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Handbook of Radiobiology

for Postgraduate Students of Medical Physics, Radiology & Imaging and Radiation Oncology



Thayalan Kuppusamy

Foreword
PG Gopalakrishna Kurup

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Interaction of Radiation with Cell

CHAPTER 3

SEQUENCE OF RADIATION EVENTS

Radiation can damage cell and result in biological effects in a multicellular organism. Therefore, the study of the radiation effects at cellular level is more useful to know about the radiation damage. The time interval between the radiation exposure and manifestation of biological effects can be divided into three stages (Fig. 3.1), namely:

- Physical stage
- Chemical stage
- Biological stage.

Physical Stage

The physical stage refers to the interaction between charged particles and the atoms/molecules of tissues. In this stage, the atoms or biomolecules absorb the radiation energy and undergo ionization and excitation, and release electrons. These electrons are called secondary electrons and they transfer energy to the surrounding by further excitation, ionization and thermal heating in the medium. Its duration is about 10^{-7} s and the deposition of energy is rapid and random.

The secondary electrons may produce additional low-energy electrons, known as delta (δ)-rays. About 1,000 δ-rays are produced by a single 30 keV electron. These δ-rays may cause additional excitation and ionization in the medium. Thus, very low-energy electrons are produced, whose energy is < 7.4 eV, the first excitation potential

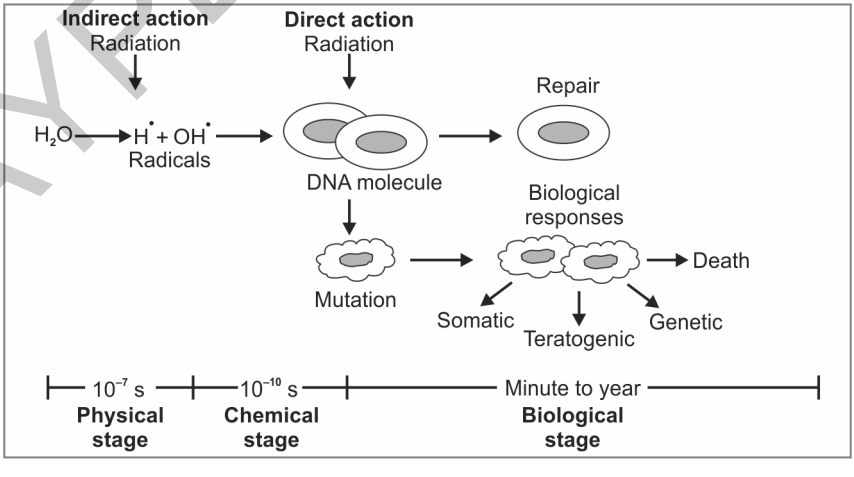

FIGURE 3.1: Interaction of radiation in cell; physical and biological response of ionizing radiation

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of liquid water. They transfer their kinetic energy to water molecule by vibrational, rotational and collisional energy exchanges.

The low-energy electrons of δ-rays form a unique ionization pattern in their track over a short range of 4–12 nm. The energy deposition of about 100 eV in the shorter track is called spurs, whose diameter is about 4–5 nm. The energy deposition of about 300–500 eV in a pear-shaped track (12 nm) is called blobs. It is believed that there are spurs and blobs along the tracks of the charged particle. A spur may contain 3 ion pairs, whereas a blob may contain 12 ion pairs and deposit energy non-uniformly in the medium.

Since the dimensions of spur and blobs are similar to that of a deoxyribonucleic acid (DNA) (2 nm), there will be an overlap between them. This initiates active and multiple radical interactions with DNA strands, resulting in variety of damages. A spur can produce damage in multiple locations in the DNA, which are close to each other. The types of damages are clustered damage, complex damage and locally multiple damaged sites. In the case of low-linear energy transfer (LET) such as X- and gamma (γ)-rays, about 95% of the energy deposition is due to spurs. These spurs and blobs produce free radicals that increase the probability of damage.

Chemical Stage

In the chemical stage, the damaged atoms and molecules further reacts with the cellular components through chemical reactions. The exposed biomolecules rearrange themselves, which results in formation of primary lesions in them. Primary lesions are transformed into bioradicals, resulting in molecular alterations. Bioradicals can also be formed with the interaction of radicals with biomolecules. The structural changes include:

- Hydrogen bond breakage
- Molecular degradation
- Inter- and intra-molecular crosslinking.

The duration of the above event is about 10^{-10} s.

Biological Stage

The biological stage starts with enzymatic reactions, which act on the residual chemical damage. Majority of the lesions are repaired and lesions that are not repaired properly result in cellular death or mutations in cells. Increased number of cell death may lead to organ death that appears as clinical changes. The biological effect includes lethality, mitotic inhibition, division delay, chromosome aberrations and induction of mutations. These effects can create radiation sickness, resulting in delayed somatic effects in humans.

Cell may take time to die and may undergo mitotic division before death. The death of stem cells appears as early effects. After the cell killing, compensatory cell proliferation occurs in the tissue. Mutation can occur in both:

- Germ cells
- Somatic cells.

The mutation in germ cells may result in hereditary effects, whereas cancer induction is the outcome in somatic cells. The time schedule for biological effects is few minutes to several years. Presence of chemicals such as radioprotectors and sensitizers during the radiation exposure may alter the above response. Protectors mostly reduce indirect action by scavenging radicals, whereas sensitizers enhance the damage of the biomolecules. At the biological stage, the altered DNA molecule may be repaired by cellular repair process. This may influence post repair processes, which can modify the magnitude of the effects.

