SECTION 1

I N T R O D U C T I O N

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IONISING RADIATIONS AND LIVING SYSTEMS

Ever since the discovery of natural radioactivity as well as of X-rays, the profound manner in which this form of energy can influence cells and organisms has been fully recognised. Early studies related to purely descriptive observations such as change in colour of skin and loss of hair and have been followed by reports of deleterious changes of considerable physiological significance such as growth inhibition, mutations and even cell death. In course of time, the new multidisciplinary science of radiobiology has evolved; from grossly empirical beginnings, it has been transformed into a fairly well defined and quantitatable subject of scientific enquiry. The phenomenal progress in our understanding of the effects of ionising radiations on life processes has been made possible by the searching investigations of biologists and microbiologists alike, genetecists and biochemists, and physicists working often in close collaboration with each other. With the advent of the atomic age and the even-increasing applications of nuclear energy for peaceful purposes, radiobiology has assumed considerable significance and taken its rightful place among the physical and natural sciences.

All radiobiological effects obviously stem from the deposition of ionising energy within the cell and the subsequent efforts of the cell to cope with it. A great deal of work is being done to understand how the absorption of this minute amount of energy can alter a wide variety of functions of living organisms.

It is now recognised that a series of chemical and biochemical events intervene between the purely physical primary step of energy deposition and the ultimate manifestation of a biological effect.

Hit and Target theories:

The earliest attempt at rationally explaining /action /the of radiation on living cells was the 'hit theory' of Dessauer (1) and this was later developed into the 'target theory' by Crowther (2 - 4). This theory postulates that ionising radiations hit vital targets within the cell causing their inactivation and consequently resulting in death of the organism. Such inactivations can be visualised to occur from ionisations or excitations of chemical molecules on direct interaction with ionising energy.

Relevance of indirect action to radiobiology:

There is increasing recognition in recent years that the mechanism of indirect action, in which there is transfer of energy from reactive species formed by direct action to other molecules by chemical interaction, could be of considerable significance in the development of radiation injury. With some variations, cells consist of about 70 - 80 per cent water and the various metabolites are either dissolved or suspended in this medium. The water molecules therefore present the largest number of targets when cells are exposed to ionising radiations and the radiolytic products of water consist of several highly reactive

species. These products, some of which are transient free radicals, are known to react with biological molecules and cause significant alterations in their structure which is often enough crucial to their function.

The significance of the indirect action of ionising radiation on biological systems has been emphasised in recent years from observations on the response of cell cultures to prior irradiation of their medium (cf 5). Such radiation effects, at times become apparent long after the actual irradiation and are believed to be brought about by stable toxic radiolytic products that eventually diffuse to functionally or structurally critical centres of the cell.

In the current state of our knowledge, it is best to assume that there is a superimposition of effects due to direct and indirect actions of radiation.

The term 'indirect' has also been used to denote systemic biological effects. Such effects mediated by physiological or neural mechanisms are best described by the term 'abscopal' (6).

BIOCHEMICAL MECHANISMS UNDERLYING RADIATION EFFECTS

The sequence of events resulting from the dissipation of ionising energy within the cell can cause chemical alterations in several bio-molecules contained in the subcellular elements, the cytoplasm and the cell membrane; these changes affect the

structure and function of the cell as a whole and are reflected in biochemical changes which appear in irradiated living systems. The irradiation of living system produces a multitude of biochemical changes (7). Some of these biochemical changes are detrimental to the normal functioning of the living system and could even lead to death, whereas some others are not of much consequence or are repairable (Fig. 1). The cellular responses to radiation vary widely with the doses of radiation required to elicit them as well as in the time intervals required for their manifestation. Many of the observed effects are secondary to radiation-induced changes elsewhere in the organism.

Radiation effects on DNA and its synthesis:

There is considerable evidence to suggest that DNA is perhaps the most important as well as the principal target of radiation effects (8, 9). Ionising radiations are known to lead mainly to strand scissions in the DNA molecule (10 - 13). The existence of repair mechanisms of differing efficiency explains to a large extent the considerable variations in radiosensitivities among microorganisms (14). The enzymes responsible for DNA repair have since been identified also in mammalian cells (15).

That X-irradiation inhibits precursor uptake into DNA of many animal tissues was first shown by Ahlstrom et al. (16). Since then, several investigations have confirmed the fact that radiation inhibits DNA synthesis in dividing cells. There is no

Fig. 1. Sequence of events in the cell following exposure to radiation*

*Reproduced from Pradhan, D.S. and Sreenivasan, A., J. Sci. Ind. Res. 30, 704, 1971.

