REVIEW OF LITERATURE
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IONIZING RADIATION AND ITS BIOLOGICAL EFFECT

Life has evolved in a world in which a major source of energy essential for biological processes is in the form of radiant energy or radiation. The term "radiation" usually indicates a physical phenomenon in which energy travels through space, even though that space be empty of matter. Radiation is utilized by living material in a variety of ways. For example, sunlight provides heat, light and the energy for photosynthesis; radio-waves give a means of communication etc. Most of these radiations are not only harmless in ordinary doses but are actually essential for life. Certain types of high energy or ionizing radiations, however, are not so harmless and are known to produce deleterious effects in all forms of life (Casarett, 1968).

Sources of Ionizing Radiation

Ionizing radiation is high energy radiation that interacts with matter principally by ionization, and it is on this criterion distinguished from other types of radiation that can damage living organisms, such as infra-red, laser, micro wave and sound. Ionizing radiations of higher and lower energy are called hard and soft respectively. They are of two types, particulate and electromagnetic. The former type consists of a stream of various kinds of atomic or sub-atomic
particles, which can transfer their kinetic energy to anything they strike. It includes the alpha and beta particles, neutrons and heavy ions produced in particle accelerators, such as protons, deuterons, etc. The electromagnetic radiations consist of self propagating electric and magnetic disturbances, which affect the internal structure of matter and thus dissipate their energy. They move with the speed of light and extend from long radio waves to shortest gamma rays and consist of, from long to short wave length side, radio waves, radar waves, infra red rays, visible rays, ultraviolet rays, x-rays and gamma rays. Perhaps the earliest source of ionizing radiation, in relation to radiobiology, had its origin in the year 1895, with the discovery of x-rays by the German professor Wilhelm Conrad Roentgen. The next important source of ionizing radiation came into existence with the discovery of natural radioactivity. Radioactivity get its existence since the creation of the earth, but its story belongs almost entirely to the twentieth century. It started with the discovery of radioactivity by the French Physicist Henry Becquerel, a member of the Academie des sciences in Paris. In this connection, Rutherford in 1898 found that uranium emitted rays of two distinct kinds. One, which he called alpha, had enormous ionizing power, but would not penetrate through a sheet of paper, while the other kind, beta, ionized much less but travelled further. Villard discovered a third kind in 1900, gamma,
which are even less ionizing and far more penetrating. During the subsequent period a number of these radiations were obtained as artificial sources particularly with the development of the Coolidge hot filament tube in 1914 and nuclear fission as demonstrated in the University of Chicago in 1942. Thus during the first three decades of the twentieth century, several types of radiations were recognised and many sources of radiation of biological interest had been identified.

Characteristics of Ionizing Radiation

Alpha particles are positively charged helium particles, emitted by radio-active disintegration of natural or artificially radioactive elements. Polonium is considered as an ideal source of these radiations, since it gives off only alpha particles. Alpha particles lose energy by excitation and ionization of atoms in the material through which they pass. These particles produce intense ionization, especially at the tail of the ionization trail and are absorbed superficially and may be completely stopped by a thin sheet of paper, 0.06 mm Al or 0.1 mm of epithelial tissue. Alpha particles are thus important sources of internal hazard in biological system. These particles may have a velocity of about $3 \times 10^9$ cm per second and energy corresponding to about 5 Mev. They produce such a high density of ionization that reduction in oxygen tension is without effect.
Beta particles are negatively charged particles. They vary in speed and energy, depending on the voltage by which they are accelerated. Beta particles are also absorbed superficially but are relatively more penetrating than alpha particles. As regard biological hazard they occupy an intermediate position, both as external and internal source. The ionization produced by beta particles is much sparser than that produced by alpha particles. Usually they are polyenergetic, but monoenergetic particles can be produced.

Neutrons are uncharged particles produced by bombarding low molecular substances with protons or deuterons. The most common source of large quantities of neutrons is the uranium fission in nuclear reactors. Fast neutrons are highly energetic particular radiation. They may give rise to gamma rays on inelastic collision with some matter, but in tissue or organisms (which consist of nuclei of atoms of low atomic weight) collisions give rise to protons. Protons are positively charged particles of about the same mass. Protons pass through tissue, forming a high density of ions. Because of the density of ionisation, neutron effects are independent of oxygen. Since neutrons are absorbed in proportion to the number of atomic nuclei, paraffin, water, etc., are more effective absorbers than sheets of lead. As neutrons are themselves uncharged they may penetrate deeply into tissue of organisms before absorption brings
about ionisation and as such may be potentially hazardous. In contrast, slow neutrons, also called thermal neutrons, are much less energetic (about 0.03 Mev); consequently, they do not cause ionisation or dislodge atomic nuclei but react with atomic nuclei to form a new isotope which may disintegrate to give rise to ionizing particles.

X-rays and gamma rays are photons. Gamma rays produced by radio-active disintegration of elements have energies of between 0.3 and 5 Mev, and x-rays have now been produced as energetic as gamma radiations. The energy of both varies considerably with the voltage. Ordinary x-rays have much lower voltages than gamma rays. If the energy is less than 0.3 Mev the photoelectric effect is observed, and the absorption of the quantum causes ionization of the atomic nucleus with the emission of an electron containing the energy of the quantum minus the binding energy of the electron. When more energy is available the photon and free or loose electrons collide resulting in deflection of the electron and the photon, the latter now possessing a longer wavelength, having given up some of its energy. This kind of interaction is known as Compton Scattering. For quanta of energy of 5 Mev or more, absorption is usually followed by positron and electron formation (Glasstone, 1958). The process is known as pair production.

Gamma rays and x-rays penetrate deeply into material of low
density, the depth depending upon the voltage. Consequently the density of ion pairs per unit path length varies, being quite sparse for hard or short wave length radiations. These radiations penetrate deeper than the charged particles because they do not react at once with matter into which they penetrate. They are recognized as important sources of external hazard. Once a photoelectron is ejected from a particle of absorbing material, its energy is quickly dissipated in formation of ion pairs, the density of which is greatest at the end (tail) of the ionization trail.