DIRECT AND INDIRECT ACTION

Direct Action

Biological effects resulting from the direct interaction of radiation with the target sites is called the direct action. There are critical sites or targets within the cells, which must be damaged in order to kill the cell. DNA is considered as critical target for radiation exposure. In a

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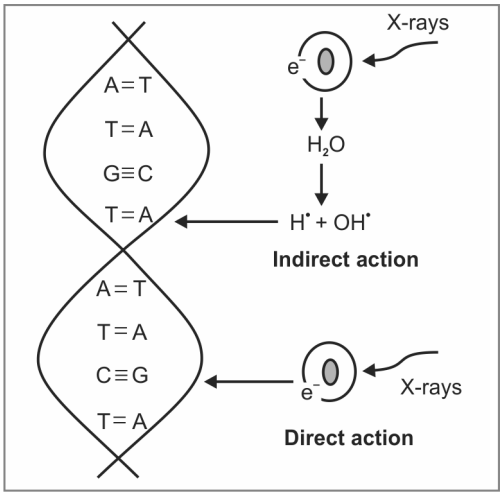

FIGURE 3.2: Direct and indirect action of radiation on a deoxyribonucleic acid (DNA) molecule

direct action, the radiation interacts directly with the critical target and creates biological effects (Fig. 3.2). It is a dominant process with high-LET radiations such as alpha (α), neutrons, etc. It involves rupture of cell membrane and breaking of chromosome structure, resulting in DNA strand breaks. The fragments of chromosomes produced in a direct interaction, which can join together in a wrong manner, result in chromosomes with abnormal structures. This is known as chromosomal aberration. The frequency of chromosomal aberrations increases with radiation dose, and hence, the magnitude of aberrations is a biological indicator of radiation dose absorbed in human body. Chromosomal aberration analysis (CAA) is useful in determining the radiation dose received by a person who is accidentally exposed to high radiation dose (> 100 mGy).

Indirect Action

Alternatively, the radiation can interact with other molecules (e.g. water) and forms free radicals. The interaction of free radicals with critical sites may also result in biological effects, which is called indirect action. For example, radiation interacts with oxygen and water molecules present in the cell. These interactions produce a large number of free radicals, which are atoms or molecules, or ions with an unpaired electron in the outermost shell, and hence, are highly reactive. Since electrons are spinning, a pair of electron spins both clockwise and anticlockwise, giving stability. Unpaired electron spins in one direction and is unstable. Free radical has high degree of chemical reactivity and interacts strongly with biomolecules. This is a major source of radiation damage. Human body tissue is composed of 80% water and the major interaction is indirect action (66%) through water. The effects of X- and γ-rays in macromolecules of living system are mainly due to indirect interactions.

RADIOLYSIS OF WATER

Absorption of radiation by water molecule (radiolysis) leads to ionization and results in ion pairs (H_2O^+) and electrons (e^-). The electron will leave the parent molecule and get trapped among other water molecules. The water molecule due its polar nature, tend to have its positively charged hydrogen atom facing the electron and the negatively charged oxygen atom away from the electron (Figs 3.3A to C). The electron, which is surrounded by a sphere of polar water molecules, is said to be hydrated, and hence, it is called aqueous or hydrated electron (e^-_{aq}). The e^-_{aq} is a transient species with a life time of about 1 μs in pure water and absorption band of 720 nm. It reacts with another water molecule to form negative water ion (H_2O^-) and attains stability with a life time of few microseconds.

Ionization

$$H_2O \longrightarrow H_2O^+ + e^- \longrightarrow H_2O^+ + e^-_{aq} \quad \text{----- (1)}$$

$$H_2O + e^-_{aq} \longrightarrow H_2O^- \quad \text{----- (2)}$$

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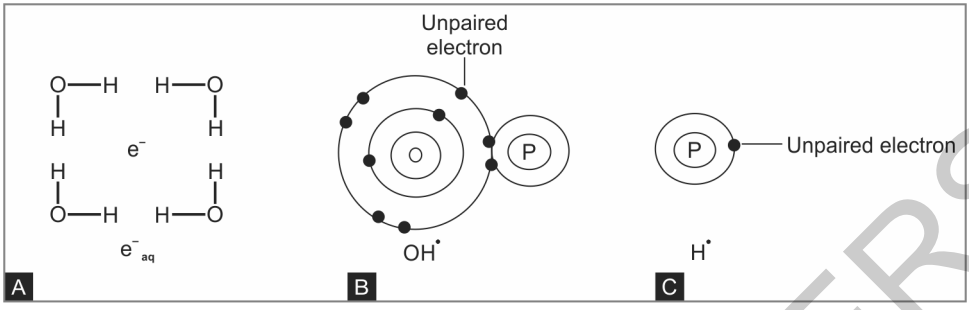

FIGURES 3.3A to C: Radiolysis of water and reactive species.
A. Aqueous electron; **B.** Hydroxyl radical; **C.** Hydrogen radical.

The H_2O^+ and H_2O^- are said to be radical ions, due to their charge and unpaired electron. The H_2O^+ is extremely reactive chemical species and performs variety of reactions. It acts as a strong oxidizing or reducing agent by combining with macromolecules. Its life time is too short, of the order of 10^{-10} s. The H_2O^+ reacts with another water molecule and produce hydroxyl radical, OH°. The H_2O^+ itself split into H^+ and OH°:

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH°$$
(hydroxyl radical) ----- (3)

$$H_2O^+ \longrightarrow H^+ + OH° \text{ (hydroxyl radical)}$$
----- (4)

The OH° has nine electrons and one electron short of the number required to give stability. It is highly reactive and has a life time of 10^{-9} s and its absorption band is about 260 nm. It can diffuse to a distance equal to twice the diameter of DNA double helix. Similarly, H_2O^- ion radical is also unstable and rapidly forms ions and H-free radicals:

$$H_2O^- \longrightarrow H° \text{ (hydrogen radical)} + OH^-$$
----- (5)