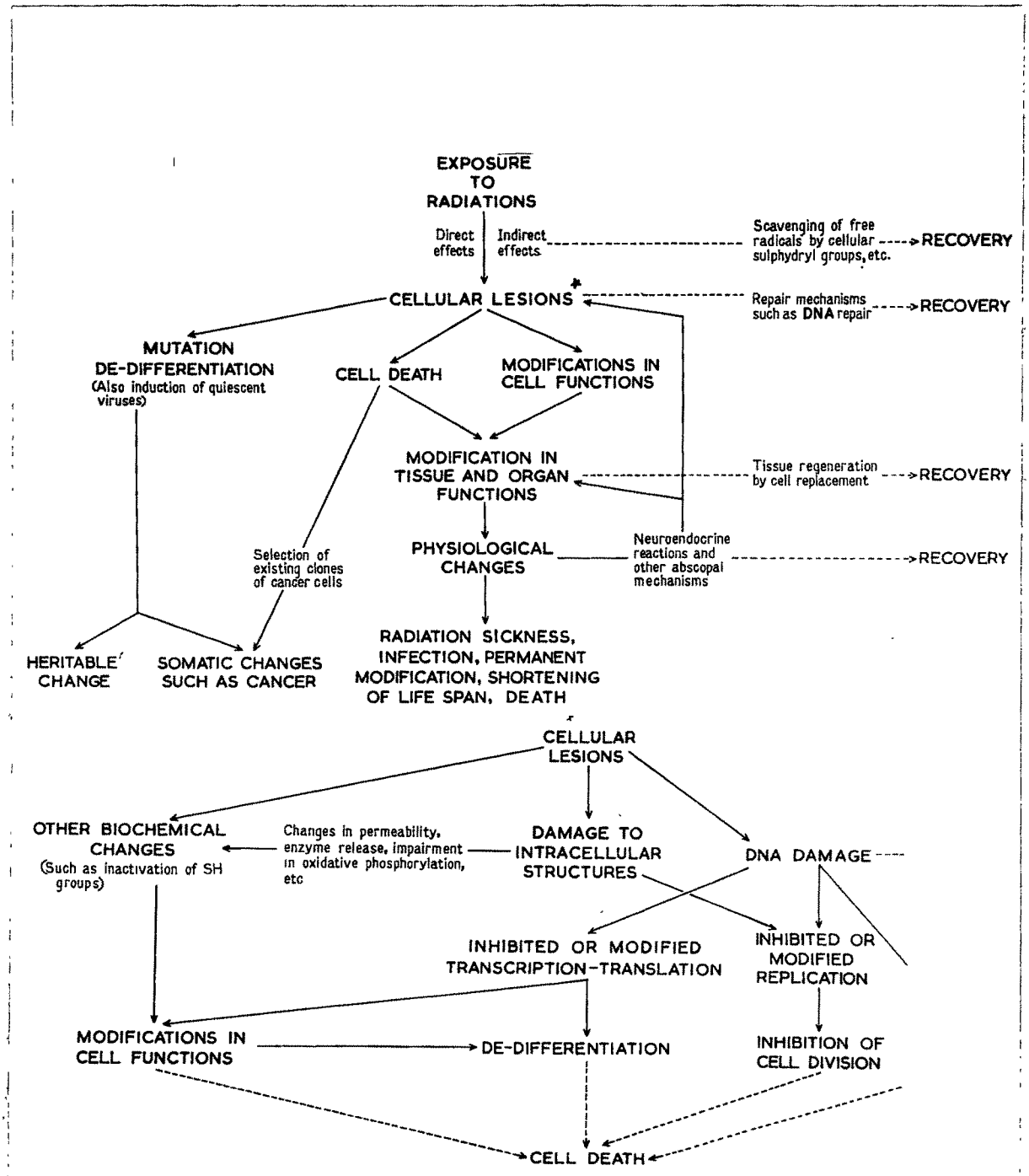
EXPOSURE TO RADIATIONS
Direct effects
Indirect effects
Scavenging of free radicals by cellular sulphydryl groups, etc.
RECOVERY
CELLULAR LESIONS
Repair mechanisms such as DNA repair
RECOVERY
MUTATION DE-DIFFERENTIATION
(Also induction of quiescent viruses)
CELL DEATH
MODIFICATIONS IN CELL FUNCTIONS
MODIFICATION IN TISSUE AND ORGAN FUNCTIONS
Tissue regeneration by cell replacement
RECOVERY
Selection of existing clones of cancer cells
PHYSIOLOGICAL CHANGES
Neuroendocrine reactions and other abscopal mechanisms
RECOVERY
HERITABLE CHANGE
SOMATIC CHANGES SUCH AS CANCER
RADIATION SICKNESS, INFECTION, PERMANENT MODIFICATION, SHORTENING OF LIFE SPAN, DEATH
CELLULAR LESIONS
OTHER BIOCHEMICAL CHANGES
(Such as inactivation of SH groups)
Changes in permeability, enzyme release, impairment in oxidative phosphorylation, etc
DAMAGE TO INTRACELLULAR STRUCTURES
DNA DAMAGE
INHIBITED OR MODIFIED TRANSCRIPTION-TRANSLATION
INHIBITED OR MODIFIED REPLICATION
MODIFICATIONS IN CELL FUNCTIONS
DE-DIFFERENTIATION
INHIBITION OF CELL DIVISION
CELL DEATH

Figure 1

evidence of a deficiency of precursors for DNA synthesis. In fact, most studies show an accumulation of deoxyribonucleosides and deoxyribonucleotides in irradiated tissues (17, 18).

Several studies have also shown radiation-induced defects in DNA replication and transcription (9, 19 - 22). Of interest in this respect are the studies (21, 22) which suggest that on exposure of E.coli cells to X-irradiation, the sequence of chromosome replication gets altered. After irradiation, new cycles of DNA replication are initiated even before the earlier cycle is completed.

The availability of the necessary enzymes following radioexposure has been studied in some detail in regenerating rat liver since this is a somewhat synchronous system. Irradiation at 6 to 12 hours after partial hepatectomy, when there is a rapid synthesis of kinases and polymerase, prevents the formation of these enzymes and thereby inhibits DNA synthesis.(23, 24). When synthesis of DNA in regenerating liver is underway, higher doses of radiation are required to achieve an inhibitory effect (25, 26). Inhibition of DNA synthesis may result from impaired capacity of irradiated DNA to 'prime' DNA synthesis which in turn may be due to inability of double-stranded DNA to separate into a single-stranded form. Alterations in the DNA molecule following irradiation can also render it incapable of acting as template. The appearance of deoxy-cytidine and increased amounts of β-amino-isobutyric acid in the urine of humans and rats after whole-body

irradiation has been noted (27, 28) and is believed to be due to increased breakdown of DNA or to an interference with DNA synthesis. Radiation effects on nuclear proteins or hormones, which may regulate nucleic acid synthesis (29, 30) may also be responsible for inhibition of DNA synthesis. Although most of the observations on DNA synthesis relate to changes occurring several hours after irradiation and may therefore represent secondary damage, very early effects on DNA synthesis have also been reported (31).

The results of recent studies on the effects of radiation on DNA synthesis are consistent with the idea that whole-body radio-exposure produces functional damage to DNA template (9).

Myer and Ram (32) have reported that early inhibition of DNA synthesis following irradiation could be discerned in all tissues of the rat irrespective of whether they are radio-resistant or -sensitive.