Exposure of Biological Species to Radiations

The fact that biological species including man are continuously exposed to ionizing radiation both from artificial and natural sources was reported by U.N. Scientific Committee, in 1958 (United Nations Report of U.N. Scientific Committee, 1958). The Committee evaluated the 'natural sources of ionizing radiation', the mean yearly total dose found to be 0.1 rem (Table I).

The natural background radiation has two components: Cosmic radiation is primarily protons, with smaller proportions of other heavy nuclei; their average energy is about 6000 Mev. They react with the atoms of the atmosphere to produce secondary cosmic rays, of which neutrons, mesons, and gamma radiation reach the surface of
the earth. High-flying aircraft may encounter relatively intense cosmic radiation, and a further possibility of cosmic radiation exists in space-expeditions and obviously on the moon's surface.

**Terrestrial radiation** sources are found both within and without the bodies of organisms. External sources give about 50 mrad/year of gamma radiation, depending on the rocks and minerals in the locality; the dose from granite is higher than from limestone or sandstone, since it contains potassium-40 in the feldspar, and from uranium-bearing or thorium-bearing rocks the dose is, of course, higher still. The principal internal radiation source is potassium-40, which gives about 15-20 mrad/year; the dose from carbon-14 is about 0.7 – 1.6 mrad/year, from radium and polonium in bone about 0.3 and from radon in the lungs about 0.3. In round figures, the total background radiation average about 100 mrad/year and there may be a considerable variation between different parts of the world (about 400%) (Bacq & Alexander, 1966).

**Artificial radiations** are the 'man-made radiations' which arise chiefly from medical exposure and from fallout, with some other minor or localised sources. Medical radiation is extremely variable. Almost everyone at one time or another receives a diagnostic chest x-ray, but only comparatively few undergo radiation
therapy, so that while diagnostic doses can be expressed with some meaning as an average over the whole population and included in overall estimates of radiation exposure (Penfil & Brown, 1968), this is not possible for therapeutic exposure. An average annual dose from man made sources has been calculated out by the U.N. Scientific Committee in the year 1958 (Table II). The estimation of average exposure from fall-out is little better than a rough guess work, because of the varied pattern of deposition over the world. The estimated dose commitment from 1954 to the year 2000, arising from weapons testing up to 1962 is shown in Table III. Occupational exposure and other radiation exposure cannot account for a remarkable dose-factor although occupational exposure limited to radiation workers may cause radiation casualty with high dose value.

Harmful Effects of Ionizing Radiation

With the advent of discoveries of radio-activity, efforts were being made to utilize them for useful purposes and in great many cases successful applications were reported. But these radiations soon revealed their damaging effects when within four months of Roentgen's announcement of x-rays, a report appeared of hair loss following irradiation of the skull. Becquerel himself found the hazardous effect of radiation when he observed a burn developed on
his skin below the phial of radium which he used to carry in his breast pocket. Many of the "Luminisers" who used to apply luminous paints to watches and clocks and were in the habit of shaping their brushes to a fine point between their lips suffered from fatal mouth and tongue cancers due to radioactivity in the paints containing radium; they had radon in their breath and radium was found in their bones after they died. Over the succeeding years it was gradually realized, more and more clearly, just how unpleasant ionizing radiations could be. However, this realization came too late for many of the pioneer radiologists; because they paid a heavy price, in pain, cancer, disfigurement and even death. It is reported that by 1936, more than 110 radiobiologists had lost their lives through radiation injury (Thornburn, 1972). Reports of destruction of life on a very large scale following acute exposure to ionizing radiation came from atomic bomb explosions in Hiroshima and Nagasaki during last war in 1945. The radiation was a mixture of gamma rays and neutrons, the dose varying from 1000 rads down to one to two rads depending upon the distance from the centre of explosion. The possibilities of acute exposure may also occur during test explosions of nuclear weapons as happened in 1954 when 239 Marshallese Islanders, 28 United States military personnel and 23 Japanese fishermen were exposed to radiation in Mid-Pacific, as a result of fall-out from a thermonuclear device.
Death of one fisherman was reported in this case. Case studies on several accidents involving exposure to high level of radiations in laboratories, hospitals, and industry have also been reported which involved more than 50 people of whom 13 died within a few weeks of the exposure. Severe symptoms of radiation sickness have been encountered in patients treated with radiation for leukaemia and other cancers or to suppress the immune system (Coggsa, 1971) for transplantation purpose.

Irradiations of human population are, now-a-days, viewed from the standpoint of whole body exposure or partial exposure exhibiting acute and chronic effects. Possibilities of whole body exposure occur majorly in atomic explosion, in accidental exposure etc., whereas partial exposures are the events in routine therapeutic use or sometimes during radiation works in the laboratories.

Interactions of Ionizing Radiation with Body Tissues

When ionizing radiation falls on matter, three types of interactions occur: namely, scattering of the Compton type, photoelectric absorption and pair production. When the energy of radiation is low and the material with which the interactions occur is of low atomic weight, it is the Compton process which predominates (Fig.1). This means that in biological tissues which consist mainly of low atomic materials, the Compton process dominates, since the
energy of the radiation used for such work has a low level ranging between 0.1 to 3 Mev. approximately.

Compton interaction takes place with the loosely bound electrons of atoms. During the process the electron is ejected out of the orbit with an initial velocity, the energy being derived from the incident radiation. The electron, so ejected is called recoiled or Compton electron. Since the radiation surrenders a part of its energy to the recoil or Compton electron, it gets scattered in a direction different from its initial direction. The scattered photon necessarily has a longer wavelength and lesser energy than the initial photon. The balance of energy is utilised partly for ejecting and partly for imparting the initial velocity to the recoil electron.

