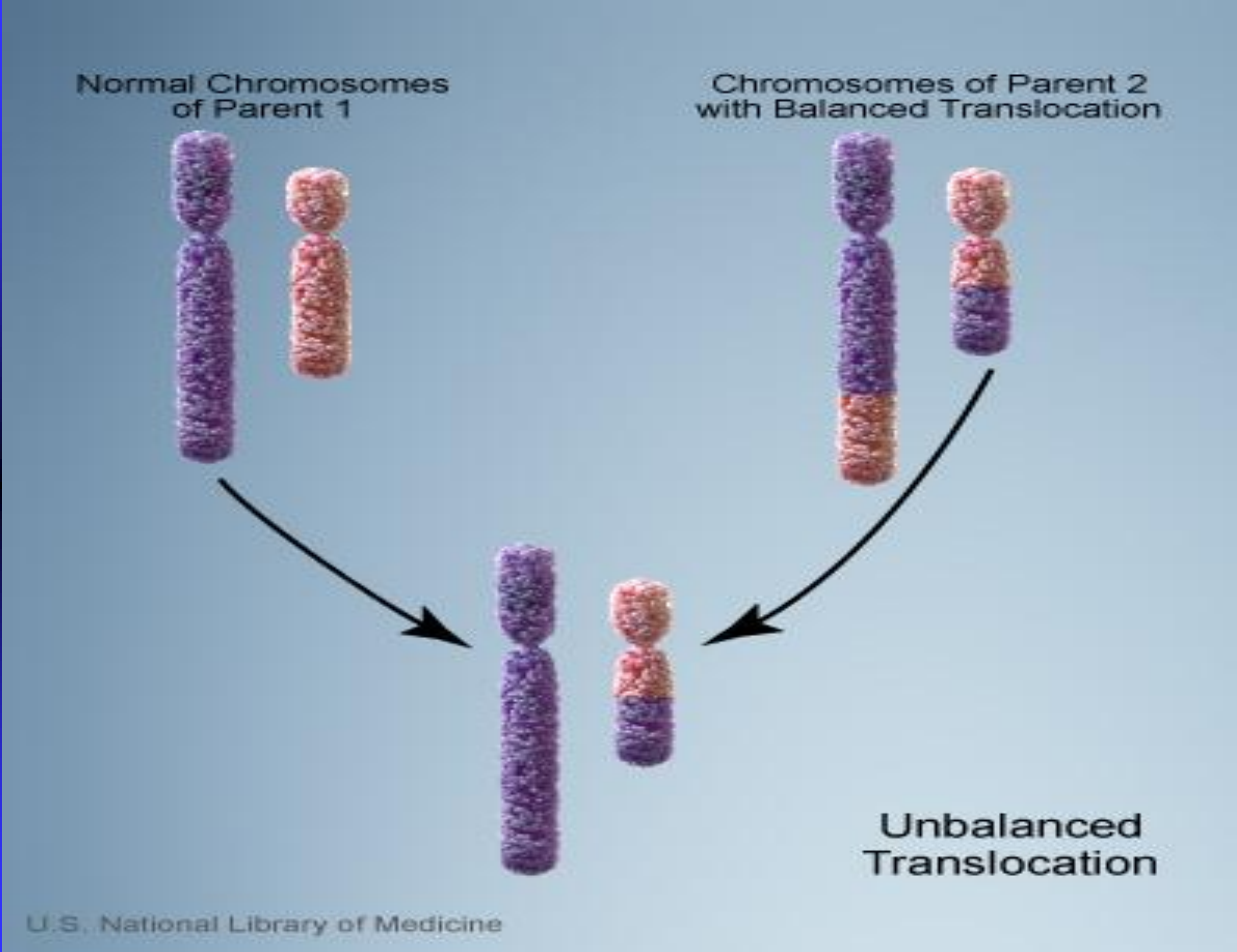
High-resolution microscopic image of an **X-irradiated human cell** which progressed into mitosis despite the presence of unrepaired DNA double-strand breaks (**red: DNA; green: double-strand breaks). An Anaphase bridge and micronuclei detached from the bulk of the genome display unrepaired double-strand breaks**.



- a) **Rearrangement** of the broken **ends without visible chromosomal damage**;
  - - the genetic material has been rearranged, but the chromosome **looks fine**. Examples are **translocations** and **inversions**.
  - - such changes result in rearrangement of the genes on the chromosomes. In this case, a **mutation** has been produced.