Excitation

Radiation exposure may also excite water molecules (H_2O^*), which in turn get dissociated and produces hydrogen (H°) and hydroxyl radicals (OH°), in a limited proportion:

$$H_2O \longrightarrow H_2O^* \longrightarrow H° + HO°$$ ----- (6)

Thus, ion radicals (H_2O^+ and H_2O^-) and free radicals (H° and OH°) are produced by radiolysis of water. Among this, e^-_{aq}, OH° and H° are the important reactive species and their relative yield are about 45%, 45% and 10%, if observed immediately after the event. These are rapidly reacting species, which can carry out variety of chemical reactions. Free radical can diffuse up to 4 nm in cells and can cause damage at distant sites. The life time of H° and OH° is about 10^{-10} s only and they have tendency to recombine with water rather than causing biological damage, which will be discussed in the following paragraphs.

Free Radical Reaction

The radicals can recombine with other free radicals and forms non-reactive chemical species such as H_2O and H_2O_2:

$$H° + OH° \longrightarrow H_2O \text{ (water)}$$ ----- (7)

$$OH° + OH° \longrightarrow H_2O_2 \text{ (hydrogen peroxide)}$$
----- (8)

The formation of water does not make any biological damage, whereas hydrogen peroxide is highly toxic to organisms. The H° radical is a powerful reducing agent, whereas OH° and $HO_2°$ are powerful oxidizing agents and easily combine with macromolecules.

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They can attack biomolecules (DNA) via hydrogen abstraction, OH radical addition and cause damage.

The presence of oxygen enhances free radical damage. The oxygen combines with hydrogen radical to form hydroperoxyl radical ($HO_2°$):

$$H° + O_2 \longrightarrow HO_2° \text{ (hydroperoxyl radical)} \quad \text{----- (9)}$$

In the case of low-LET radiation, free radicals are the primary agents that cause the biologic effects. Approximately, two third of all radiation-induced damage is considered to be caused by the hydroxyl free radical in low-LET radiations. However, in the case of high-LET radiations the direct action dominates over the indirect action of radiation.

Repair of radiation damage is possible at molecular, cellular and tissue levels. Enzymatic mechanisms and cofactors do exists within cells, which repair the radiation damage and return to pre-irradiated state within hours to days. Repair of biomolecules can occur by hydrogen donation from thiol compounds. This is followed by the production of thiol radical, which is less damaging. For example, DNA single strand break and base damage can be repaired by specific endonucleases and exonucleases that are present in the cell. If the damage is severe, the DNA molecule may undergo mutation. However, it depends on the DNA location and its environment, radiation dose, dose rate, special distribution of energy and radiosensitivity of the system.

Cells are more radiosensitive during the phase of cell division known as mitosis, when the chromatin material is being distributed to daughter cells. Hence, rapidly dividing cells such as intestinal epithelium, bone marrow cells and reproductive cells are more radiosensitive. Highly differentiated tissues, such as muscle and brain, are least radiosensitive to radiation.

CHARACTERISTICS OF ACTIONS OF RADIATION

Direct and indirect interactions of radiation with cell ultimately result in:

1. Cell modifications such as gene mutation, cell transformation and chromosome aberrations.
2. Cell death.

Mutation in germ cell may lead to hereditary effects that carry over to many generations. Oncogenic transformation of the cell may be delayed even up to 40 years. Cell death has time range and it is expressed in hours to days.

Indirect Action

The effect of radiation by indirect action is characterized with:

- Dilution effect
- Effect of freezing
- Presence of second solute
- Solute pH.

The effect of ionizing radiation on aqueous solution depends upon the concentration of the solute. The number of molecules transformed per 100 eV of energy absorption is called G value. At low solute concentration, the radical concentration is more than the solute concentration. Radicals interact with each other and lose their number. As the solute concentration increases, the radical interacts mostly with solute and the G value increases. Thus, the G value increases with solute concentration. At a particular solute concentration, it becomes saturated. This is known as dilution effect.

The radicals have to be mobile in order to migrate and react with solute molecules. By freezing the solution, the mobility of the radicals is reduced, resulting in reduced irradiation effect. When more than one solute is present, the reactions of the one solute will be affected by the presence of radicals. Hence, there will be decrease in yield due to

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radical reactions. If the pH decreases more and more, e^-_{aq} is transformed into H° radicals, this may cause increased inactivation especially in proteins.

Direct Action

Ionizing radiation can interact directly with biological molecules by causing ionization and excitation. One or more chemical bonds may be broken and leave the atoms or molecules with unpaired electrons. This process of radical formation takes about picosecond time. Radical-radical interaction may result in crosslinking. Radical can also react with oxygen and produce chain reactions. Further reaction with DNA, lipid and protein molecules may produce crosslinking. About one third of the radiation interaction is only by direct action. This process is dominant with high-LET radiation.

IRRADIATION OF MACROMOLECULES

The irradiation of macromolecules can produce three effects namely (Fig. 3.4):

- Main chain scission
- Crosslinking
- Point lesion.

The molecules are more radiosensitive, if irradiated in vivo. Macromolecule synthesis is more important for cell survival and reproduction. In protein synthesis, messenger ribonucleic acid (mRNA) is transcribed from DNA and it is transferred to cytoplasm. The genetic code from mRNA is then translated into protein with the help of transfer RNA (tRNA). In the case of DNA synthesis, the deoxyribose, phosphate and base molecules accumulate in the nucleus, during G1 phase. They form a single large molecule. During the S phase, the parent DNA is duplicated into two daughter DNA molecules. Thus, synthesis is carried out with series of events, irradiation of macromolecules may disturb any one of the event, in turn, the synthesis.