The electron so ejected now loses its energy in the tissue in five different ways: namely, bremsstrahlung, excitation, ionization, breaking of molecular bonds and heat. Normally, thirty such interactions are necessary for the complete absorption of the photon, when the whole of the energy of the incident photon gets deposited in the tissues in a way as described above. Of the five interactions of the recoil electrons, the three, namely, excitation, ionization and breaking of molecular bonds produce all the chemical changes and remain responsible for the biological damage. The radiation, as it enters the body tissue, exerts its primary direct action on the macromolecules due to their large size and water molecules due to their abundance.
A. Effects at Molecular Level

Radiation damage of biological molecules is brought about by two distinct mechanisms: (a) Direct action, the alteration of a biological molecule through deposition of energy in it as a result of a primary interaction with the radiation, (b) Indirect action, which takes place when primary action of radiation is on water, resulting in production of highly reactive products of radiolysis and then transfer their acquired energy to other molecules. Biological molecules are attacked largely by those products. Indeed the biological systems suffer mainly from indirect action since it is mostly water.

Interactions with Water Molecules: Radiolysis of Water:

The interaction of radiation with water is important in biological systems, since water makes up a large part of living things. The most likely reaction for the radiolytic breakdown of water is ionization of water followed by the capture of the electron by another water molecule with a subsequent decomposition into an ion and a free radical. This constitutes a sequence of reactions resulting in formation of H\(^+\) and OH\(^-\) radicals. The ultimate products are highly oxidizing free radicals such as hydroxyl and perhydroxyl and oxidizing compounds such as hydrogen peroxide or organic peroxide as shown in the Table IV (Blois, 1961; Alexander, 1965).

All of these agents may attack and damage sensitive biological
molecules depending on their positions to the site where the free radicals are created. The end products may be formed in a single step, or by means of a series of reactions involving several intermediates - the 'Chain reaction':

\[ \text{RO}_2^+ + XH \rightarrow \text{ROOH} + X^*; \quad X^* + O_2 \rightarrow XO_2^*; \quad \text{etc.} \]

Much damage may be caused before the chain ends. In aqueous inorganic solutions, however, the primary reactions are reduction of cations and anions by \( H^+ \) and oxidation by \( \text{OH}^- \) and \( \text{HO}_2^- \) radicals and by \( \text{H}_2\text{O}_2 \) (Casarett, 1968).

**Interactions with Macromolecules:**

When an aqueous organic solution is irradiated, there is usually the loss of a hydrogen atom or an entire radical group (e.g., \(-\text{CH}_3\) group) from the molecule. Due to the presence of several hydrogen atoms in most organic molecules a number of different organic radicals (\( R^* \)) can be formed in the following way:

\[ \text{RH} + \text{OH}^- \rightarrow R^* + \text{H}_2\text{O} \]

This is further complicated by the process of condensation, rearrangement, splitting and combination with molecular oxygen. The resultant molecule thus formed may be stable, and exhibit a different molecular species exerting a different chemical and biological effect (Casarett, 1968).

As a result of such chemical changes, very large molecules
common in the biological systems may undergo a variety of structural changes which ultimately tend to an altered function. In general, it is found that: first, aromatic compounds are more resistant to radical attack than aliphatics, because of their resonant structures; secondly, a ring stabilizes a chain; and thirdly, energy may react in a molecule elsewhere than at the site where it was absorbed, or be given off as heat or it may be transferred to other molecules. Macromolecules may undergo structural changes with associated physical changes, although the chemical nature of the changes can not generally be determined at this level. There might be changes in viscosity, alteration in the zeta potential or surface changes of colloidal solution etc. (Casarett, 1968).

"Degradation", or breaking into smaller units, has been shown to occur when large molecules are irradiated (Schultz & Dovey, 1956; Alexander et al., 1954, 1955). In molecules containing series of identical or repeating units, the break is usually in the same bond which ultimately results in number of smaller molecules with different functional ability. "Cross-linking" is another common change associated with the formation of chemical bonds (Alexander & Charlesby, 1955, 1957). In dilute solution, the cross-linking is predominantly intra-molecular (becoming attached to itself) when a chemically active locus is produced on it and when this spot can come in contact with another reactive area. At higher concentrations
of the solute, the cross-linking is mostly intermolecular (attachment between different molecules). As the radiation dose increases, the number of cross-links increases, and the solution gradually becomes more viscous, eventually turning into a 'gel'. The resulting 'gel' represents an entirely different physical-chemical state. Still another change associated with macromolecules during irradiation is "disruption of secondary structure". This type of effect has so far only been encountered in proteins and DNA (Bacq & Alexander, 1966). 

Proteins are much more affected by the direct action of radiation than indirect (Bacq & Alexander, 1966; Casarett, 1968). Indirect action may also produce important changes, e.g., in solubility. All enzymes can be inactivated in solution although the doses required vary widely (Collinson et al., 1950; McDonald, 1954, 1955). However, alteration of protein structure and function does not seem to be greatly important in primary radiation effects on biological systems.

In nucleic acids, several significant changes take place, both in vivo and in vitro. Possibilities of mutation exist when there is loss or alteration of bases (Scholes & Weiss, 1954; Ord & Stocken, 1960): adjacent pyrimidines are susceptible in the formation of dimers, particularly thymine. There is breakage of
hydrogen bonds; but the inherent rigidity of the structure, unlike proteins, holds it together while hydrogen bonds are broken and allows them to reform correctly. Breakage of the main strands may occur; but a break in single chain is difficult to detect since DNA helix is inherently rigid and will hold together. The broken ends may rejoin if not peroxidised in the presence of oxygen. Breaks may join forming cross-links, either within the same DNA molecule, between two DNAs, or between DNA and proteins. A "double break" in the nucleotide sequence may occur depending upon the number of nucleotides at which the first break occurs. But chances of 'double break' are rare.

The chief action of radiation on lipids involves the polyunsaturated fatty acids, and affects the carbon atom between the double bonds: hydrogen is removed and resonating structure is formed. Radiochemical studies on carbohydrates are few and inconclusive. However, some authors indicated polymer production of aqueous sugar in the absence of oxygen. Extremely high doses produce a variety of degradation products from sugars.