Deletion
Breaks in Chromosome
Deleted Genetic Material
U.S. National Library of Medicine


Paracentric Inversion
Centromere
Breaks in Chromosome
inversion
Reinserted Piece of DNA
Pericentric Inversion
Breaks in Chromosome
Centromere
inversion
Reinserted Piece of DNA with Centromere
U.S. National Library of Medicine


Normal Chromosomes
of Parent 1
Chromosomes of Parent 2
with Balanced Translocation
Unbalanced
Translocation
U.S. National Library of Medicine

- A change in a chromosome means corresponding **changes in DNA**.
  - \- Changes in DNA either an alteration in the amount of DNA resulting from a deletion or an alteration in the sequence of bases on the DNA molecule, result in a change in genetic information in the cell (**mutations**).
  - \- The **type of cell** (somatic or germ cell) in which the change occurs has **important implications**.
  - \- **Mutations in somatic cells** do not have an effect on the general population.
  - \- **Germ cell mutations** on the other hand **do affect the general population**.

Fate of irradiated cells

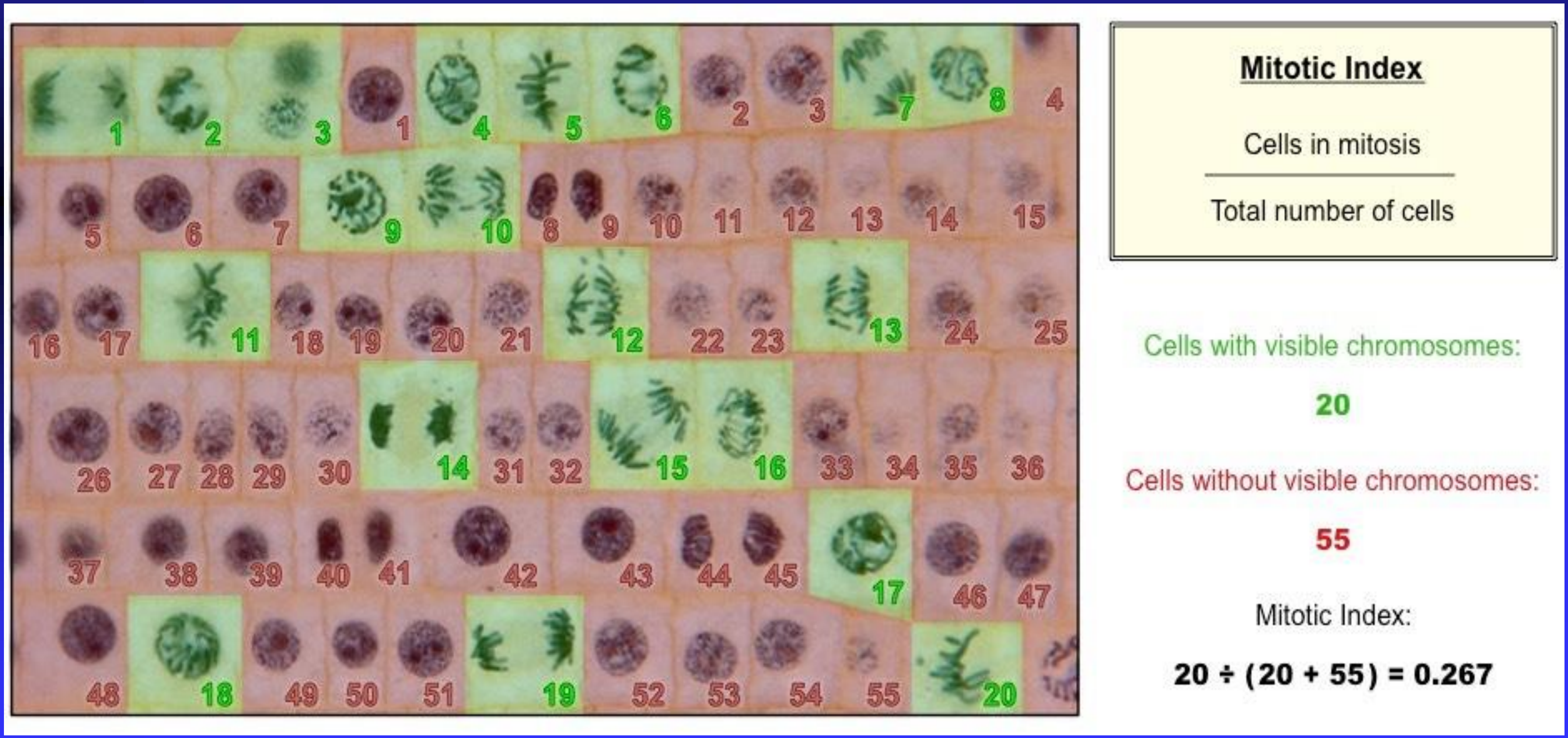
- One of **three things** can happen to a cell after irradiation:

1. **Division delay**
2. **Interphase death**
3. **Reproductive failure**

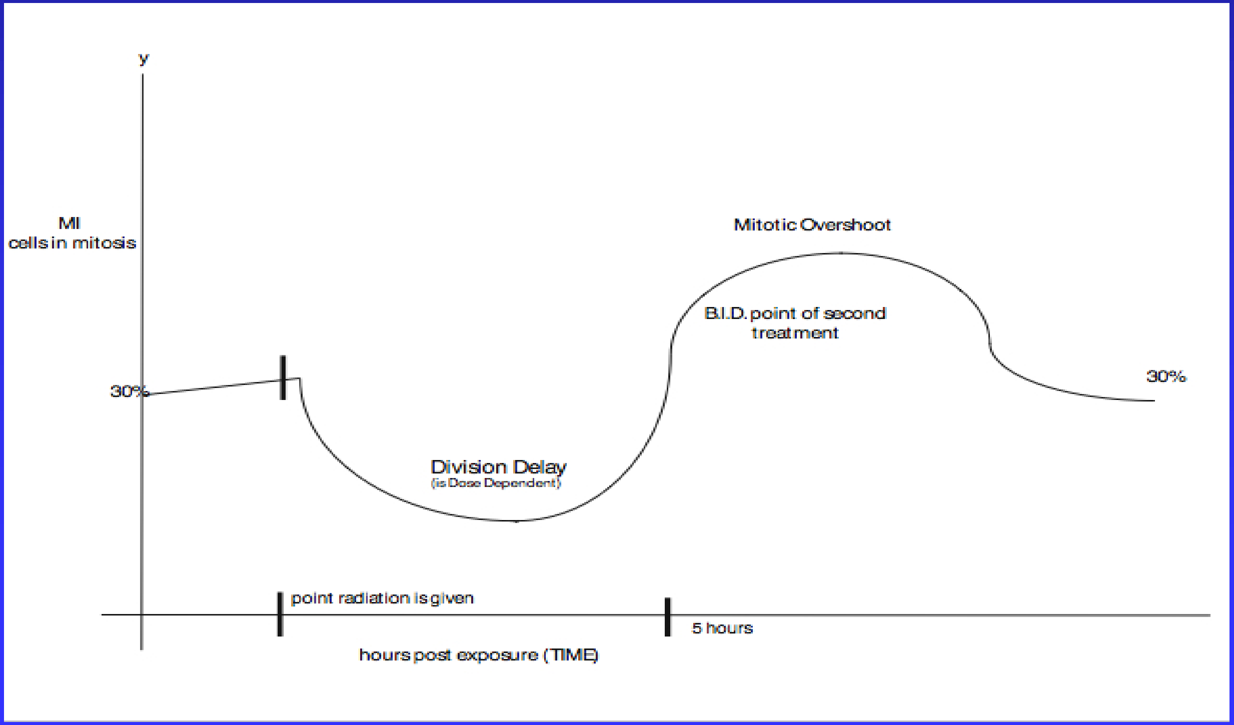
Division delay occurs in both non-lethally and lethally damaged cells.

Interphase death and reproductive failure occur **only in lethally damaged cells**.

- **The cellular response to radiation** involves mitosis. Irradiation of the cell population disturbs the ratio of mitotic to nonmitotic cells. **The ratio of the number of cells in mitosis at any one time, to the total number of cells in the population is termed the mitotic index.**



- Cells in mitosis at the time of irradiation complete division, but those about to enter division are delayed in this process. The mitotic index, therefore, **decreases** for a period of time as some cells are stopped from proceeding through mitosis at their appointed time.
- If the dose is low enough, these cells recover from this delay and proceed through mitosis - resulting in an increased number of cells in mitosis, termed **mitotic overshoot**.



