SECTION 2

MITOCHONDRIAL OXIDATIVE METABOLISM IN RAT TISSUES FOLLOWING WHOLE BODY RADIO-EXPOSURE

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INTRODUCTION

Although it is generally recognised that whole body irradiation of rat can result in a significant decrease of mitochondrial oxidative phosphorylation, there has been some disagreement as to whether the comparatively radioresistant tissues such as the liver and brain are also affected. Van Bekkum (1), Scaife and Hill (2) and Thomson et al. (3) have reported that no uncoupling is evident in the liver of rats following whole body radio-exposure. On the other hand, several investigators have observed a significant deleterious effect of radiation on coupled phosphorylation, in the liver (4 - 13). The divergent observations of various researchers are traceable in many instances to apparent differences in the experimental protocols employed. Thus, studies have been carried out employing a variety of oxidisable substrates and the radiation effects have been assessed with a range of doses and at different post-irradiation periods from a few hours to days. Some of the more important factors, apart from individual differences between animals, that could contribute to variations in the response of this facet of cell metabolism to radiation have been critically discussed by Yost et al. (13).

The earliest report on the differential radiation sensitivities of the three sites of phosphorylation coupled to the electron transfer chain is that of Clarke and Lang (14). These investigators reported that the first site of phosphorylation is far more sensitive

than the other two, when mitochondria were irradiated *in vitro*. It would, however, appear from other published findings (13) that whole body irradiation has a greater effect on the terminal and the second sites of phosphorylation.

A review of the literature on radiation effects on mitochondrial oxidative phosphorylation reveals lack of intensive investigations to understand the underlying mechanisms in terms of currently accepted concepts of electron transport and coupled phosphorylation. The investigations presented in this section have as their major objectives: (i) an assessment of the effects of whole body irradiation on rat liver mitochondrial oxidative phosphorylation in relation to the dose and the time following exposure; (ii) a comparison of the damage to oxidative phosphorylation in different tissues following whole body irradiation, *vis a vis* their known radiation sensitivity; and (iii) a quantitation of the differences, if any, in the response of the three coupled phosphorylation reactions in rat liver mitochondria, to whole-body irradiation.

MATERIALS AND METHODS

Chemicals:

Adenosine triphosphate (sodium salt), sodium succinate, L-glutamic acid, sodium ascorbate, cytochrome c, hexokinase, bovine serum albumin, antimycin A, phenazine methosulfate, heparin and Tris-hydroxymethyl aminomethane were obtained from

Sigma Chemical Co., St. Louis, Mo., U.S.A. The various inorganic chemicals, of 'AnalaR' grade, as well as EDTA, glucose and sucrose were obtained from British Drug House Laboratory Chemicals Division of Glaxo Laboratories, Bombay, India.

Animals:

Male albino rats, of the Wistar strain, weighing about 125 gm and reared on a nutritionally adequate laboratory stock diet, were used.

Whole-body x-irradiation:

The rats were confined, four at a time, in a rectangular wooden box and exposed to x-rays from a Siemens Stablipan unit operated at 250 kV and 15 mA, using a 2 mm Al filter. A dose rate of 100 R/minute was obtained at a distance of 63.5 cm from the source, where the animals were placed and the total dose delivered was 800 rads. The control rats were sham irradiated.

Isolation of liver and spleen mitochondria:

The animals were sacrificed by stunning and decapitation; the organs were quickly removed and suspended in ice-cold 0.25 M sucrose. A 10% homogenate was prepared in 0.25 M sucrose with a Potter-Elvehjem type glass homogeniser fitted with a teflon pestle with two up- and-down motions. The mitochondrial fractions were obtained as described by Dingle (15). Cell debris and nuclei were removed by centrifuging at 600 g for 5 min and the supernatant was

spun at 10000 g for 10 min in a Sorvall superspeed centrifuge. The loosely packed upper layer was discarded and the pellet was washed four times by suspension in 0.25 M sucrose and recentrifugation. The mitochondria were finally suspended in 0.25 M sucrose (1 ml suspension ≡ 1 g liver).

Isolation of brain mitochondria:

Mitochondria from brain were isolated according to the method of Beattie et al. (16). The excised brain was suspended and homogenised in medium A containing 0.4 M sucrose, 0.001 M EDTA, and 0.02% heparin with the pH adjusted to 6.8 - 7.0 with KOH (1 g tissue ≡ 2 ml homogenate). The homogenate was stirred at 0 - 4°C for 15 min with a magnetic stirrer and the pH maintained during this period by the addition of 2 M-Tris (pH 10.8), if necessary. The suspension was diluted ten-fold with medium A and centrifuged at 184 g for 20 min followed (without transfer of supernatant) by centrifugation at 1153 g for 20 min. The residue was discarded and the supernatant centrifuged at 12000 g for 15 min. The residue was gently homogenized in medium A (6 ml per g of original tissue) and centrifuged at 12000 g for 30 min. The supernatant was discarded and the mitochondrial fraction was washed by homogenization in medium A (4 ml per g of original tissue) and centrifuged at 12000 g for 15 min to yield the final mitochondrial fraction. The washing was repeated three more times and the final pellet was suspended in medium A (1 ml/g of brain).