B. Effects at Cellular Level

It is not surprising that massive doses of radiation can break and destroy the structural components of a cell and produce cell
death (Duryee, 1949; Trowell, 1953; Oakberg, 1955). Exposure to lower amounts of doses can also cause cell death, but by less obvious mechanisms. Cellular effects are divisible broadly into three categories of interference: with cell's vegetative function, with its information store and with the process of its division.

Large doses of radiation (3000 to 5000 rads) produce changes in the membrane system. There is rupture of the plasma membrane and dilatation of the membraneous structure of the endoplasmic reticulum. Mitochondria often appear swollen with disorganization of the cristae. Changes in the nuclear membrane has also been observed, but they seem to be somewhat more resistant to physical alteration than the cytoplasmic membraneous structure. Altered permeability of the plasma membrane has been reported by Goldfeder (1963) which permits the extracellular fluids to enter the intracellular space. There are also changes in the permeability of the mitochondrial membrane permitting an excess "leakage" or shift of the enzyme within the intracellular space. These views are in support of the "Enzyme-Release Hypothesis" which explains an increased accumulation of enzymes in the cell sap following a large dose (Bacq & Alexander, 1966). Radiation also breaks down the lysosomes or other membraneous barriers, allowing an interaction of enzymes and substrates. Due to an overall change in the protein-lipid sandwich structure, an alteration in the transfer rates of Na⁺.
K\(^+\) and Ca\(^{++}\) may also occur (Bacq & Alexander, 1966).

A decrease in the rate of DNA synthesis following irradiation has been reported by many authors (Conard, 1956; Kay & Entenman, 1959b; Maisin, 1966). There is also a delay or depression of RNA synthesis (Nygaard & Potter, 1960). Enzyme synthesis is also found to be depressed after irradiation (Casarett, 1968) but precise information on the subject is, however, still lacking.

Radiation has profound effects on energy metabolism of cells. Phosphorylation or ATP production is reduced in many cells following moderate doses of radiation. Disorders in the electron transport system has been reported by many authors (Vön Bekkum, 1956; Moss, 1957; Thomson, 1964). Oxygen consumption is also reduced in individual cells.

Enzyme activities are reduced in many cases following a high dose of radiation, but no such reduction in enzyme activity has been found in lower doses. In lower doses enhanced enzyme activities have also been reported (Bacq & Alexander, 1966). In fact, alteration in the quantity or activity of existing enzymes does not appear to be an important initial effect of radiation, although it may be a secondary indication of other types of radiation damage (Casarett, 1968; Altman et al., 1970).
Chromosomal Aberration and Mitosis:

Radiation is well known to induce chromosomal aberrations for many years (Revell, 1959). Two types of aberrations have been discussed, namely, chromosome-type and chromatid type, the first showing 'breaks' through the thin filaments of chromosomes whereas the later shows "breaks" through chromatids. Irradiation of cells in G₁ (presynthetic phase) portion of interphase results in the appearance of chromosome-type aberration at the next metaphase. During the synthetic period (S), either chromosome or chromatid types occur, whereas irradiation in G₂ (postsynthetic) leads to chromatid aberrations. A lesser degree of alteration is what is described as mutation and may be genetic or somatic.

Small doses of radiation produce a temporary alteration in the pattern of cell division, the mitotic delay. With higher doses it stops completely. The cycle is most sensitive to radiation when division is about to start, in the G₂ stage of interphase. With higher doses, however, there is mitotic death which follows deletion of fragments of chromosomes. Formation of 'Giant cells' has also been reported.

Primary site of radiation damage:

A variety of morphological and functional changes thus occur in irradiated cells and it was suggested that cell nuclei are a major
site of the radiation damage leading to cell death. A series of studies by Ord & Danielli (1956), and some other workers have found marked correlation between cell sensitivity and nuclear parameters such as chromosome number, nuclear volume, interphase chromosome volume, and DNA content. These factors support the above hypothesis that nucleus is a major site of nuclear damage.

C. Effects at Tissue and Organ Level

Since the discoveries of radiation in 1895, extensive studies have been made so as to analyse the nature of radiation damage in living organisms. In 1897 Walsh first described nausea, vomiting and anorexia as symptoms of clinical radiation sickness. Degeneration of the intestinal mucosa of irradiated animals was subsequently reported by Krause and Ziegler in 1906. Bergonie and Tribondeau (1906) while looking into the effect of radiation on the rat testes discovered that the dividing (germinal) cells were markedly affected by the radiation, whereas the non-dividing (interstitial) cells appeared undamaged. This observation was later stated in the form of a law after their name which led to the generalisation that actively dividing tissues are "radiosensitive" and non-dividing tissues are "radio-resistant". Thus in mammals, the liver, the kidneys, muscles, brain, bones, cartilage and connective tissues have been found to be relatively radio-resistant. On the other hand, the bone marrow,
the intestinal epithelium, the gonads, the lymphocytes and the skin suffer the most damage following whole body doses of radiation. Details of their pathophysiology have been discussed by Patt and Brues (1954) and Bacq & Alexander (1966).

The modes of death in mammals exposed to whole body radiation have been studied and three specific "radiation syndromes" have been described to be the causes of 'radiation death' in mammals (Coggle, 1971; Thornburn, 1972). Death in animals following whole body doses of radiation between about 200 and 500 rads is due to the 'Bone marrow syndrome'; while damage to the intestinal epithelium is known to be the main cause of death of animals following doses between 500 and 1000 rads and is said to be due to the Gastrointestinal syndrome; radiation damage to the central nervous system leads to the CNS syndrome, and is recognised to be the main cause of death of animals following large doses of radiation, in excess of 10,000 rads.

In CNS syndrome, most mammals die within 48 hours. In brief, the symptoms that such heavily irradiated animals show are irritability, hyperexcitable responses, epileptic type fits and coma. These symptoms are associated with pathological changes in the nerve cells and blood vessels of the brain, an immediate change in the fluid and electrolyte balance. The animals are agitated and irritable, but this is soon followed by apathy, vomiting, salivation,
repeated defaecation and diarrhoea. Soon the animals are unable to coordinate their voluntary movements (ataxia) and become disoriented, involuntary rolling of the eyes occurs, tremors and frequent seizures are followed by the final phase of convulsions, prostration, coma, respiratory failure and death (Coggle, 1971).