- During this time, cells entering mitosis consist of two classes: these normally progressing through mitosis that was not delayed by irradiation and those that were delayed by irradiation. This cellular response to radiation is termed **division or mitotic delay**

- The overshoot was followed by a **return on the mitotic index to its pre-irradiation value** (100% value after exposure to low doses).
  - \- However, after higher doses, the mitotic index falls bellow the pre-irradiation value and remains there.

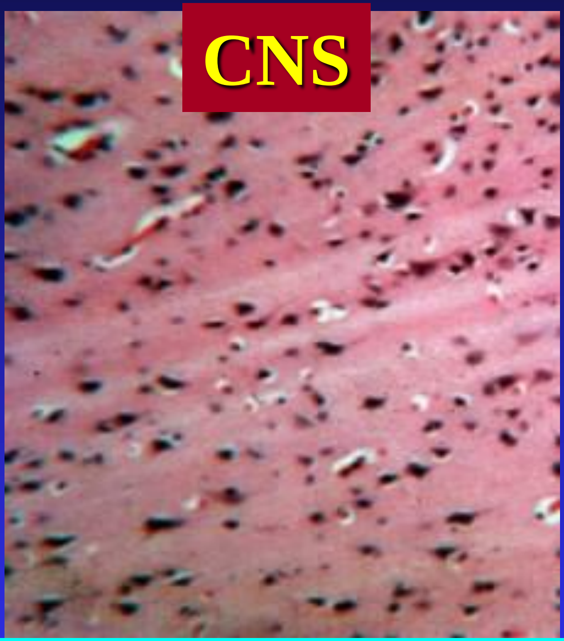
- At these doses another mechanism of damage was **operational**: **the cells divide but died** after division. This response is termed **reproductive failure**.
- Following an irradiation of dose of **10 Gy the length** and **magnitude** of delay greatly **increase**, **but no mitotic overshoot occurred**.
- This phenomenon indicates that the **cells are not able to overcome the block imposed by the radiation**, and therefore died before division. It is the **interphase death.**

- **The interphase death** morphologically is not a degenerative process, like cellular necrosis. In interphase death the cell condenses and breaks up into pieces, but the **cytoplasmatic organelles remain intact**. These pieces are then phagocytosed by other cells.
- Chromosomes are not the only organelles affected by radiation.
  - \- **Permeability of the cell membrane** is altered after irradiation, affecting the transport function of the membrane and its ability to keep molecules in or out the cell.
  - \- Alteration of membrane by radiation can also affect organelles in the cell that are membrane bound e.g. **mitochondria and lysosomes.**

III. EFFECTS OF RADIATION ON NORMAL TISSUES

- In general, **inflammation, edema, hemorrhage** and **denudation of mucosal surfaces** characterize **acute damages** in most organs.
- **Chronic changes** consist of **fibrosis, atrophy, ulceration, stricture, stenosis, and obstruction**.
- The radiosensitivity of the various tissues may be estimated approximately by **the law of Bergonie and Tribondeau.**
  - **Less differentiated cells are more radiosensitive than highly differentiated ones.**
  - **Proliferating tissues are more radiosensitive than non-proliferating ones.**
- with the except of **lymphocytes**, which are very radiosensitive in spite of the fact that they are highly differentiated and are not dividing.

Radiosensitivity of tissues



Highly radiosensitive

- Lymphoid tissue
- Bone marrow
- Gastrointestinal epithelium
- Gonads
- Embryonic tissues

Moderately radiosensitive

- Skin
- Vascular endothelium
- Lung
- Kidney
- Liver
- Lens (eye)

Least radiosensitive

- Central nervous system (CNS)
- Muscle
- Bone and cartilage
- Connective tissue

Haematopoietic system

Bone marrow

a) Bone marrow

- **The primary effect** of radiation on the bone marrow is the **decrease the number of stem cells.**
- **Low doses** result in a slight decrease with recovery occurring within a few weeks post exposure.
- **Moderate and high doses** produce a more severe depletion of cells in the bone marrow, resulting in either a longer period of recovery or less recovery, manifested also with increase in the amount of **fat and connective tissue.**