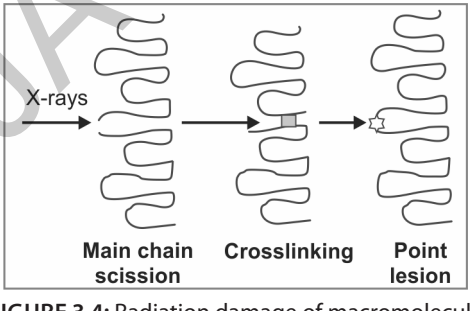

FIGURE 3.4: Radiation damage of macromolecule

Main-chain Scission

Main-chain scission is the breakage of the backbone of the long-chain macromolecule. This will result in production of multiple numbers of small macromolecules. The size of the molecule is reduced and viscosity of the surrounding solution is also reduced. The measurement of viscosity of the solution may help to detect main chain scission.

Crosslinking

Some macromolecules produce small, spur-like structures in sideways after irradiation. The spur extends laterally, away from the main chain. They have stickiness at the end and are capable of attaching to a neighboring macromolecule or another segment of the same molecule. This is called crosslinking (Fig. 3.5).

Crosslinking may be protein-protein crosslink, DNA-protein crosslink, intra- and interstrand crosslink. Intrastrand crosslinking is one in which crosslinking occurs between bases on the same stand, e.g. formation of adjacent thymine dimers (TT). Dimerization reduces the distance between the bases and results in distortion (kink) of sugar-phosphate backbone. The rupture of hydrogen bond may lead to irreversible changes in the secondary and tertiary structure of DNA molecule, which may compromise genetic transcription and translation.

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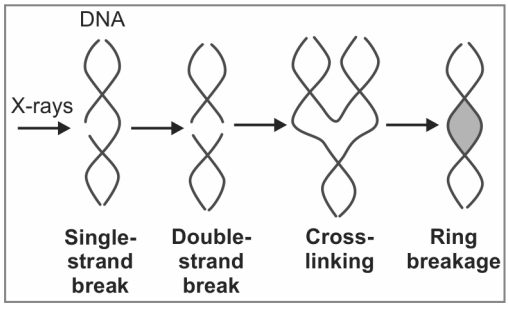

FIGURE 3.5: Radiation damage of DNA

Point Lesion

Irradiation of macromolecule may disturb a single chemical bond, known as point lesion. It is not detectable, but capable of causing slight modifications in the molecule. Hence, the molecule may malfunction within the cell.

Strand Break

Radiation exposure also causes DNA strand break in addition to base change and the DNA is degraded into smaller fragments. As a result, there will be a decrease in molecular weight of DNA. The strand breaks may be single-strand break (SSB) or double-strand break (DSB). If the break is located on one of the strand, it is referred as SSB. If two breaks are located on opposite strands, separated by five bases, then it is called DSB. The SSB between sugar and the phosphate can rejoin, if it is not separated. The SSB are mostly caused by OH radical and easily repaired compared to DSB. Presence of oxygen may cause peroxidation of base that prevents rejoining. DSB also undergo repair; if the repair is not proper then it can lead to mutation. Thus, DSB are basically genotoxic lesions that can result in chromosome alterations. This may cause activation of oncogenes, inactivation of tumor suppressor genes or loss of heterogeneity, resulting in carcinogenesis.

The DSB is the result of sparse ionization pattern of the low-LET radiation. An X-ray dose of 1 Gy, may disturb the mitotic capability in 50% of the exposed cells. All ionizing radiations are capable of producing DSB and complex DNA damages. In low-LET radiation, about one fourth of the dose is deposited in tissue via low-energy secondary electrons (0.1–5 keV). These low-energy electrons produce dense ionization tracks over a short range, equal to the diameter of double helix strand. This may result in complex DNA damages that are less likely to be repaired.

Though the amount of DSB lesions caused by the radiation is more, the number giving rise to cell killing is small. The dose of ionizing radiation that gives one lethal event per cell and leave 37% of the viable cell is called D_0. The D_0 value for low-LET X-ray lies between 1 and 2 Gy dose, which is sufficient to cause about 1,000 base damages, 1,000 SSB and 40 DSB per cell. SSB is of little importance, since its damage is mostly repaired by taking a template from the opposite strand. If incorrect repair takes place in SSB, it may lead to mutation. If both strands are broken and are well separated, it can also be repaired, since the two breaks are handled as two SSBs.

The DSB is the most important damage in DNA, after radiation exposure. The interaction of two DSB may lead to cell killing, carcinogenesis and mutation. There are many kinds of DSB, depending upon the distance between the break and kinds of end point. The DSB yield out 0.04 times that of SSB and it is induced linearly with dose. Mostly DSBs are caused by single track of ionizing radiation. DSB result in cleavage of chromatin into two pieces and is the critical lesion, responsible for cell killing. Experimental studies reveal that DSB produced initially correlate with radiosensitivity and survival at low dose. Whereas, unrepaired or misrepaired DSB correlate with survival at higher doses. High-LET radiations are capable of producing complex DSB lesions. The clusters of ionization and excitations that take place at the end of the secondary electrons tracks are capable of producing multiple lesions within 20 nm range, resulting in cell death.

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The DNA damage is a normal event in a cell's life. Apart from radiation, there are many more agents that may cause DNA damage. The extent of DNA damage depends upon the repair mechanism present in the organism. Eukaryotic cells are equipped with ability to repair DSB, whereas organisms with prokaryotic cells are not equipped. A single DSB is lethal to bacteria, whereas about 60 DSB are required to kill a mammalian cell. Similarly, few SSBs are lethal to viruses and microorganisms, whereas several hundred SSB are required to kill a mammalian cell. The above difference is due to repair capacity between different organisms.