Assay of succinoxidase activity:

The oxidation of succinate was assayed manometrically (17) by measuring the oxygen uptake in a medium consisting of potassium phosphate buffer, pH 7.4 (200 µmoles), sodium succinate (15 µmoles), calcium chloride (1.2 µmoles), $AlCl_3$ (1.2 µmoles), cytochrome c (0.04 µmole) and 2.0 - 2.5 mg of mitochondrial protein in a total volume of 3.0 ml, at 37°C for 20 min.

Oxidative phosphorylation studies:

Phosphorylation coupled to electron transfer during the oxidation of glutamate and succinate was determined according to the method of Yost et al. (13), with minor modifications. With either of these substrates, the main compartment of each flask contained: sucrose (250 µmoles); phosphate buffer, pH 7.4 (40 µmoles); cytochrome c (0.09 µmole); ATP (6 µmoles); $MgSO_4$ (15 µmoles); KF (70 µmoles); and mitochondria corresponding to 7 mg protein. In studies with brain mitochondria, the main compartment also included bovine serum albumin (5 mg). Hexokinase (10 mg), glucose (75 µmoles) and either succinate or glutamate (20 µmoles) was present in the side arm, whereas the centre well contained 0.2 ml of 10% KOH with a filter paper strip dipped in it. The total volume of the reaction mixture was 3.4 ml and the incubation was carried out at 25°C for 20 min.

The terminal site of phosphorylation with ascorbate as substrate was measured using a slight modification (13) of the method of Lehninger et al. (18).

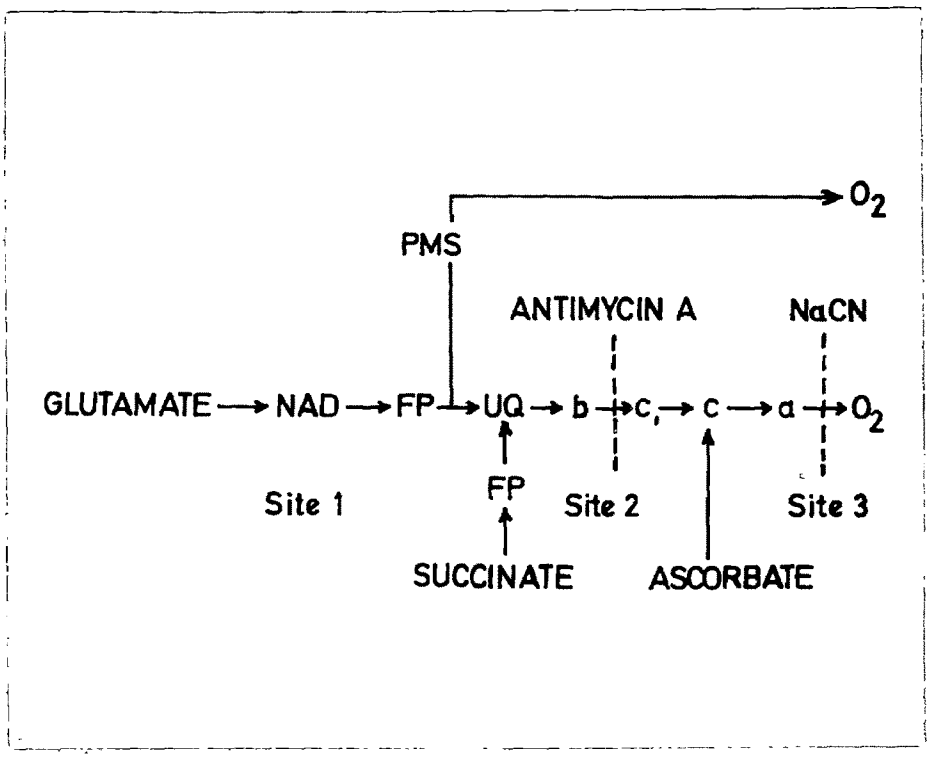
The contents of each flask consisted of mitochondria corresponding to 5 mg protein ; sucrose (38 µmole); phosphate buffer, pH 7.4 (20 µmole); $MgCl_2$ (10 µmole); EDTA (2 µmole); ATP (5 µmole); KF (26 µmole); and cytochrome c (0.03 µmole) in the main compartment and ascorbate (100 µmoles), hexokinase (6 mg) and glucose (40 µmole) in the side arm. The centre well contained 0.2 ml of 10% KOH. The total volume of the reaction mixture was adjusted to 2.0 ml and the incubations were carried out at 25°C for 20 min.