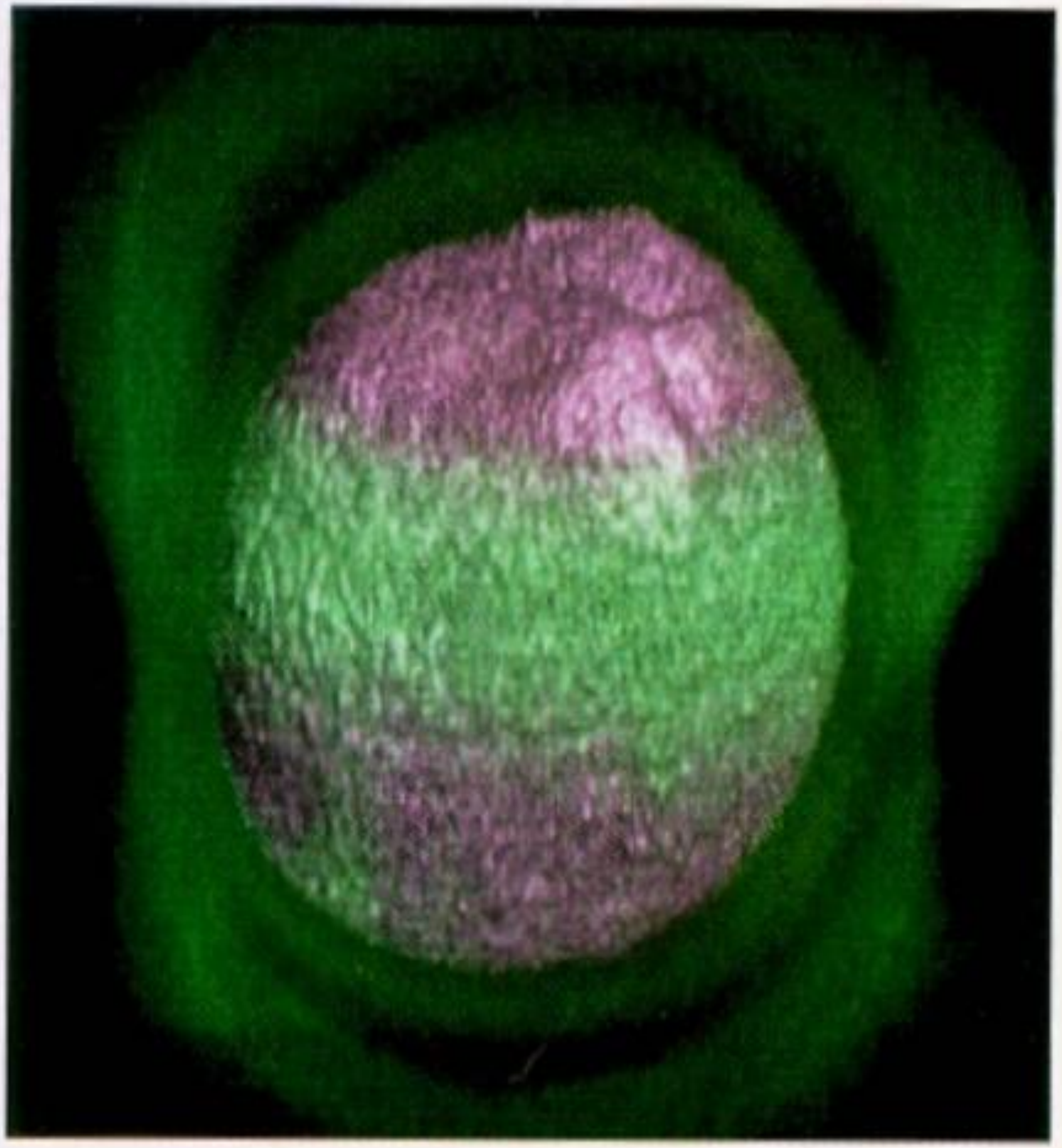
- Although all stem cells in the bone marrow are very radiosensitive, there are variations in their sensitivity.
  - **The most** radiosensitive are **erythroblasts**;
  - **Second** in sensitivity are **myelocytes**;
  - The **least radiosensitive** are **megacaryocytes**;