DNA Strand Break Measurements

The DNA strand break can be measured by the following methods:

- Pulsed-field gel electrophoresis
- Single-cell electrophoresis
- DNA damage-induced nuclear foci.

1. **Pulsed-field gel electrophoresis (PFGE):** It is used to detect induction and repair of DNA DSBs. This is basically a technique of electrophoretic elution of DNA. The damaged DNA fragment is believed to have net negative charge. If it is embedded and lysed in an agarose gel, they migrate under an electric field. The electrophoresis allows the separation of DNA fragments based on their size. The fraction of DNA is directly proportional to the dose. The speed of migration is inversely proportional to their size. A DNA-specific fluorescent dye is added to study the movement. However, to improve the separation of fragments, the field is applied in alternative direction, at 60° to the axis of migration. The separation of fragments is a measure of DSB. This is calibrated with DNA of known molecular weight, e.g. yeast.
2. **Single cell electrophoresis:** The irradiated cells are embedded in low-density agarose and lysed to release DNA. The lysis is performed under neutral buffer and alkaline buffer conditions, to quantify DSB and SSBs respectively. If the cells are not damaged, the DNA remains intact and does not migrate. If there is damage such as SSB and DSB, the DNA begins to migrate and the migration of DNA is proportional to the damage. The lysed DNA fragments take the shape of comet's tail, under electrophoresis condition. This assay has high sensitivity and specificity to SSB and a lesser degree to DSB. However, by changing the lysis condition from an alkaline to neutral pH, the sensitivity to DSB can be increased. Both methods are cell based, where the DNA is more resistive to radiation. Cell killings do not correlate with SSB, but well correlate with DSBs. This is because that DSBs are capable of causing chromosome aberrations. This technique is useful to detect DNA damage and repair, and has the advantage of finding differences in DNA damage and repair in a single cellular level. It requires only small number of cells. Hence, it has advantage for analysis of biopsy specimen, which contains small number of cells.
3. **DNA damage-induced nuclear foci:** The irradiated cells are incubated with a specific antibody, so that they signal to a repair protein. The binding of the antibody is detected with a secondary antibody, which also carry a fluorescent tag. Under the fluorescence microscope, the location and intensity of the fluorescent tag can be quantified. The commonly assayed proteins for foci formation are γH_2AX and 53BP1. Basically, the H_2AX is a histone protein, after radiation damage, it gets phosphorylated and form γH_2AX. The 53BP1 protein also gets phosphorylated after radiation damage and form nuclear foci at the sites of DSB. Thus, this technique quantifies the DNA

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damage by recruiting DNA repair proteins to the sites of DNA damage. It can be carried out on both tissue sections and an individual cell.

Mutation

Human cell contains about 30,000 genes. Each gene occupies a specific position on the chromosome. All cells have identical sets of both mother and father. An individual, therefore, receives from his/her parents, two sets of genes identical to one another. These two genes, which determine the same characteristics, are known as 'alleles'. The hereditary information contained in the gene is coded in the form of specific sequence of bases in the DNA molecule. The mutation constitutes a change in the information contained in the genes. Such changes can be brought about either by:

1. Change in the structure of the DNA molecule (base transformation, base deletion).
2. Microscopically visible changes in the structure of the chromosomes (chromosomal aberrations).
3. Changes in the number of chromosomes (aneuploidy).

Mutations of the first type are known as gene mutation or point mutation, while the latter two are called chromosome mutation. Gene mutations represent the largest category of significant genetic alterations. It can occur at random in one or both the alleles. Alleles in an unmutated state are called wild type. When one of the alleles is mutated and the other is wild type, the combination is called heterozygous. When both the alleles are of the same type, either muted or wild, it is called homozygous.

Structural and Mitotic Mutations

Chromosome mutations are involved with:

- Structural changes
- Mitotic cycle.

The first type arises from the result of breakage and reunion of the genetic material (inversions and translocations). Inversions involve breaks and rearrangements in the same chromosome, whereas in translocation, a part or full chromosome is attached to another chromosome. In the latter type, mutation occurs during the mitotic cycle and the chromosomes fail to separate from each other to form haploid germ cell. One gamete receives both chromosomes, while the other gets only one. Chromosome mutations are dominant in nature and cause both lethal- and non-lethal effects.

Thus, mutation is the term that refers the change or loss of base in a chromosome. These changes are discrete and need not have structural changes in the chromosomes. Mutations can cause heritable changes in the genetic material. Mutation can occur due to natural causes, such mutations are called spontaneous mutations. They occur at slow rate in man, which is estimated to be 1×10^5/gene/generation. The factors that influence spontaneous mutation are temperature, chemicals and natural radiation. However, radiation is capable of producing chromosome breaks, which can be viewed microscopically during mitosis.

Usually, any DNA damage can be recognized by the enzymes and gets repaired suitably. But in mutated cell, the damage is not repaired and the cell gets replicated. Though the cells have reproductive capacity, they contain altered information in their genome. The defect is passed on to the descanters of the mutated cells. However, mutations that take place in germ cells can pass the defects to the progeny of the host.

Dominant and Recessive Mutation

Mutations are classified into:

- Dominant mutation
- Recessive mutation.

In dominant mutation, a single mutant allele inherited from either parent is sufficient

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to cause altered phenotype. In this, the organism has one mutant and one normal allele of the gene, and is called a heterozygote. In the case of recessive mutation, two mutant alleles of the same gene, one from each parent are required to produce a mutant phenotype, and it is called homozygote. Mutations in genes that code for structural proteins are dominant, whereas the genes that code for enzymatic proteins are recessive.