The inhibition of DNA synthesis could be related to the action of radiation on nuclear membranes, probably involved in the regulation of DNA synthesis. This possibility is strengthened by studies which indicate that intracellular membranes are susceptible to radiation damage by mechanisms such as lipid peroxidation and oxidation of SH groups of the membranes (33).

The inhibition of DNA synthesis in many instances may be the result of blocking of the preceding mitosis. This has been

the conclusion in a number of reports (34 - 38). The necessary conditions for synthesis and the triggering mechanism may not be present as a result of some unknown radiation effect which arrests the cycle in G_2 phase.

Metabolism of RNA following irradiation:

The effects of radiation on synthesis and function of RNA has not been studied in as much detail, the major problem being the great metabolic and chemical heterogeneity of cellular RNA. It appears that certain fractions of RNA are more sensitive to radiation than others. A number of workers have reported retardation of RNA biosynthesis after irradiation (39 - 42).

Inhibition of RNA synthesis may be due to primary radiation injury to DNA. Such evidence has been presented in experiments with E.coli (43), in which radiation inhibits the induced synthesis of the enzyme β-galactosidase. The alterations of a DNA molecule necessary for the synthesis of a messenger RNA molecule which in turn is required for the synthesis of some vital protein would produce death.

It has been shown that in liver there is an enrichment of polysomes following irradiation (44 - 46). Hidvegi et al. (45) have reported that in guinea pigs this increase is solely related to a stimulus in RNA synthesis. It has been shown (45, 47) that whole-body radioexposure leads to increase in the capacity of liver nuclei to polymerize RNA. On the other hand, evidence has

also been presented to indicate that the increase in the capacity of liver nuclei to synthesize RNA is due to amplification of the template function of endogenous chromatin rather than the activation of RNA polymerase (48).

Radiation effects on protein synthesis:

The way in which whole-body radioexposure brings about changes at the translational level in the liver is not clear.

Unlike the liver which is a fairly radioresistant tissue, the thymus show decreased protein synthesis following irradiation and this is attributable to a significant lowering in the proportion of polysomes. This is accompanied by increase in alkaline RNase activity in this tissue and a concomitant fall in the level of alkaline RNase inhibitor (49). It is likely that, in thymus, disaggregation of polysomes has its origin in the radiation-induced lowering of the activity or the levels of this inhibitor.

The normal activities of the protoplasm of irradiated amoeba can be restored when unirradiated protoplasm is allowed to fuse with it (50). Polyribosomes or large subunits of them are believed to be the restorative agents. When a lethally irradiated amoeba receives unirradiated polysomes, it may synthesize proteins again, including enzymes needed for the repair of the damaged nuclear DNA.

Alterations in diverse metabolic pathways following radioexposure have been shown to result from an influence on activities of the enzymes involved therein. Protein synthesis in irradiated cells has been reported to exhibit either general or specific effects. In fact, alterations in protein synthesis patterns in a number of tissues (9, 61, 62) are some of the early signs of responses of animals to radiations. Protein synthesis in some animal tissues has been reported to be retarded following whole-body exposure to radiation, whereas in others a significant elevation in protein synthesis is discernible (56,61-66).

Total body irradiation has been reported to enhance incorporation of labeled amino acids into proteins of normal liver (51 - 60). These studies are indicative of enhanced protein synthesis in liver between 12 and 36 h post-irradiation (48, 49, 52, 55, 57). Recent experiments have established that net biosynthesis of certain specific proteins increases in isolated rat liver, perfused with rabbit blood 4 - 6 days after irradiation (66). The way in which whole-body radioexposure (18 h post-irradiation) brings about changes at the transcriptional level and in turn in protein synthesis in the liver is not clear. Radiation-induced secretion of adrenal steroids, resulting from pituitary-adrenal overactivity (61), could be one of the factors underlying the observed changes in RNA and protein synthesis.

However, in regenerating liver, a decrease in amino acid incorporation into proteins has been observed. The incorporation of labeled amino acids into both cytoplasmic and nuclear

proteins is depressed 24 h after partial hepatectomy if the animals are exposed to radiation between 2 and 6 h after surgery (67). It is difficult to determine whether such changes truly represent alterations in rate of protein synthesis, or in amino acid pools, or both.

While whole-body exposure stimulates the synthesis of proteins in liver, protein synthesis in the more radiosensitive tissues, such as thymus, is considerably lowered within a few hours following irradiation (49, 62 - 65). Even in the case of thymus, it appears that there occurs a very early stimulation of nuclear protein synthesis and this is followed by inhibition at 2 h after whole-body irradiation. The decrease in protein synthesis in thymus is associated with a significant lowering in the proportion of polysomes (49).

Immediately after irradiation at a dose of 100 R, the *in vivo* incorporation of valine into proteins is enhanced, but decreases later in lymphoid organs (63). The early increase could be an indication of stimulation via adrenals, since it is not observed in adrenalectomised animals. With doses up to 2000 R, there is a dose-dependent decrease of incorporation of valine into nuclear proteins *in vitro* (63).

NUCLEUS VS. CYTOPLASM AS PRIME TARGET OF RADIATION

Most of the early investigations on the biological effects of ionizing radiation had emphasised that derangement in

cellular processes was a consequence of structural and functional alterations occurring in the cell nucleus. It is easy to visualise that, in view of the primary importance of DNA as the genetic material, several metabolic activities of the cells could be significantly influenced as a result of damage to DNA. In addition to explaining the diverse nature of radiation injury, it has been pointed out that the large differences in radiosensitivity of different cell types may also be related to some inherent character of the nucleus. Nuclear volume has been postulated as an important criterion determining the response of cells to radiation - the larger the nuclear volume, the more radiosensitive the cell. Radiosensitivity has been shown to be correlatable with nuclear volume in several plant cells (68). Since the size of the nucleus may be expected to be proportional to its DNA content, it has been suggested that the amount of DNA per cell may account for the variability in radiation sensitivity (68). In particular it has been pointed out that the highly radiosensitive lymphocytes contain larger quantities of nucleic acid than do most radioresistant cells (69).