In gastrointestinal syndrome death within several days has been reported after massive dosages of radiation delivered either to the abdomen or to the whole body (Hall & Whipple, 1919; Moon et al., 1941; Quastler et al., 1951). The clinical radiation sickness characterised by nausea, vomiting and anorexia was first described by Walsh in 1897 in this connection. Following a dose range of 1000 to 12000 r, the mean survival time was recorded to be 3–4 days in mice. An early death was also reported only when a large portion of the intestine was irradiated (Quastler et al., 1951). Exposure of the liver, kidney, spleen and adrenal does not lead to early death, at least in mice. Malabsorption and diarrhoea followed by severe loss of fluid and sodium through the gut have been shown to be the cause of death in great many cases (Thornburn, 1972). Damage to the epithelium in the small intestine with the resultant systemic infection from intestinal flora is taken to be the most critical characteristic of the gastro-intestinal syndrome by many authors (Patt & Brues, 1954; Coggle, 1971).
although damage to the bone marrow cells does play a part. Irradiation of the isolated intestine, however, showed many, though not all, of the signs of the gastro-intestinal syndrome.

Animals having received a dose of 200-500 rads may die, within 25 days of radiation, from bone marrow or haemopoietic syndrome. The radiosensitivity of blood forming tissues and the consequent hazard of blood damage have attracted considerable attention since the classical work of Heineke in 1903. The signs and symptoms that precede death are anaemia, haemorrhage and infection. The vast early literature on this subject has been reviewed by Dunlap (1942). Profound changes in peripheral blood have been shown to be due to the extreme radiosensitivity of the "stem cells" in the marrow. In haemopoietic death following radiation at relatively high dose, a considerable overlap of the gastrointestinal syndrome has also been reported. In the initial period after such radiation exposure between 200 and 1000 r, animals exhibit signs of intestinal disorders such as vomiting and diarrhoea. Following a latent phase of about 2 days, the destruction of the bone marrow cells and the resultant depletion of the circulating blood cells have been observed. The results of red cell, platelet and granulocyte loss lead to anaemia, haemorrhage, infection respectively. The latent period is followed by a period of extreme illness. Severe diarrhoea, which is often bloody, heralds serious intestinal
disorder that leads to fluid imbalances. The fluid imbalances, together with the haemorrhage that occurs in all the organs and the infection, are recognised to be the ultimate cause of death. Most haemopoietic deaths occur in less than 25 days after whole body radiation (Coggle, 1972).

In most of the cases, the cause of death following acute exposure of animals as well as man to ionizing radiation have been ascribed to be due to "intestinal deaths" or "Intestinal Syndrome" (Altman et al., 1970; Coggle, 1971), and results from destruction of the intestinal epithelium (Warren & Whipple, 1922). It is expected that a systematic study of the intestinal epithelium following whole body radiation will yield valuable informations on mechanism of radiation damage, yet largely unknown.

The Gastrointestinal Syndrome

The gastro-intestinal syndrome is characterised by nausea, vomiting, anorexia and diarrhoea and with severity leading to haemorrhage, dehydration and death. This is due to depletion of precursor cells of the intestinal crypts which supply matured non-dividing functional cells of villi.

Many of the manifestations of irradiation of the whole body are due to the above syndrome and are referable to the gastro-
intestinal tract, which constitutes a sensitive locus for radiation action. The nausea, vomiting, and anorexia of clinical radiation sickness were first described by Walsh in 1897, and degeneration of the intestinal mucosa of irradiated animals was reported by Krause and Ziegler in 1906. The most significant of the early observations was made by Regaud et al. in 1912 who pointed out that the small intestine of the dog is more sensitive to direct x-irradiation than the stomach or colon and that the duodenum and jejunum are the most sensitive regions of it. The effects of x-irradiation of the abdomen were also shown to be more severe than those following exposure of other portions of the body (Moon et al., 1940; Quastler et al., 1951).

What follows is a short review of the morphological, biochemical and physiological alterations in intestine following irradiation as described until recently.

**Morphological changes:**

Destructive changes in the gastrointestinal tract are noted as early as $\frac{1}{3}$ hour after exposure to a moderately large dose (Pierce, 1948), the damage being greater in the small intestine than in the stomach or colon, greatest in the crypts, most pronounced at 8 hours, and repaired within 4 weeks. The duodenum is the most sensitive part of the intestinal tract (Lesher, 1957). Thus following 800 rads of total body x-irradiation, marked degenerative changes in the duodenal epithelium of rabbits are seen by 30 minutes after exposure. They consist in varying degrees
of nuclear swelling and clumping of chromatin in the cells of the villus, crypts, and Brunnerian epithelium. The basal cells of the crypts of Lieberkuhn have been described to be the most sensitive locus of such radiation damage. Nuclear fragmentation and karyolysis occur in such areas. The Paneth cells lose their staining properties within 2 hours after irradiation and display irregular granulation. The epithelium of the Brunner's glands have been described as 'more radio resistant' than the villus or crypt epithelium.

A whole body exposure to 800 r causes less severe injury to the ileum than to the duodenum. Qualitatively the changes are similar, but recovery is more rapid in the ileum. Early effects seen in the lamina propria are oedema and absence of inflammatory cellular reactions. The effects on the lymphatic follicles of the ileum and the appendix are similar to those encountered in other lymphatic tissues. Although, the crypt epithelium is the most sensitive part of intestine, destruction of the entire intestinal lining can occur after lethal doses, leaving fragmented crypt cells, denuded villi, oedema, haemorrhage and ulcers. In young animals, the extent of radiation damage to the small intestine is even greater, and the injury occurs earlier than in older animals (Lesher et al., 1958).

Doses of 50 r or less have no effects on rabbit intestine.
After 100 r there is only a depression of mitosis and occasional dead crypt cells could be seen. A dose of 400 r causes less severe damage than 800 r, with fewer cells affected and a more rapid recovery. Large doses (2000 r) to the abdomen of dogs lead to extensive intestinal damage with severe haemorrhage and ulceration and death of animals (Warren et al., 1922; Regaud et al., 1914).

