S Y N O P S I S

Most early investigations on the biological effects of ionizing radiation had emphasised on the structural and functional alterations occurring in cell nucleus in attempts to explain derangements in various cellular processes. Thus, nuclear volume was postulated as an important criterion determining the response of cells to radiation - the larger the nuclear volume, the more radiosensitive the cell.

In recent years, considerable amount of evidence has been obtained indicating that cellular components other than the nucleus also play a significant role in the development of radiation injury, particularly in cells in interphase. An increase in the size of the nucleus is associated with a diminution of cytoplasmic volume and consequent paucity of other cytoplasmic organelles. Extensive researches have shown that, with few exceptions, there exists an apparent correlation between relative mitochondrial content and their quality on one hand and the degree of radioresistance on the other, in a variety of cell types.

Even if mitochondrial damage does not represent the prime radiation lesion, impairment of energy metabolism in mitochondria will seriously interfere with repair processes in the cell and could, therefore, be a critical factor influencing radiation injury.

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.

The literature pertaining to investigations on the effects of whole-body irradiation on oxidative phosphorylation in mitochondria is reviewed and the scope of the present investigations brought out in Section 1 of the thesis.

Experimental data on the comparative effects on coupled phosphorylation in various tissues, at different post-irradiation periods following whole-body radio-exposure at sub-lethal and lethal doses are presented in Section 2. It is observed that the impairment of oxidative phosphorylation in liver mitochondria following exposure of the rats to 400 rads is reversible beyond 96 h. At the higher dose of 800 rads, however, the P/O ratio declines progressively till no coupled phosphorylation is seen beyond 168 h in surviving rats. Brain mitochondria show a decrease in P/O ratio of less than half that observed with liver mitochondria, whereas spleen mitochondria show a greater decrease. Studies using different oxidisable substrates with or without specific inhibitors of electron transport and artificial electron acceptors, reveal

that the terminal site of coupled phosphorylation in liver mitochondria is far more sensitive than the other two.

In Section 3 are presented experimental investigations designed to throw some light on the direct or abscopal nature of the radiation effect and on the influence of radioprotective agents. Lead-shielding of the liver does not totally abolish the decrease in P/O ratio although it is less marked than when the liver is also exposed. These findings point to the indirect nature of the mitochondrial damage following whole-body irradiation; the finding that very large doses are required when mitochondria are irradiated in vitro to obtain significant lowering of P/O values also supports this contention. The studies using adrenalectomised rats rule out the possibility of the involvement of adrenal hormones in the radiation-induced impairment of oxidative phosphorylation. The radioprotective compounds, serotonin and AET (2-aminoethyl isothiouronium bromide hydrobromide) afford significant protection against decrease in P/O following irradiation. Administration of the hypocholesterolemic drug, ethyl-α-p-chlorophenoxy-isobutyrate, which is known to increase the mitochondrial content of rat liver, is also effective in protecting against the inhibition of oxidative phosphorylation. Paired feeding experiments have indicated that the inhibition of P/O ratio following whole-body irradiation in rats is not due to inanition.

Investigations to locate the possible site and to define mechanism of impairment of oxidative phosphorylation in liver

mitochondria of animals exposed to whole-body irradiation form the basis of Section 4. Studies on reduction of NAD^+ by reversed electron flow reveal that the formation of high energy intermediates proceeds normally in liver mitochondria from irradiated rats. Thus, the rates of reduction of NAD^+ in liver mitochondria from control and irradiated rats are identical, when the energy for the process is derived from oxidation of ascorbate. However, with exogenous ATP as energy source, the irradiated mitochondria reduce NAD^+ much more slowly, suggesting decreased transphosphorylation function. Using a reconstituted phosphorylating system containing electron transport particles and coupling factors isolated from control and irradiated rat livers, it is observed that whole-body irradiation results in considerable loss of activity in factor F_1 (ATPase). There is also a progressive loss of mitochondrial Mg^{++} following irradiation which parallels the inhibition of oxidative phosphorylation. These data indicate that loss of activity of coupling factor F_1 and of Mg^{++} following irradiation may contribute to impairment of coupled phosphorylation.

Some observations on mitochondrial protein synthesis in vivo and in vitro as well as on the activities of some lysosomal enzymes following whole-body irradiation are presented in Section 5 of the thesis. The protein content of liver mitochondria from irradiated rats is more and is also reflected in increased in vivo incorporation of ^{14}C-leucine into mitochondrial proteins. Similar increased labeling by ^{14}C-leucine is also observed with liver slices, but isolated mitochondria incubated in vitro actually show

decreased incorporation. These and other data indicate that whole-body irradiation has differential effects on protein synthesis in the cytoplasm and in the mitochondria, the former being stimulated while the latter is inhibited. By fractionation of the mitochondrial proteins, after labeling with ^{14}C-leucine, it is shown that, following irradiation, a fraction established to be entirely of mitochondrial origin has decreased radioactivity.

Studies on lysosomal cathepsins and RNAse show an increase in the free form in the livers of irradiated rats indicating lysosomal membrane damage; this may have a role in the radiation induced damage to mitochondrial structure and function.