However, these early suggestions have not withstood critical experimental assessment over the years (70). The amount of DNA has been found to be almost the same in every type of cell in the same organism, except in a few polyploid cells of some tissues (70). An increase in the size of the nucleus is associated with a diminution of cytoplasmic content and consequent paucity of other organelles. In recent years, considerable evidence

has been obtained indicating that cellular components other than the nucleus may play a significant role in the development of radiation injury, and may be of prime importance in cells in interphase. These cytoplasmic factors could either enhance the resistance of the cells to the destructive action of radiation or could help the cell recover from radiation damage.

There is evidence in literature of alteration in the ultrastructure of the cell following exposure to ionising radiation (71); the electron microscopic studies of Goldfeder deserve particular mention in this connection (71). These extensive investigations have brought out that morphological alterations are not limited to any particular organelle but that there is widespread disruption of intracellular structural organisation. Following irradiation of mice, there occurs disruption of plasma membranes, dilation of vesicles such as the cisternae of the endoplasmic reticulum, swelling of mitochondria as well as destruction of mitochondrial cristae in both lymphocytes and transplanted tumour cells (72). A significant aspect of these studies relates to the fact that in cells in which there was evidence of such extensive cytoplasmic damage, often enough the nucleus and the nuclear membrane remained intact. The existence of extra-nuclear radiation sensitive sites located in the cytoplasm has also been clearly indicated in studies with the giant unicellular algae, Acetabularia mediterranea (72, 73), It has been observed that anucleated cells of this organism, which are capable of growth and morphogenesis, are as sensitive to radio-exposure as are the

whole cells. On the basis of these findings, it has been contended that the nucleus cannot (74) be the main target of radiation injury in this single cell organism.

Evidence for mitochondrial damage following irradiation:

That the mitochondria are quite susceptible to radiation damage has been recognised as early as in 1930 (75), although in quite a few subsequent reports either no deleterious effects were observable in mitochondria following irradiation (76 - 78) or these were found not to precede morphologically discernible alterations in nucleus (78). At the present time, there is extensive evidence in literature of the damages suffered by mammalian mitochondria due to radioexposure as also an increasing recognition of its significance to the development of radiation injury, as a whole.

The exposure of mice to whole-body radiation at doses of 500 - 1200 R has been shown to result in globulation, fragmentation and relocation of mitochondria; such effects were hardly noticeable prior to 8 h following a dose of 500 R, but were significant at such early periods with higher doses (79). These changes were more pronounced at 24 - 48 h. The number of mitochondria in ascites tumor cells showed an immediate decrease when mice were irradiated at 500 - 2000 R (80). The mitotic index decreased subsequent to the fall in mitochondrial number and the recovery of mitosis was slower than the recovery of mitochondrial count. A similar decrease in mitochondrial population has also been reported in lymphocytes

and reticulocytes from spleen of irradiated mice (81). It appears likely that the lowering of mitochondrial number may result from their fragmentation and disintegration.

The decrease in mitochondria is also accompanied by increased vacuolation, clumping and interestingly enough, an enlargement in size (82). There are several reports relating to such ultrastructural changes in mitochondria, including dilation of outer membrane, breakdown and fragmentation of cristae, swelling, loss of matrix and other abnormalities (83 - 85). The electron microscopic observations reported in these studies have been made from very early periods, such as 15 min to several days after irradiation and likewise the doses used also fall in a wide range from a few hundred to several thousand R. The quantitative electron microscopic studies of Bahr (86) are of particular interest. Following whole-body irradiation of young rats with a dose of 1000 R of x-rays, there occurs a parallel increase in volume and dry mass of mitochondria within a few hours. These studies have led to the concept of 'unit mitochondrion'.

That a poor mitochondrial content may be responsible for extreme susceptibility of some types of cells was first suggested by Schjeide et al. (87). Mitochondria have been shown to be relatively radiosensitive (88, 89) and could be important factors in determining the response of cells to radiation. The difference in the degree of radioresistance of differentiated cells as compared to undifferentiated cells, which has been known

for a long time, has also been attributed to the significant variations in respect of their mitochondrial content (90). Undifferentiated cells, such as embryonic tissue are comparatively poor in mitochondria and are highly radiosensitive (75).

Systematic studies correlating mitochondrial character with radiation damage at cellular level have been carried out by Goldfeder and colleagues (8, 71). These studies, with a variety of normal and cancer cells, have made possible certain generalisations correlating composition and integrity of cytoplasmic ultrastructures, especially of mitochondria, with cellular radiosensitivity. The epithelial tumour cells are less damaged by irradiation than the spindle cell tumour; the mitochondria in the former are more numerous and contain more internal membranes. Likewise the cells of the kidney and heart are very rich in mitochondria and these organs are very radioresistant; conversely, the cells of lymph node contain few mitochondria and are extremely susceptible to radiation damage. In analysing the greater radioresistance of cells which may be considered superior in terms of their mitochondrial content, it may be pointed out that this may be related to one or more of the following: greater content of catalase; increased ATPase activity; higher capacity for oxidative phosphorylation; and increased chances of a greater population of undamaged mitochondria.

Based on the structural and functional integrity of mitochondria, it seems reasonable to conclude that the quantity

and quality of mitochondria play a significant role in determining the radiosensitivity of cells.

BIOCHEMICAL LESIONS IN IRRADIATED MITOCHONDRIA

Although the deleterious structural alterations in mitochondria, following the absorption of ionising energy, have been recognised for a long period, studies to understand the biochemical nature of the radiation damage have been carried out only during the past decade.

Among the most apparent of the biochemical changes is the decrease in mitochondrial soluble proteins paralleled by an increase in cell sap protein (91). This release of proteins from mitochondria probably arises from altered membrane permeability (91). Among enzymes of the respiratory chain, succinic dehydrogenase has been observed to show a marked increase at early periods (less than 24 h) followed by a return to normal values and a renewed increase with maximum activity at 4 and 8 - 9 days following irradiation (92). The levels of NADH-cytochrome c exhibited a regular oscillation around the value in normal animals (92). A drop in the concentration of NADPH in liver mitochondria of rats exposed to 1000 R of x-rays has been noted at 3 and 24 h after irradiation (93).