Ionizing radiation can cause mutations in both germ cells and somatic cells. When radiation-induced mutation occurs in germ cells and passed on to the progeny, heritable diseases occur. The mutation in the germ cell leads to inherited effects in the offspring, known as genetic mutation. Alternatively, the mutation in the somatic cells results in effects in the individual, called somatic mutation, e.g. cancer. Radiation cannot produce new inheritable effects due to mutations, instead increases the frequency of mutations that are already occurring naturally.

EFFECTS OF RADIATION ON CELL DIVISION AND GROWTH

It is well-known that radiation results in proliferative integrity. In addition, radiation exposure can also cause delay in mitosis and cell division, which are usually termed as mitotic delay and division delay respectively. At the end of the delay period, these cells can divide indefinitely and give rise to colonies. Those which do not survive may:

1. Lyse before entering into first mitosis.
2. Divide only a limited number of times and give rise to abortive colonies containing only a few cells.
3. May continue the synthesis of cellular material in the absence of cell division, giving rise to multinucleated cells called the giant cells.

Mitotic Inhibition

Cultured mammalian cells exposed to doses above 0.5 Gy show a decrease in the percentage of cells in mitosis (mitotic index), but this change cannot be detected as in control cells. The mitotic index (MI) is defined as the ratio of the cells in mitosis to the total number of cells (N) in the sample:

$$M1 = 100 \times \frac{(P + M + A + T)}{N} \quad \text{---- (10)}$$

where,

P, M, A and T are prophase, metaphase, anaphase and telophase respectively.

The cells irradiated to 0.83 and 3 Gy, show an increase in MI after a lapse of 24 hours and finally show MI higher than that of controls. Cells irradiated to a higher dose of 10 Gy do not show an increase above those of controls. The decrease in the MI seen during the first 1–2 hours is due to the blocking of the cells in G2 phase of the cell cycle. The cells in G2 phase are delayed the most by radiations as compared to cells in G1 and S phases of the cell cycle.

Further, the cells in early mitosis (prophase) revert back to G2 phase and stay there for a few hours before proceeding through division. Cells, which are in later phase of mitosis, progress without significant delay and complete division. The differential delay in different phase of the cell cycle is referred as G2 block. As soon as the cells are released from this block, they proceed through division in a synchronous fashion. This explains the increase in MI seen after 4 hours. At higher doses, however, cells in all the phases of the cycle are inhibited, and hence, the MI remains less than that of controls.

The MI is an indicator of reproductive capacity of a group of cells and is related to radiation sensitivity. Generally, cells of high MI are more sensitive to radiation. Based on the MI, cells can be divided as:

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1. Zero MI, e.g. neurons, sensory organs, cornea, adrenal medulla and red blood cells.
2. Low MI, e.g. thyroid, connective tissue and vascular endothelium.
3. High MI, e.g. epidermis, bone marrow, intestinal epithelium and gonads.

Law of Bergonié and Tribondeau

The radiosensitivity of different cell line varies widely; in turn the nature of deterministic effects also varies with different organs. In 1906, Bergonié and Tribondeau (French radiobiologists) have proposed a law correlating radiosensitivity and the proliferation rate of the cells. The law states that the cells are more sensitive to radiation, when they are:

- Rapidly dividing
- Undifferentiated
- Have long mitotic future.

The law came well before the discovery of DNA and its mechanism. Though law has certain exceptions, still it serves as a rule of thumb to determine relative radiosensitivity of the cells. Mitotic rate is one of the important factors, which influence the radiosensitivity of cells. It is found that rapidly dividing cells are killed with relatively low radiation exposure. Based on the criteria of cell death, cells that have low mitotic rate are more resistant. Certain types of damage are produced during the irradiation, but cells die only when the cells enter division. If the mitotic rate is high, there will not be enough time for the cellular lesions to be repaired.

The cell with long mitotic future is the one which can undergo many cell divisions. In normal circumstances, the liver cells do not multiply, but if a small part of the liver is cut off, the cells multiply and the liver is regenerated. On the other hand, if the nerve cells are damaged they cannot be regenerated. Hence, liver cells have a greater mitotic future than the nerve cells and are more sensitive towards radiation. Mammalian cells, which are less differentiated, are more radioresistant than the highly specialized cells. However, there are exceptions to the above law, as seen in lymphocytes, neoplasms and oocytes. Though they are non-dividing cells, they are most radiosensitive. Based on radiosensitivity, tissues are divided as given below with decreasing order:

- Vegetative intermitotic cells, e.g. erythroblasts
- Differentiating intermitotic cells, e.g. myelocytes
- Reverting postmitotic cells, e.g. liver
- Fixed postmitotic cells, e.g. nerve cells.

Effects on Cell Cycle

Cells are more sensitive during M and G2 phase of the cell cycle, and more resistant during the S phase. Experiments have shown that there is a depression of DNA synthesis, after radiation exposure. This is mainly due to G2 arrest, which reduces the number of cells moving around the cycle and entering the S phase. The extent of radiation-induced depression of DNA synthesis is proportional to the oxygen tension. It is also found that cells are most sensitive for 3 hours proceeding mitosis, which corresponds to G2 period. In general, radiation slows down all stages of the cell cycle; the most important delay is the G2 arrest, which results in division delay. The magnitude of division delay is dose dependent. Further, cells irradiated in G2 phase show a greater delay compared to cells irradiated in S phase, suggesting that most of the division delay is caused by the inhibition of mitosis. During the delay period, the cells may be repairing the damage induced by radiation, which may be a very important requirement of the cells to survive the crucial test of mitosis.

The rapidly dividing cells spend more time in M phase; if the MI is higher, there is more possibility of cell death. The sulfhydryl compounds are the natural radioprotectors.