b) Circulating blood

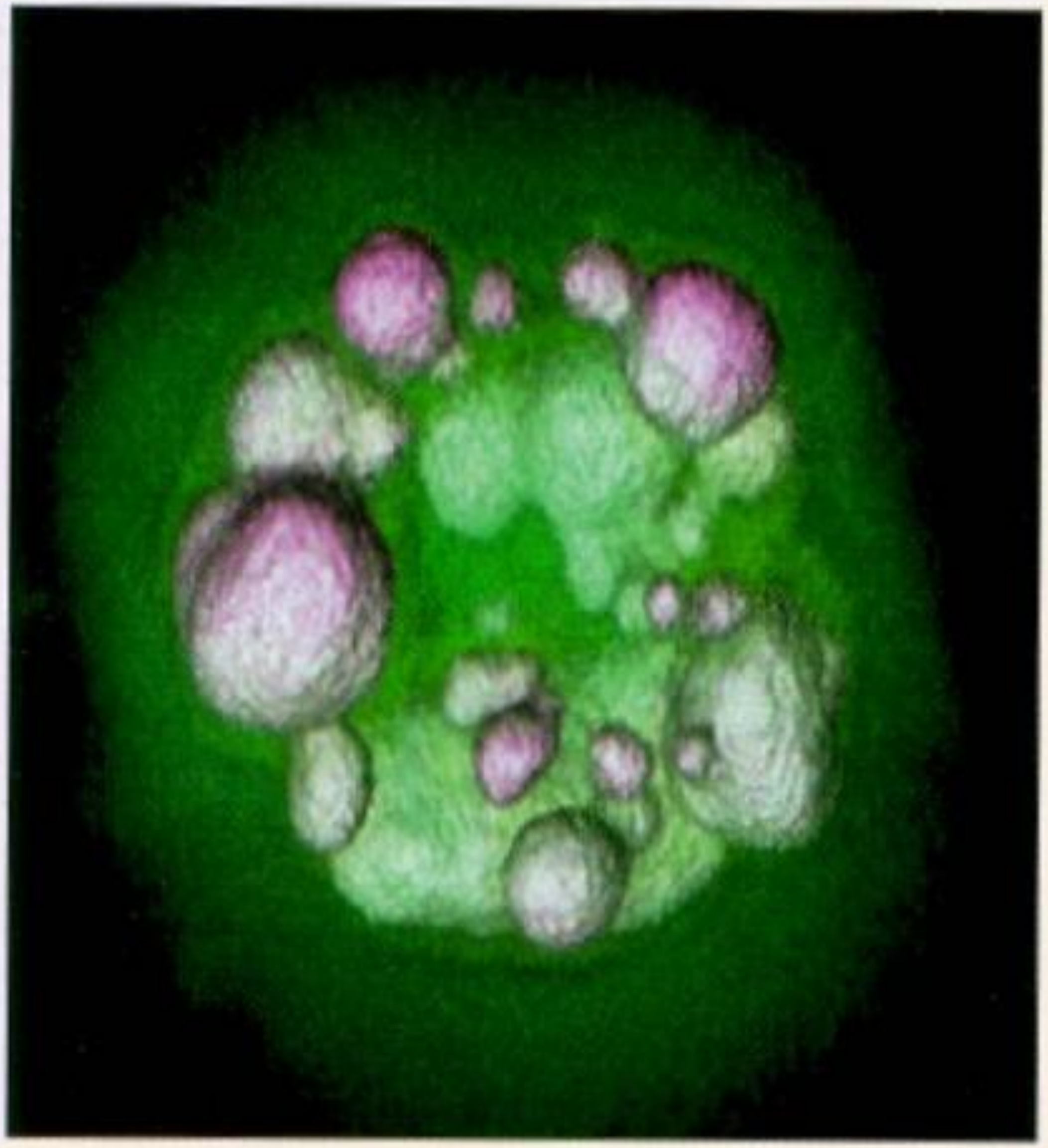
- With the **exception of lymphocytes**, the cells in the **circulating blood are resistant to radiation** (they are non-dividing and differentiated cells).

- The circulating blood reflects radiation damage of the marrow and depends on two factors:
  - **The sensitivity of the different stem cell.**
  - **The lifespan of each cell type in circulating blood.**
- **First decreases the number of the lymphocytes** (by doses as low as **0,1 Gy**).
- **Second decrease the neutrophyls** (doses over **0,5 Gy** produce decrease).
- **Third decrease the platelets and RBS$_s$** (at doses greater than **1 Gy**).
- **Lymphocyte counts** will approach **zero** within **a few days** following moderate doses, with **full recovery** occurring **a few months** post exposure.
- **Granulocyte** counts **fall to minimal** values approximately **1 week** following **a moderate dose**. **Their recovery** begins soon and approaches normal values **within a month** post exposure.
- **The lower doses** in the moderate range will have a minimal effect on **platelets** and **RBS**$_s$, but **the higher doses** at this range result in marked **depression** of these cells.