Evidence has been presented indicating an increase in total lipid phosphorus and a change in distribution of phospholipid in liver mitochondria from irradiated rats (94). There were

qualitative variations in the phospholipid composition. Irradiation has also been reported to produce an increase in total lipids of mitochondria, with a decrease in the amount of strongly bound lipids (95). The phosphatide content of the loosely bound lipids showed a decrease.

With the recognition that mitochondria contain their own protein synthetic machinery with its own characteristic DNA, attention has also been focussed on the effect of radiation on mitochondrial nucleic acid and protein metabolism. Whole-body irradiation of rat depressed the incorporation of tritiated thymidine into mitochondrial DNA of both normal and regenerating liver (21 h post-hepatectomy). The degree of inhibition is proportional to the rate of synthesis at the time of irradiation and is therefore greater in the regenerating liver which shows an enhanced synthesis of mitochondrial DNA (96). A comparative study of the incorporation of thymidine into nuclear DNA and mitochondrial DNA in normal and regenerating (17 or 24 h post-hepatectomy) rat liver and the effect of whole body irradiation (500 or 1500 R) has revealed some interesting observations (97). The synthesis of mitochondrial DNA decreased following irradiation until a minimum value was reached (4 h in case of normal and 12 h in the case of regenerating liver), after which it increased at an exponential rate. In yet another study also, it has been reported that mitochondrial DNA content increased rapidly in regenerating rat liver after partial hepatectomy and that irradiation (600 R) of the animals blocked mitochondrial DNA biosynthesis (98).

Although there has been some argument as to whether the radiation-induced inhibition of thymidine labeling of mitochondrial-DNA reflects a damage to the enzyme system involved in the overall synthesis, from present evidence it does appear to be substantiated. The degree of radiation inhibition of mitochondrial DNA synthesis is dose dependent in both normal and regenerating rat liver. The extent of the radiation effect on mitochondrial DNA synthesis does not depend as much on the time of irradiation following partial hepatectomy, as it does on the time interval between irradiation and sacrifice. This observation is in conformity with the concept of a relative independence of mitochondrial DNA synthesis from the mitotic cycle.

In the unicellular organism, Acetabularia mediterranea, where mitochondrial damage has been shown to be of prime importance in the manifestation of radiation injury, mitochondrial DNA is destroyed at radiation doses that have no significant effect on chloroplast DNA (73). It is interesting to note that radiation damage to mitochondrial DNA is also capable of repair; studies with Tetrahymena pyriformis have shown that the strand scissions in mitochondrial DNA following radioexposure are efficiently repaired (99).

Reports on whole-body irradiation effect on protein synthesis in mitochondria of different tissues show divergent trends and it is difficult to generalise on this aspect of radiation effect. A week following localized exposure to 300 R of x-rays, mitochondria

isolated from rabbit neurons and neuroglial cells show an increased labeling of the proteins by radioactive leucine (100). Liver mitochondria from mouse subjected to whole body irradiation at a supralethal dose of 2000 R also exhibit enhanced protein synthesis as assessed by *in vivo* and *in vitro* labeled amino acid incorporation studies (101). This enhanced labeling of protein may possibly arise from increased permeability, as evident from the swelling, leading to greater availability of precursors. In contrast to these findings, a decreased protein synthetic activity of liver mitochondria of rats exposed to a dose of 800 R has also been reported (102).

RADIATION EFFECTS ON MITOCHONDRIAL ENERGY GENERATION AND CONSERVATION

Mitochondrial function, especially in relation to electron transport and coupled phosphorylation, is dependent on maintenance of structural integrity of its membrane (103, 104). In view of the key role of mitochondria in energy generation, an interference with the prime function of this particulate fraction may result in impairment of several cellular processes that are dependent on chemical form of energy. Aspects of mitochondrial energy metabolism *vis-a-vis*, the development of cellular injury due to ionising radiations have interested investigators for over two decades.

A brief review of currently accepted concepts and mechanisms of mitochondrial electron transfer and coupled phosphorylation is presented in the following paragraphs for a better appreciation of radiation effects on cellular energy generating processes.

Mitochondrial electron transport chain:

By far the quantitatively most important aspect of biological energy production occurs through the mediation of a multienzyme system which is associated with the inner membrane of mitochondria. It involves the transfer of electrons from oxidiseable substrates through a chain of carriers, that include flavoproteins, cytochromes and a lipid quinone, down the steps of a thermodynamic ladder to molecular oxygen which acts as the final electron sink. The conservation of the energy derived from this sequential oxidation takes place at three specific steps of electron transfer, through the formation of adenosine triphosphate (ATP). Chemical energy needed for various biosynthetic pathways is derived from the hydrolysis of the terminal phosphate group of ATP, the amount of energy available being 10 - 12 Kcal. per mole of ATP.

Our knowledge of the assembly and sequence of the catalysts of the oxidation chain and of the mechanisms of electron transport and coupled phosphorylation (103, 104) is still incomplete. Lipmann, Lehninger, Slater, Chance, Boyer, Cohn, Lardy, Green, Pullman, Racker and Mitchell are some of the major contributors to the unravelling of the mechanisms of electron transport and

coupled phosphorylation. Based on the experimental evidence available at present, the most acceptable scheme of the respiratory chain is shown in Fig. 2. Extensive studies that have led to the proper sequencing of these respiratory catalysts have also been responsible for the development of a number of highly specific artificial electron acceptors as well as site specific inhibitors that enable the bypassing of normal route of flow of electrons.

Between DPNH and oxygen there occurs about twelve successive oxidoreductions. Although, approximately a similar number of oxidoreductions take place between succinate and oxygen, the nature of the first three of these is different. The potential drop in three segments of the electron transport assembly, viz., between DPNH and coenzyme Q, reduced coenzyme Q and cytochrome c and reduced cytochrome c and molecular oxygen, is more than sufficient to 'power' in each case the synthesis of one pyrophosphate bond of ATP per pair of electrons transferred. But this is not true for the potential difference between succinate and coenzyme Q.