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Their intracellular level is related to radiosensitivity, since the level vary with cell cycle phase. In the S phase, the DNA is replicated and all biochemical compounds for replication and repair are available. Hence, it is suitable site for repair and all the radiation damages are repaired. Therefore, it is most radioresistant. On the other hand, the M phase involves series of steps that includes condensation, alignment, attachment of spindles and separation of sister chromatids. Failure of any one step, make the cell fail to divide. Thus, the radiation damage has greater potential to kill the cell in M phase, compared to other phases.

Research reveals that the cell cycle progression is controlled by a family of genes called molecular checkpoint genes. The function of checkpoint genes is to ensure the correct order of cell cycle events. A specific gene halts the cells in G2, so that the inventory of chromosome damage is taken, repair is initiated and completed before mitosis. A mutant cell, does not have the above G2 checkpoint gene, moves directly into mitosis and has higher risk of dying.

Alternatively, these types of cells are more sensitive to radiation, since they have damaged chromosomes. The checkpoint controls and monitors the spindle function during mitosis. If the spindle is disturbed, the progression through mitosis is blocked. The G2 checkpoint gene involves cyclin-dependent kinase 1 (Cdk1), i.e. p32 protein kinase. The levels of the gene control the passage through mitosis. The cells that lack checkpoint genes are also prone to carcinogenesis. This G2 block causes inverse dose rate effect at low-dose rates. That is, as the dose rate decreases, the cells are more radiosensitive as seen in the range 0.3–0.4 Gy/h in brachytherapy.

Giant Cell Formation

Cells survive after a high dose of radiation above 10 Gy, generally form colonies with most of the cells considerably larger than normal cells. Cells exposed to < 10 Gy, generally form colonies containing both normal cells and large cells called the giant cells. Giant cells vary in size and sometimes grow 100 times larger than normal cells. Protein and nucleic acid content of giant cells are proportional to their volume. Hence, formation of giant cells is due to some difficulties associated with cell cycle division. Inhibition of cell division and endoreduplication (synthesis in the absence of cell division), and several sequences of progression in the absence of mitosis are suggested as the cause for giant cell formation. Finally, the giant cell undergoes death by lysis.

SYNCHRONOUS CELL CULTURES

In general, cell population is asynchronous during irradiation, i.e. the cells are distributed throughout in all the phases of the cell cycle. To study the radiosensitivity of the cell cycle, the cells must be made synchronous; they should be in the same phase of the cycle, at a given time. There are two methods by which cells can be made as synchronous population namely:

- Mitotic harvest
- Use of drug.

The mitotic harvest is used only for cultures that grow monolayers attached to the surface of the growth vessel. When the cells are close to mitosis, they round up and get loosely attached to the surface. If the growth medium is shaken, the mitotic cells detach from the surface and float in the medium. Now, the medium is removed from the culture vessel and plated in a new petri dish. The dish now consists of only the mitotic cells that are incubated at 37°C. This causes the cells to be synchronous and moves together through the mitotic cycle. The hydroxyurea is the most commonly used drug for making synchronous population of cells, which can be used both in culture and in tissue. If the

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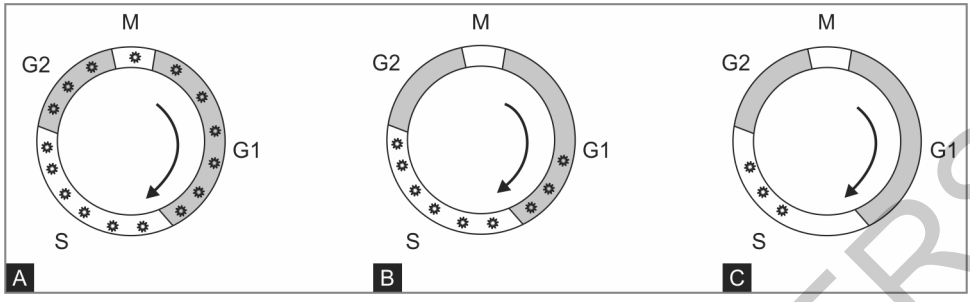

FIGURES 3.6A to C: Formation of synchronous cell population by the drug hydroxyurea. **A.** The drug kill the cells in S phase and imposes block at the end of G1 period; **B.** Cells at the G1, M and G2 phase gets accumulated at the block; **C.** Block is removed, the synchronous cells move through the cell cycle (G1/G2, interphase; M, mitosis; S, synthesis).

drug is added to the population of dividing cells, it has two effects (Figs 3.6A to C):

- The cells in the S phase are killed
- The drug impose block at the end of G1 period.

The cells in the G2, M and G1 phase progress through the cell cycle and get accumulated at this block. The drug is left in the cell for a period equal to the combined period of G2, M and G1. At the end of the period, all the cells, which are ready to enter S phase, are brought to the end of G1. If the drug is removed, then the synchronous cells progress through the cell cycle.

RADIATION AND CHROMOSOME DAMAGE (Figs 3.7A to E)

Irradiation can damage chromosomes and delay the cell's entry into mitosis. This delay is dose dependent. If the cells are irradiated during the interphase, they begin to divide and undergo aberrations. Chromosome aberration refers to their appearance in the first metaphase after irradiation. The stable aberrations may be carried out through number of cell divisions, generally called chromosome aberrations. Unstable aberrations may lead to cell death. If cells are exposed to radiation, DSB occurs in chromosomes. The broken ends are sticky due to their unpaired bases. These broken ends may:

- Join with their original chromosome
- Fail to rejoin, which leads to aberrations
- Rejoin with other broken ends.

These aberrations may be chromatid or chromosome aberrations. Chromatid aberrations are due to irradiation of cells, while they are in late interphase (G2). In this, one arm of the sister chromatid is broken. The DNA has already doubled and the chromosomes consist of two strands of chromatin, during irradiation. If the cells are irradiated, while they are in early interphase (G1) and if unrepaired, leads to chromosome aberrations. In this case, the chromosome is not duplicated and the damage is a SSB of chromatin. During the S phase, an identical strand is synthesized. This is visible in the mitosis as identical breaks in the pair of chromatin strands. Irradiation of cells, while they are in S phase may bring both types of aberrations.