Normal human lymphocyte: chromosomes uniformly distributed



Apoptotic cell: chromosomes and nucleus fragmented and collapsed into apoptotic bodies



2. Reproductive system

a) Male

- Most of the tissues of the male reproductive system, with the exception of the testes, are **radioresistant**.
    - The testes contain both nondividing, differentiated, **radioresistant cells** (**mature spermatozoa**) and dividing undifferentiated **radiosensitive cells** (**immature spermatogonia**).
- The primary effect of radiation on the male reproductive system is damage and depopulation at the spermatogonia, eventually resulting in depletion of mature sperm in the testes, a process termed **maturation depletion**.

a) Male

- A variable **period of fertility** occurs after irradiation, attributable to the radioresistance of the mature sperm, and it is followed by **temporary or permanent sterility** (depending of **the dose).**
- **2.5 gray to testis causes temporary sterility**
- **5-6 gray to testis causes permanent steritity**
  - **Sterility is due to a** loss of the immature spermatogonia, which divide and replace the mature sperm lost from the testes.
- Another potential hazard of testicular irradiation is the production of **chromosome aberrations** that may be passed on the succeeding generations.

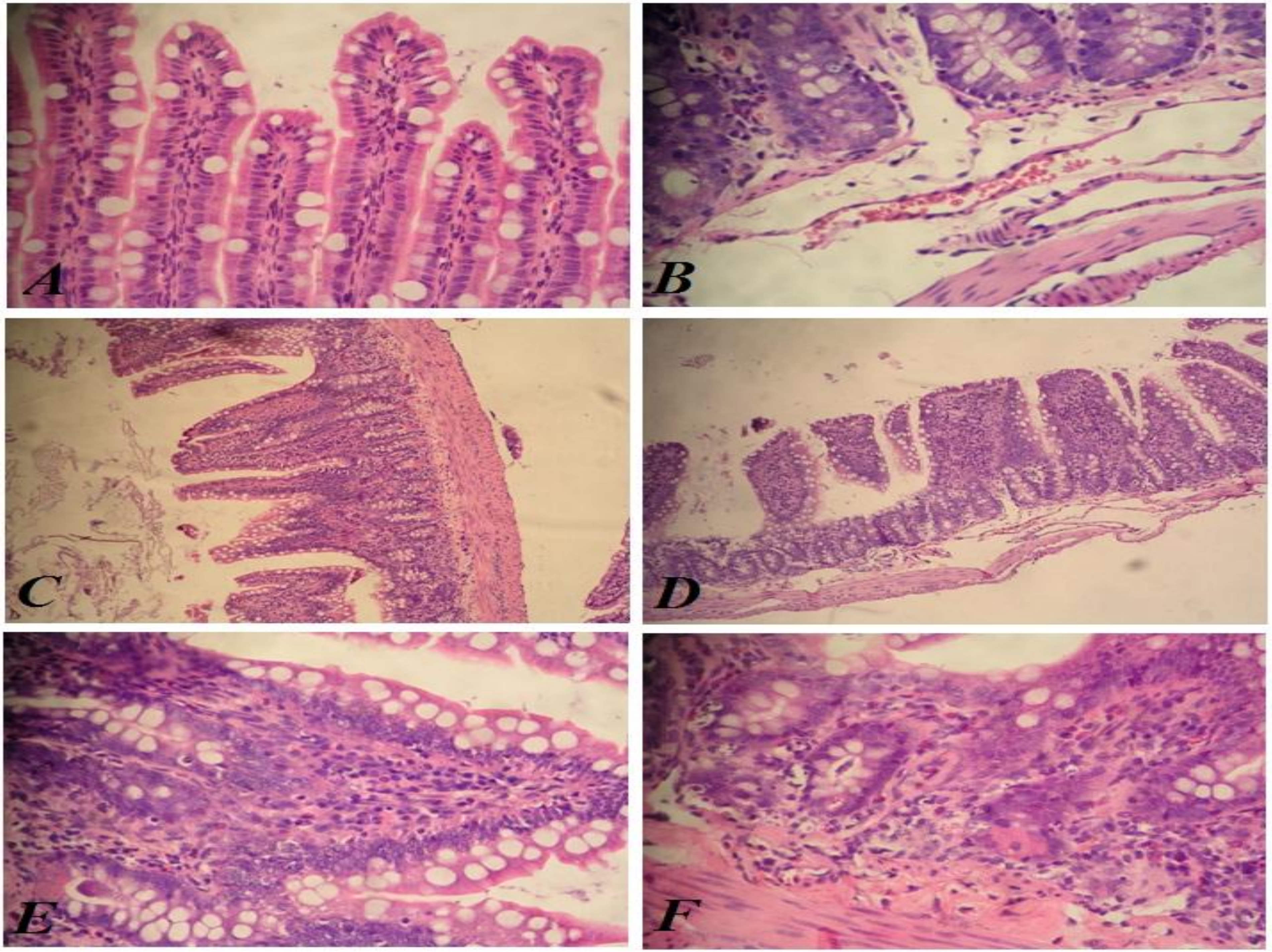
b) Female

- The radiosensitivity of the **follicles** is as follow:
  - **small follicles** are most **resistant**;
  - **intermediate follicles** are **most sensitive**;
  - **mature follicles** are **moderately sensitive**;
- An **initial period of fertility** occurs after moderate doses of radiation to the ovaries, due to the presence of moderately resistant mature follicles that can release an ovum.
  - This fertile period is followed by **temporary** or **permanent sterility** from damage to ova in the **radiosensitive intermediate follicles**.
- The doses which are greater than **6,25 Gy** produce sterility in women. **Oocytes do not divide** – thus no repopulation.

3. Digestive system

- Moderate to high doses of radiation produce **inflammation** in the **mucous membranes of the oral cavity** and **esophagus**.
  - Healing occurs, with minimal chronic changes, after moderate doses, while high doses results in **atrophy, ulceration, fibrosis** and **esophageal stricture**.
- The **stomach** appears to be **more sensitive than the esophagus**. The moderately high doses produce ulceration, atrophy and fibrosis.
- The **small intestine** is the **most radiosensitive** portion of the gastrointestinal (GI) tract.
  - The radiation damages mainly the cells of the crypts (of Lieberkuhn) - a rapidly dividing, undifferentiated stem cell population.