No changes were observed in the intestines of mice which were exposed daily to 8.8 r of gamma rays during an examination period of 2 to 16 months (Pierce, 1948). Daily total-body exposures to 80 r of x-irradiation caused mild damage after the first few exposures, but after twenty to thirty-five exposures the mucosa was normal. Recovery thus was complete even after a total dose of more than 2800 r. After fractionated doses some radio-resistance may be acquired by the crypt epithelium (Bloom, 1950).

Intestinal cell population kinetics have been studied by Patt and Quastler (1963), Hagemann et al., (1971). A drastic derangement in the cell renewal system was the result in all those cases following radiation doses from 600 to 14000 r. A dose of 800-1000 r has been described to be enough to render the crypt cells incapable of dividing and to cause complete denudation of the villi (Hornsey and Vatistas, 1963; Wilson, 1964; Vatistas et al., 1968).
Biochemical Changes:

Macromolecular changes in the intestinal mucosal layer have been studied by Conard (1956), Gerber et al (1963), Kay & Entenman (1959a) and several other authors and it has been shown that weight as well as DNA and protein content of the intestine diminish after irradiation. The incorporation of radioactive precursor into DNA is reduced 10 minutes after exposure (Abram, 1951; Nygaard and Potter, 1957, 1960, 1962; Sherman and Quastler, 1960; Looney, 1966). Autoradiographic studies indicated that the number of cells synthesizing DNA remained largely the same at that time but the cells in 'S' phase incorporated less radioactive precursors into DNA (Looney, 1966). Indeed, degradation of DNA has been observed in the intestine after irradiation (Fienstein & Butler, 1952), but data on DNA breakdown are less extensive for the intestine than for other organs, e.g., lymphoid tissue. As in the case of lymphoid organs, the fate of macromolecules in the irradiated intestine has been studied by administering labeled precursors before exposure. DNA (Gerber et al, 1963) and RNA (Gerber et al, 1961) present in the intestine at the time of exposure are lost to a certain extent during the post irradiation period when cells die and are lost.

Incorporation of precursors into RNA (Abram, 1951; Toal et al., 1958; Maisin, 1966) was reported to be less sensitive to the early
action of radiation than that into DNA. In the later stage, following irradiation, RNA synthesis diminished and the RNA precursors accumulated.

The synthesis of protein is not altered immediately after exposure (Abram, 1951; Toal et al., 1958; Looney, 1966) but diminishes during the second and third day in the intestine (Lipkin et al., 1963), when the epithelial cells have become abnormal and contain only a few ribosomes. During regeneration, protein synthesis increases above normal 6 days after irradiation with 700 r (Edwards et al., 1964).

Metabolic dysfunctions involving different enzymes in the irradiated intestine have not been studied as extensively as those in liver or lymphoid tissue. Moreover, many studies have been carried out using only semi-quantitative histochemical techniques (Altman et al., 1970). The enhanced proteolytic activity in the intestines of irradiated dogs was reported by Warren as early as 1922. The oxygen consumption of the intestinal mucosa diminishes after irradiation (Barron et al., 1954). Aerobic lactate formation (Perris et al., 1966; Perris, 1968), glucose oxidation (Wesemann et al., 1962) and perhaps citric acid formation (Dubois et al., 1951; Perris, 1968) are reduced from 1 day after exposure to 650-1200 r. The synthesis of glucosamine increases (Cescon & Cavina Pratesi, 1962) whereas that of glucuronides decreases after irradiation (Hartiala et al., 1961). The synthesis of lipids from acetate (Coniglio et al., 1956), the esterification of fatty acids, and the transformation of fatty acids to phospho-lipids (Maisin, 1966) are
also impaired 3 days after exposure. The studies on the different enzyme activities in intestine have been reviewed by Altman et al. (1970) which summarises some of the metabolic reactions involved.

Physiological Changes:

Profound changes in the physiological functions of the gastrointestinal system have been observed with relatively small doses of radiation, of the order of one-third of the LD-50(30) or greater (Conard, 1951, 1956). Immediately following exposure, changes in the tonus and motility can be observed, sometimes preceded by a period of excessive intestinal peristalsis and anti-peristalsis. Anorexia may be a prominent symptom, and gastric emptying time is markedly increased in the rat in the period of hours to days following exposure (Goodman et al., 1952; Swift et al., 1955). The delay in gastric emptying time has been reported to be an abscopal effect, as direct radiation of the bowel was not necessary to produce such effect (Swift et al., 1955). Periods of marked retention of intestinal contents are observed as a result of serious disturbances in the propulsive motility of the intestine (Conard, 1953). There is a marked weight loss following exposure to radiation and the degree of loss is considerably greater than that seen in starved control animals (Conard, 1954), the maximum loss of weight being observed on approximately the third day following exposure over a wide dose range.

The degree of damage in the gastrointestinal tract is in direct
proportion to the dose-administered; as the dose of radiation is increased, the effects become more pronounced. The reduction in gastric emptying time appears to be a direct result of dose (Swift et al., 1955). Anorexia becomes more marked and early vomiting is noted in species capable of vomiting (Bond et al., in Press). With much higher doses, there is diarrhoea which becomes more severe with blood shedding at the terminal event. Death of animals occur usually within three to six days following exposure to large doses, with a picture of severe bowel disturbance.

Animals usually recover temporarily from the early gastrointestinal damage when exposed to smaller doses, in the LD50(30) range. A late effect is, however, observed in gastrointestinal system in the second or third week accompanied by anorexia, distension of the abdomen and diarrhoea, frequently bloody. These changes appear to be related primarily to gastrointestinal lesions secondary to pancytopenia, which is, in turn, secondary to severe bone marrow damage (Bond et al., 1955; Cronkite & Bond, 1960) accompanied by granulocytopenia and other immunological disturbances. Haemorrhages into the mucosal surface produce lesions which may act as sites for invasion of bacteria. In animals dying in gastro-intestinal syndrome, bacteria are occasionally, but not always, found at the time of death (Taketa, 1962). The destruction of cells, altered gastrointestinal motility and later diarrhoea affect the intestinal bacteria and
their metabolism and it has been observed that coliform bacteria and enterococci in the intestinal lumen increase in number at the expense of lacto bacilli three days after whole body exposure of rats to 1400 r (Kent et al., 1968).