Theories of oxidative phosphorylation:

The coupling mechanism, which enables the transformation of oxidative energy into bond energy of ATP also appears to be associated with the inner membrane of mitochondria. Several proteins have been isolated during the past decade or so from mitochondria and have been designated as coupling factors to

Fig. 2. Electron transport system of mammalian mitochondria.

Legend: I, II & III refer to the three sites of coupled phosphorylation. a, a_3, b, c and c_1 denote the various cytochromes; F_p and Q stand for flavoprotein and ubiquinone, respectively.

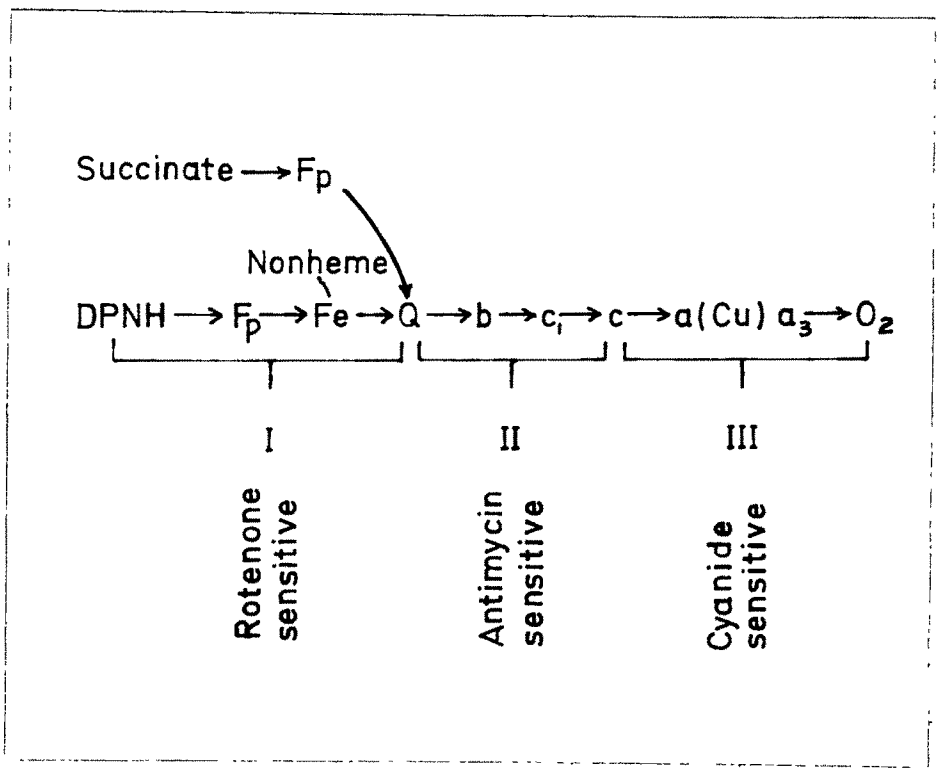
Succinate → Fp
Nonheme
DPNH → Fp → Fe → Q → b → c1 → c → a(Cu) a3 → O2
I
II
III
Rotenone sensitive
Antimycin sensitive
Cyanide sensitive

Figure 2

denote their functional role in this process. These factors may be structural components of the membrane participating in the proper organisation of the catalysts, rather than being catalysts themselves (105 - 107). It is also possible that the function of some of the coupling factors may be neither catalytic nor structural, but regulatory e.g., they may act by counteracting natural uncouplers.

The generation of ATP from ADP and Pi takes place with the removal of water, resulting in the formation of the anhydride bond of the terminal pyrophosphate group of ATP. There are two major mechanisms that have been proposed to spell out the reactions involved in this process.

The chemical theory, first proposed by Slater (108) is shown in an abbreviated form in Fig. 3A.

The chemical coupling theory involves two hypothetical nonphosphorylated intermediates A ox$\sim$X and X$\sim$Y and a hypothetical phosphorylated intermediate X$\sim$P. 'A' is a component of the electron transport chain. The hypothetical high energy intermediates involved in the coupling sequences have not yet been isolated or even identified. According to the chemical theory the mitochondrial membrane serves as an organiser of the catalysts which participates in the generation and utilisation of X$\sim$Y.

The chemiosmotic theory of oxidative phosphorylation, elaborated by Mitchell (109) envisages the pH gradient and a membrane potential created by the translocation of protons during

Fig.3. Hypothetical representation of oxidative phosphorylation

A. Chemical hypothesis

Legend: A red and A ox stand for reduced and oxidised forms of electron carrier. X and Y are hypothetical compounds involved in the process of transferring energy.