- **Moderate doses** of radiation result in **shortening of the villi** due to **killing of crypt cells**, followed by regeneration of these cells with a corresponding repopulating and healing of the villi.
- **After high** doses more cells are killed, minimal recovery occurs, the **villi** become **shortened** and **flattened** and the intestine may become **denuded**, leading to **ulcerations, hemorrhages, fibrosis** and **necrosis**.
- The **rectum** is much **more resistant** to radiation.


A
B
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4. Skin

- **Acute changes** in the skin following a moderate or high dose of radiation are **inflammation, erythema, and dry or moist desquamation**.
- Moderate doses permit healing to occur in the epidermis by regenerative means, resulting in minimal chronic changes.
- **Chronic changes** such as **atrophy, fibrosis**, decreased or increased **pigmentation, ulceration, necrosis**, and **cancer** may be observed after exposure to **high doses**.
- **Hair follicules** are **radiosensitive**. The moderate doses cause a temporary **epilation**, while the high doses may cause permanent epilation.
- **Sebaceous** and **sweat glands** are relatively **radiosensitive**.
- Damage after high doses produce **glandular atrophy** and **fibrosis**, resulting in minimal or **no function**.

5. Eye

- The lens of the eye contains a population of actively dividing cells that may be damaged and destroyed by irradiation.
  - Because there is **no mechanism for removal of injured cells**, those damaged cells **form a cataract**.
  - Moderate doses of radiation produce **cataract**
    - Seen with doses as low as **2 gray**
    - Very likely at **4 gray**
  - but an acute dose of **7 Gy – 100%**

Cataracts after Radiation Treatment (dr Paton's case)

6. Central nervous system

- In general the cells of the various parts of the nervous system are **nondividing differentiated cells**, rendering them **relatively radioresistant**.
- In fact, the nervous system is considered **the most radioresistant system** in the **adult**:
  - therefore, low and moderate doses will result in minimal, if any, **morphological damage**.
  - however, some authors have reported early functional changes in low doses.
- **Early changes** in CNS after high doses include **inflammation**, progressing to **necrosis and fibrosis** of the brain or spinal cord.
  - These early changes are thought to be due to **loss of glial cells**, whereas the chronic changes are suggested to be of vascular origin.

Radiosensitivity of Various Cell Types	
Radiosensitivity	Cell Type
Low	Nerve cells Muscle cells
Intermediate	Osteoblast Endothelial cells Fibroblast Spermatids
High	Spermatogonia Lymphocytes Stem Cells Intestinal mucosa cells Erythroblast