Lethal Type Chromosome Aberrations

The types of chromosome aberrations that are lethal to cells are:

- Dicentric
- Ring
- Anaphase bridge.

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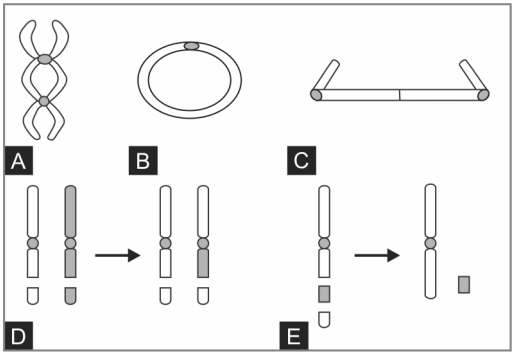

FIGURES 3.7A to E: Radiation-induced chromosome damage. **A.** Dicentric; **B.** Ring formation; **C.** Anaphase bridge; **D.** Symmetric translocation; **E.** Small interstitial deletion.

Dicentric

Two separate chromosomes undergo breaks at the early interphase. If the broken ends are close to each other, they get united illegitimately and replicated during the S phase. After the synthesis, a pair of sister chromatids appears with two centromeres, which is called dicentric. It is an unstable aberration lethal to the cell, not passed on to the progeny. It decreases slowly over a period of time after radiation exposure. Since spindle has two centromeres to grab during the metaphase, it gets disturbed, while pulling the chromatids during the anaphase.

Ring

Radiation induces breaks in each arm of a single chromatid, at the early part of the cell cycle. The breaks may rejoin and form ring and fragments. During synthesis, it gets replicated and appears as overlapping rings and acentric fragments without centromere. Since the ring or the acentric fragment do not have properly constructed centromere for the spindle to attach, chromosomes are unable to divide properly. The fragments will be lost during next mitosis.

Lethal Type Chromatid Aberrations

Anaphase Bridge

Anaphase bridge formation is the result of radiation-induced breaks at the later part of the interphase (G2), after replication. In this case, the break occurs in each chromatid of the same chromosome. The damaged sister chromatids do not separate properly, but stretch to the extreme poles of the cell. The broken ends join incorrectly and form a sister union. This results in a dicentric chromatid known as anaphase bridge. This type of aberration prevents the separation of parent into daughter cells, resulting in reproductive death. In addition, a fragment is formed without centromere, which will be lost during mitosis. Anaphase bridge type of aberration is usually lethal to the cell.

Non-lethal Type Aberrations

The types of aberrations that are not lethal to cells are:

- Symmetric translocation
- Small interstitial deletion.

They basically arises from DSB, remains intact with two chromatids and centromere, and carryout mitosis normally. However, there may be some loss of genetic information that may be passed on to the next generations.

Symmetric Translocation

In symmetric translocation, breaks occur in two different chromosomes at the early interphase (G1). The broken ends are exchanged between two chromosomes and get rejoined. This type of aberration can be easily seen with fluorescent in situ hybridization (FISH) technique or chromosome painting. Using the FISH technique, translocations can be scored on the exposed individual, even after 50 years of radiation exposure. Thus, translocations

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Thayalan Kuppusamy MSc DipRp Mphil PhD FUICC FIMSA FUSI is a senior medical physicist in India, and presently serving as Head, Medical Physics Division, Dr Kamakshi Memorial Hospital, Chennai, Tamil Nadu, India. Earlier, he served as Professor, Department of Radiology Physics, Madras Medical College, Chennai. He has 33 years of experience both in clinical and teaching.

He was born in Vattathur village, Cuddalore district of Tamil Nadu, to parents of K Kuppusamy and Arukkaniammal. He completed his master degree in physics with first rank at the Annamalai University (1978) and did his postgraduate diploma in hospital physics and radiological physics at the Bhabha Atomic Research Centre (BARC), University of Bombay (1985), Mumbai, Maharashtra, India. He is a certified Radiological Safety Officer (RSO) for medical, industrial and research institutions. He got his MPhil degree in Radiation Physics from the Loyola College, University of Madras (1983), Chennai. He obtained his doctoral degree (PhD) on high dose rate brachytherapy from The Tamil Nadu Dr MGR Medical University (2003), Chennai.

He underwent an advanced clinical medical physics training (1997) at the Harper Hospital and Wayne State University, Detroit, USA with ICRETT Fellowship of International Union Against Cancer (UICC). He is the fellow of the UICC, International Academy of Medical Sciences (IMSA) and Ultrasonics Society of India (USI). He is a member of College of Medical Physics of India (CMPI), Indian College of Radiology and Imaging (ICRI), and Indian College of Radiation Oncology (ICRO). He is the member of Professional Development Committee, Asia-Oceania Federation of Organizations of Medical Physicists (AFOMP), representing India. He served as the Vice-President, Association of Medical Physicists of India (AMPI), Treasurer, Secretary and Chairman of its Tamil Nadu and Puducherry chapter. He is one of the founder members of Indian Brachytherapy Society (IBS) and currently serving as the Vice-President of IBS. He is a member in 19 scientific associations.

He has 27 publications to his credit, presented 74 scientific papers, delivered 119 invited lectures, and chaired 33 scientific sessions, in national and international conferences and an author of 6 books. He is an examiner for undergraduate and postgraduate courses, member of board of study, advisory board, ethic committee and expert panel in many Indian universities, and has been guiding students for PhD. He is the task group member of the Atomic Energy Regulatory Board (AERB), Mumbai for the preparation of safety codes and guides.

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