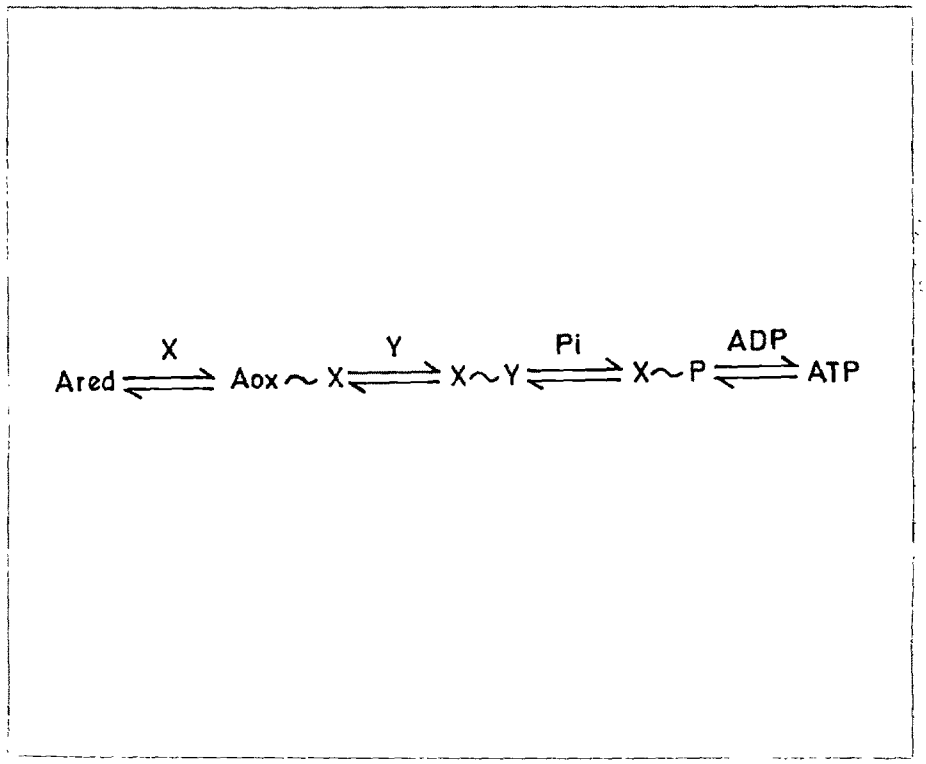

Figure 3 A.

the respiratory process as the initial driving force for the formation of chemical energy (Fig. 3B). According to both formulations there is a high-energy intermediate X~Y, but the mechanisms for its production and utilisation are quite distinct and visualise different functions for the membrane in the process.

Radiation induced changes in mitochondrial electron transport function:

The effect of irradiation on the activity of various enzymes of the respiratory chain (110) have been investigated ever since the development of the manometric techniques. The results of many of these studies have been reviewed periodically and it is still difficult to reconcile the numerous contradictions (55, 111, 112); in many instances the discrepancies are paobably only apparent and may be caused by differences in experimental conditions. It appears likely that at least some of these observations may represent secondary effects of radiation or they may be abscopal in nature as indicated by their differential response to local and total body irradiation (113, 114).

The response of different tissues to irradiation, as assessed by the functional integrity of their electron transport chain, do not reveal any parallelism to the degree of radiosensitivity (110).

Among the earliest observations is that of Maxwell and Ashwell (115) relating to the unimpaired ability of spleen mitochondria

B. Chemiosmotic hypothesis

Legend: The respiratory chain is arranged in three loops, so that during the passage of two electrons from the NAD-linked dehydrogenases to oxygen, a total of six protons are ejected (or moved from the matrix to the outer compartment). The reduced forms of NAD^+, flavoprotein and coenzyme Q are assigned the role of hydrogen carrier in this formulation. The anisotropic ATPase is operating in the direction of ATP synthesis driven by the left-to-right (inward) movement of protons. The mechanism involves the formation of an anhydride ($X \sim Y$) as a consequence of the removal of protons from the right side of the membrane (by the formation of H_2O at the high pH), and the addition of protons and the removal of water on the left. The anhydride effectively becomes a high-energy intermediate in the electrochemical conditions which exist on the inner side of the membrane. When this enzyme system is operating as an ATPase, the reactions are all reversed and protons move from right to left (outward). R and L stand for right and left sides of the membrane respectively. X and Y are energy carriers.

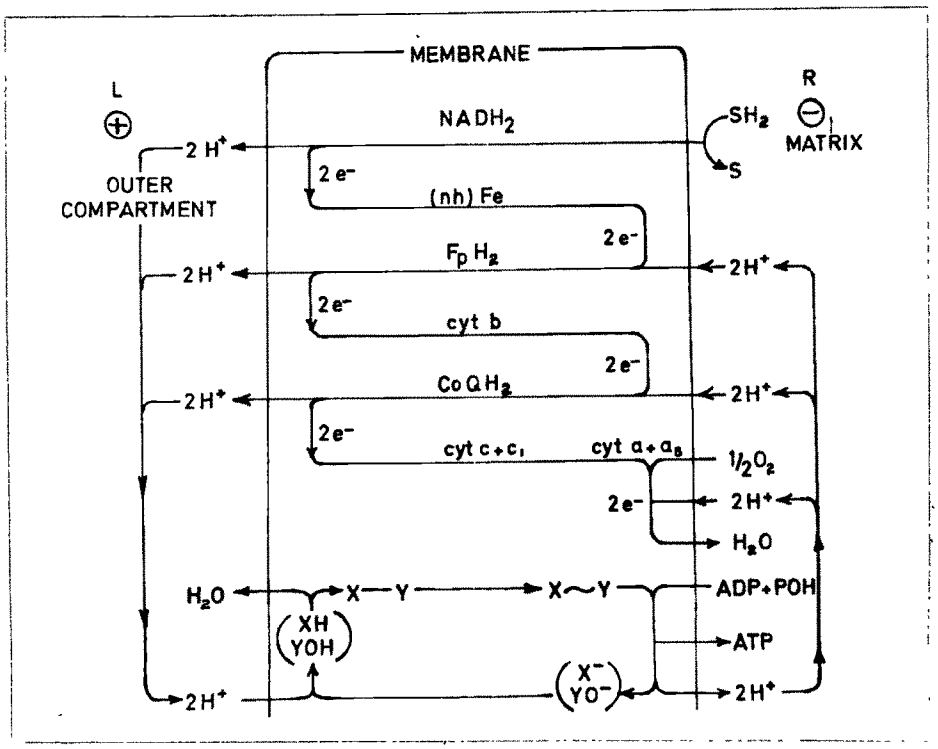
MEMBRANE
L
R
NADH2
SH2
MATRIX
2 H+
2 e-
OUTER
COMPARTMENT
(nh) Fe
2e-
Fp H2
2H+
cyt b
CoQH2
cyt c+c1
cyt a+a3
1/2O2
H2O
X—Y
X~Y
ADP+POH
XH
YOH
ATP
X-
YO-

Figure 3 B.

from whole body irradiated animals to oxidise succinate. A significant decrease in endogenous respiration of spleen from irradiated mice has been reported (116) and is believed to be an abscopal effect since radioexposure of the exteriorized spleen, with the rest of the body shielded, failed to show a similar effect. With another highly radiosensitive tissue, the thymus, the NAD dependent step in the oxidation of α-ketoglutarate has been observed to be damaged by *in vivo* irradiation; respiration is unaffected when the thymus mitochondria are irradiated *in vitro* (117).