Changes in the enzyme content of the bowel have been discussed by Conard (1956). Synthesis of enzymes by the gastrointestinal system and its accessory glands is not affected greatly by intermediate doses of radiation, but is impaired after doses which damage the morphological structure of the organs (Altman et al., 1970). Synthesis of amylase in the pancreas remains unaltered 4 hour after a dose of 2000 r (Hokin & Hokin, 1956), but is depressed after doses of 5000 r and more (Volk & Weilmann, 1968). Production of saliva and of gastric, pancreatic or intestinal juices undergoes clinical variation after irradiation with only 200 r (Kurtsin, 1963), but it is uncertain how these changes affect the animals during radiation illness.

Profound alterations in the intestinal absorptive function have also been observed by a number of workers. A complete review of the subject has been done by Bond (1963). The absorption of carbohydrate has been found to be decreased markedly in patients undergoing radiation therapy as well as in animals after whole body exposure, but the changes do not become pronounced until 2-3 days after irradiation (Moss, 1957; Sullivan, 1961). The transport of glucose is depressed
during the initial period after irradiation (Perris, et al., 1966; Perris, 1966) and then comes to normal or is enhanced after 24 hours (Perris, 1968); during the following days glucose transport diminishes markedly again (Perris, 1966). Besides the active transport of glucose, the passive diffusion of xylose is also impaired (Sullivan, 1961) after irradiation.

Changes in the absorption of fat was reported as back as 1922 by Mottram et al., and has been confirmed by a number of investigators in patients undergoing radiation therapy (Reeves, et al., 1963; Dalla Palma, 1968). The same change have been observed in animals by Morehouse & Searcy (1956), and Schwartz & Shapiro (1961). On the other hand introduction of fat directly into the duodenum did not show any reduction until the third day after irradiation (Dulcino et al., 1957; Sullivan, 1961). Studies with everted intestinal sac technique showed absorption of fat more on the first day and less on the second day after exposure to 1200 r, a behaviour which corresponds to that of glucose absorption (Wesemann et al., 1962).

The intestinal absorption of many other substances has been studied after irradiation. A decreased absorption of vitamin B groups namely thiamine (Detrick et al, 1961), pyridoxine (Detrick et al., 1964), vitamin B12 (Sullivan, 1963) have been observed. Reduced absorption of amino acids, alanine (Mehran & Blais, 1966, 1967), methionine
has also been reported; whereas no change in absorption was reported for protein and vitamin A (Bennett et al., 1950). A reduced absorption of bile acids (Sullivan, 1965), iron (Coniglio et al., 1956; Prosad and Osborne, 1963) and iodide (Acland, 1967) has been observed following irradiation whereas an increased absorption of strontium and calcium two hours after exposure to 400 r has been reported by Lengemann and Comar (1961) which is decreased two-three days after exposure to 1150 r (Marcus & Vos, 1966).

Loss of substances from the animal via the intestine appears to be a more crucial factor in the gastro-intestinal syndrome than the impaired absorption of food. Protein leakage through the intestinal wall on the third day after exposure and thereafter has been reported by Vatistas and Hornsey (1966) and there was a consequent fall in serum proteins. Other macromolecules, e.g., Polyvenyl Pyrolidone (Sullivan, 1960) as well as low molecular substances (N-methylnicotinamide) (Detrick et al., 1963) are also lost in increased amounts via the intestine after irradiation.

Diminished uptake and loss of electrolytes during the terminal phase of the gastrointestinal syndrome precede and probably cause death. Perris et al. (1966) found that the active transport of sodium ions and water in the intestine is depressed within the first hour after
irradiation, returns to normal after 24 hours, and progressively diminishes later (Curran et al., 1960; Vaughan & Alpen, 1961; Mckenney, 1968). There is no marked alteration in the passive flux of Sodium ions. The changes in active transport, however, result in a marked reduction in the net uptake of sodium ions on days 3 and 4 after exposure (Curran et al., 1960). Excretion of sodium ions into the intestine becomes more after irradiation, the concentration of sodium in the wall and the contents in the intestine increase markedly after irradiation of rats with 600 to 1500 r (Caster, and Armstrong, 1956; Maisin & Popp, 1960; Baker et al., 1963). The potassium content in the intestinal wall diminishes, on the other hand, 2 days after a dose of 1500 r, but the potassium level remains normal or elevated 3 days after a dose of 600 r (Barker et al., 1963). It has further been reported that animals irradiated with doses causing gastrointestinal death excrete more sodium and potassium ions into the urine and the faeces than do starved controls (Jackson et al., 1958).

Modification of Radiation Effects

Restoration after Radiation Injury:

All forms of life, from micro-organisms to mammals, can recover from radiation damage. The existence of recovery mechanisms has been shown by the sparing effect of split-dose technique. Several other
techniques have shown that there are two kinds of damages, one repairable and the other not - the latter is, therefore, cumulative and irreversible, and often long-term and/or delayed in action. The work of Stapleton (1955), Hollander (1953), Sheldon Wolff (1955) and others have shown that recovery from radiation injury is facilitated by sub-optimal temperature, a different metabolic state and certain other suboptimal conditions. No report on mutation restoration has been obtained in weeks and months after irradiations (Brookhaven, 1967). All these however, do not mean that there is no repair under normal physiological conditions, but only that the time after irradiation, during which repair occurs, is relatively short (Elkind & Sutton, 1959).

The existence of repair in mammals that have been irradiated is obvious and symptomatic recovery from radiation sickness often appears to be complete. The repair processes may not start immediately after the end of irradiation; there may be a time lag. Mole (1956) found marked fluctuations in the LD$_{50}$ of mice during the first 24 hours after a single dose of 400 r, an early fall being followed by a rise. It has been observed (Hagen & Simmons, 1947; Kohn & Kallman, 1957) that injury from radiation decreases exponentially as far as rats and mice are concerned. If one irradiates continuously at small dose rates, the increase in the total dose necessary for death is also a measure of recovery.
The Oxygen Effect:

Oxygen has a prominent role in radiation injury due to its intimate association with radiochemical reaction (Bacq & Alexander, 1966). If the oxygen concentration of cells or tissues is reduced sufficiently, the damage done by x- and gamma-radiation is diminished though not abolished altogether, i.e., hypoxia or anoxia decreases radio-sensitivity (Hall et al, 1966; Tribukait, 1968; Hall, 1969; Hornsey, 1970). A possible explanation is that, though the formation of an organic free radical R* may be reversed in the absence of oxygen by combination with hydrogen radical and no damage results (R*+H*—RH), in the presence of oxygen, damaging peroxyl-radical may be formed and the organic molecule irreversibly altered:

\[ R^* + O_2 \rightarrow RO_2^* \quad \text{and} \quad H^* + O_2 \rightarrow HO_2^* \]

Further, possibility of free-radical chain reactions exists in the presence of oxygen. Legrys et al., (1968) and Gerald et al., (1969), in fact, reported an increased radiosensitivity at high atmospheric oxygen pressure.

Hydration:

Radiolysis of water is well known to produce toxic radicals which are responsible for damage in most biological systems as all of them contain abundant quantities of water. Hydration, thus, constitutes
a single most important factor which influences the physiological factors that develop the radiation injury. Works on dry seeds showed less damage when they are soaked in D₂O than in H₂O before irradiation, the effect being highly dependent on oxygen (Gaur et al., 1969). Works on dehydration is necessarily limited to micro-organisms and dry seeds of plants, and is not applicable to higher animals.

Temperature:

Radiosensitivity of tissues at various temperature has been studied by a number of workers and it was found to be of interest in different biological systems (Etoh et al., 1963; Hyodo, 1965; Etoh et al., 1965; Egami, 1969 and Levan, 1970). Hallaender (1953) and Stapleton (1955) showed that normal recovery of E. coli from radiation damage was highly facilitated at a suboptimal incubation temperature. Subsequent studies confirmed that a lowered body temperature has a marked effect in reducing radiosensitivity in animals (Cook, 1939; Patt and Swift, 1948; Storer and Hengelmann, 1952; Hornsey, 1956, 1957; Philips and Carrol, 1970; Musacchia & Barr, 1971). A dose reduction factor has been established in irradiated animals maintained at low temperature (Hornsey, 1957).

Metabolism:

The essential role of metabolism in the development of
radiation lesion has been discussed by Bacq & Alexander (1966); a decreased metabolic activity has been found to show a reduction in radiosensitivity whereas an increased metabolic activity before irradiation has an opposite effect. Decreased radiosensitivity under such condition has also been reported by Musacchia et al., (1969). Hibernators, having a depressed metabolic state show greater radio-resistance (Smith and Grenan, 1951; Doull and Dubois, 1953; Kushkin et al., 1959; Musacchia & Barr, 1969) than non-hibernators.

External agents: Chemicals and others:

Addition of a number of other chemical compounds modifies the 'radiochemical yields'. Radiosensitivity might be increased by a number of chemicals, such as synkavit (tetrasodium salt of 2-methyl 1:4 naphtho-hydroquinone di phosphates), Actinomycin D, iodoacetate, tetracyclines (Mitchell, 1954). Such substances are believed to decrease the oxidation-reduction potential and thus alter the oxygen effect.

A number of other chemical substances, on the other hand have been found to show a radioprotective effect. As early as 1949-50 several independent series of investigations by the Leige School, Pett and his collaborators demonstrated the radioprotective actions of chemical agents (Bacq & Alexander, 1966). Of all, the most
important radio protective chemical yet found is cysteine and its
decarboxylated product cysteamine (β-marcapto-ethylamine). These
sulphur containing aminothiols and their derivatives afford a
radio-protection (Pennington et al., 1968; Modig, 1968) probably
by reducing the intracellular oxygen concentration, or scavenging
free radicals or repairing the damage they cause or by contributing
mixed disulphides, linking an –SH group in a protein. Another
belief is that sulphur protectors induce a profound physiological
and biochemical shock, with the radiation process: first, a very
rapid activation of the hypothalamic-pituitary-adrenal neuro-
endocrine systems, secondly, cardiovascular disturbances and
thirdly inhibition of carbohydrate metabolism leading to a decrease
in oxygen consumption and respiratory quotient, which correlate
very well in time with the intensity of protection and might link
with ultrastructural changes of mitochondria. They also perhaps
act by delaying mitosis and DNA synthesis (Brookhaven, 1967).

Amines (aromatic) are also taken as good radio-protectors
(Bacq & Alexander, 1966; Prosad et al., 1969), of which, serotonin
(5-hydroxy tryptamine) is the most important (Alexander et al.,
1975). This, also, is believed to act by causing tissue hypoxia
by vaso-constriction. The detailed mechanism is, however, still
obscure (Streffer, 1974). Adrenaline, noradrenaline and also his-
tamin show radio protection by the same way. A variety of other
substances like para-amino benzoic acid (PABA), dimethyl sulphoxide (Ashwood-Smith, 1967), sex hormones, anoxic agents, anaesthetics, hydroxy compounds, etc., are also weakly active. Chelating agents are also very effective, but are toxic due to their interference with calcium. Cyanides and nitriles in non-lethal doses are also weakly protective. Mixtures of protectors show a better protection as a result of a synergistic action on each other (Maisin et al., 1968).

There are still other agents (e.g., geranyl hydroquinone, bone marrow and spleen extracts) which show a post-irradiation therapeutic action by acting through restoration and replacement mechanisms. The complete mechanisms are, however, not known (Thornburn, 1972).