Evidence obtained from different studies on radiation effects on hepatic mitochondrial electron transport system are conflicting. Inactivation of liver mitochondrial succinate oxidase following *in vivo* or *in vitro* exposure of x-rays has been reported (118). Likewise, an inhibition has also been observed with cytochrome oxidase activity of liver mitochondria of rats exposed to 1000 R of x-rays (119). On the other hand, oxygen uptake has been reported to be increased with pyruvate, β-hydroxybutyrate, citrate or glutamate in liver mitochondria of rats, 6 h after exposure to whole body irradiation (120). An increase in respiration in liver has been reported following a lethal whole-body x-ray dose of 1300 R (121). Observations of Clark *et al.* (121) on loss of respiratory control with a variety of substrates following *in vitro* irradiation (up to 100 KR) of liver mitochondria would also seem to be in line with the *in vitro* findings discussed above.

Mitochondria from heart of animals subjected to whole body irradiation reveal decreased oxygen consumption (119) which may be related to the reported inhibitions of cytochrome oxidase activity and NADH oxidation (119). Among other tissues studied, brain and kidney (120) mitochondria are also reported to show decreased respiratory rate following whole body irradiation of the animals. Irradiation of rat had opposing influences on two enzymes of the electron transport chain in adrenal mitochondria (122), succinic dehydrogenase increasing abruptly at 72 h post-irradiation and NADH cytochrome c reductase decreasing significantly.

Radiation effects on oxidative phosphorylation:

An impairment of oxidative phosphorylation is one of the most marked effects of radiation on the biochemistry of the living cell. In the earliest report on the subject, Potter and Bethel (123) found that spleen mitochondria of rats given whole body radiation showed a reduced capacity to carry out oxidative phosphorylation, as early as 1 h after irradiation. Similar effects on coupled phosphorylation in spleen mitochondria have since been made by several other investigators (114, 124, 125, 127 - 131). Even small doses of radiation such as 50 - 100 R resulted in significant damage to oxidative phosphorylation in spleen mitochondria a short time following whole body exposure (125). In one of these studies, it was shown that even if the spleen is shielded, oxidative phosphorylation is adversely affected (129), showing the indirect nature of the effect. Experimental

evidence has been obtained to indicate that the radiation action is indirect and mediated through a hormonal response (129).

Mitochondria of other highly radiosensitive tissues and cells such as thymus, testes and lymphocytes have also been reported to show significant lowering of the efficiency (117, 128, 132 - 136) of oxidative phosphorylation. The radiation lesion in the case of thymus mitochondria can be repaired *in vitro* by the addition of cytochrome C, bovine serum albumin or vitamin K (116). While it has been suggested that activation of ATPase in irradiated lymphoid tissue may result in breakdown of some ATP *in vivo*, depression of oxidative phosphorylation has been shown to precede the activation of ATPase (128).

There has not been general agreement among various investigators as to whether the coupling of phosphorylation to oxidation is also affected in non-radiation sensitive tissues such as liver, brain and others. Van Bekkum (128), Thomson *et al.* (137) and Scaife and Hill (117) did not find any uncoupling in liver mitochondria of irradiated animals. It has been suggested (138) that the detrimental effects on oxidative phosphorylation in hepatic mitochondria observed by some investigators could be artefacts resulting from damage to mitochondria during isolation.

There is, however, overwhelming evidence at the present time of an interference with the coupling of oxidative energy in liver mitochondria of irradiated animals (141 - 150). The effects due to radiation are considerably enhanced in the regenerating

liver (126, 129, 130, 139 - 141) and these observations lend support to the hypothesis that rapidly proliferating tissues are more radiosensitive.

The sources of variability in the response of liver mitochondrial oxidative phosphorylation to whole body irradiation have been critically examined by Yost et al. (149). These studies leave no doubt about the real nature of the radiation induced damage to coupled phosphorylation in liver mitochondria and also point to the abscopal nature of the effect. These investigations have also suggested that the uncoupling is merely part of a generalised response to stress and that it might be to the advantage of an organism to accelerate its metabolism for the restoration of damage (129). The finding that the terminal site of phosphorylation is most sensitive to radiation is in accord with their suggestion since uncoupling at this site can be expected to achieve release from 'tight coupling' control with the least possibility of damage to the rest of the phosphorylating chain.

SCOPE OF THE INVESTIGATIONS REPORTED IN THE THESIS

The alterations in metabolism following exposure of an organism to ionising radiation are multifaceted and it appears likely that there are more than one primary targets of radiation damage. Most early investigations on radiobiological effects had emphasised the damage to nucleus and nuclear function, in view of

the critical significance of the DNA for survival and growth of the cell. Notwithstanding the preeminent role of nucleus in controlling metabolic processes, it is quite conceivable that other cellular components, especially the mitochondria may also play a significant role in determining the response of the cell to radiation. Even if mitochondrial damage does not represent the prime lesion, impairment of energy metabolism in mitochondria will seriously interfere with repair processes and could, therefore, be a critical factor in influencing radiation injury.

In spite of the considerable evidence in literature on the deleterious effects of radiation on oxidative phosphorylation, there are considerable qualitative and quantitative inconsistencies between the observations of various investigations. There have also been no attempts made to discern the mechanism of the radiation induced impairment of mitochondrial oxidative phosphorylations, in the light of currently accepted concepts of electron transport and coupled phosphorylation. In view of these considerations, detailed investigations have been undertaken on the response of mitochondrial energy metabolism to whole body irradiation. These studies have aimed at an assessment of: (i) the time-dose relationship of radiation damage to oxidative phosphorylation; (ii) comparative sensitivities of mitochondria from various tissues, (iii) differential effects on the three sites coupled to electron transport; (iv) the primary or abscopal nature of the radiation effect on mitochondria; (v) the precise locus and mechanism of impairment of oxidative phosphorylation; and (vi) protein synthetic ability of the mitochondria from irradiated rats.

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