Phosphorylation at the first site alone, was determined using glutamate as substrate, together with (i) antimycin A (0.20 µg/mg mitochondrial protein) and NaCN (0.25 µg/mg mitochondrial protein) to inhibit the normal flow of electrons through the respiratory chain and (ii) phenazine methosulphate (0.1 mM) to act as an artificial acceptor of electrons from the flavoprotein region of the chain and to carry it to molecular oxygen (cf Fig.) (19). The reaction mixture was the same as that used for glutamate oxidation but contained in addition, antimycin A (1.5 µg), NaCN (1.75 µg) and phenazine methosulphate (0.34 µmole).

Measurements of O_2 consumption were made in a Warburg respirometer. The incubations were terminated by addition of 10% trichloroacetic acid (0.5 ml) and the amount of phosphorus esterified was measured by the difference between initial and final amounts of Pi present in the medium, as determined by the method of Lowry and Lopez (20).

Fig. Schematic representation of the electron transport chain, illustrating the use of inhibitors and an artificial electron acceptor to bypass the normal pathway.

Legend: Antimycin A and NaCN are used to prevent the normal flow of electrons through the second and third coupling sites of the respiratory chain. The addition of the artificial electron carrier, phenazine methosulfate (PMS), however, enables the flow of electrons beyond the first site directly to molecular oxygen.


O2
PMS
ANTIMYCIN A
NaCN
GLUTAMATE
NAD
FP
UQ
b
c1
c
a
O2
FP
Site 1
Site 2
Site 3
SUCCINATE
ASCORBATE

Figure

Estimation of protein:

The protein content of mitochondria was determined by the method of Lowry et al. (21).

RESULTS

In the first series of experiments, the effects of whole body irradiation of the rat at sub- and supra-lethal doses on the ability of liver mitochondria to carry out phosphorylation coupled to the oxidation of succinate have been investigated. The efficiency of phosphorylation was investigated at very early periods following radioexposure as well as at various time intervals up to a few days to ascertain whether the damage sustained by the mitochondria is permanent in nature or is subject to recovery mechanisms. Observations on the mortality of the rats following irradiation were also recorded. These data are presented in tables 1 and 2.

Whole body exposure of rats to 400 R did not have any marked effect on either oxidation or coupled phosphorylation; a small lowering (-12%) of the P/O ratio was noticeable after the lapse of one day following irradiation, becoming slightly more pronounced (-17%) on second and third days. There was a clearly discernible trend towards normalisation beyond this period and on the fifth day the P/O ratio obtained was similar to those of control rats. None of the irradiated animals died during a seven day observation period.

Table 1

OXIDATIVE PHOSPHORYLATION IN LIVER MITOCHONDRIA FROM RATS SUBJECTED TO SUB-LETHAL WHOLE-BODY X-IRRADIATION

Post-irradiation period (h)	O_2 uptake	Pi esterified (μ atom/min/mg protein) x 10^2	P/O ratio	Inhibition (%)
0	4.91 ± 0.62	9.50 ± 0.42	1.94 ± 0.08	-
24	5.80 ± 1.10	9.86 ± 0.36	1.70 ± 0.09	12
48	5.22 ± 0.91	8.32 ± 0.52	1.60 ± 0.05	17
72	5.00 ± 0.23	8.02 ± 0.43	1.60 ± 0.07	17
96	4.81 ± 0.88	8.16 ± 0.76	1.70 ± 0.10	12
120	5.42 ± 1.20	10.53 ± 0.32	1.95 ± 0.08	0

Rats were exposed to 400 R x-rays and killed at different intervals as shown. Succinate was used as the oxidisable substrate. The values represent averages of four independent determinations ± S.E.M. All the rats that were exposed to 400 R of x-rays survived beyond one week following irradiation.

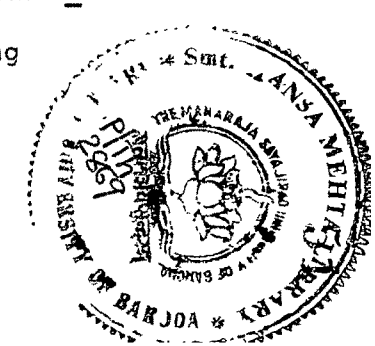


Table 2

OXIDATIVE PHOSPHORYLATION IN LIVER MITOCHONDRIA FROM RATS SUBJECTED TO LETHAL WHOLE-BODY X-IRRADIATION

Post-irradiation period (h)	O_2 uptake (μ atom/min/mg protein)	Pi esterified x 10^2	P/O ratio	Inhibition (%)	Mortality (%)
0	5.26 ± 0.24	9.99 ± 0.78	1.94 ± 0.07	--	-
4	6.10 ± 0.42	10.98 ± 0.82	1.80 ± 0.07	7	0
16	5.80 ± 0.51	8.70 ± 0.63	1.5 ± 0.09	23	0
24	6.50 ± 0.90	8.45 ± 0.41	1.3 ± 0.09	33	0
48	5.20 ± 0.82	5.30 ± 0.62	1.02 ± 0.08	47	12
72	6.92 ± 0.31	4.90 ± 0.53	0.71 ± 0.04	63	25
96	6.22 ± 0.46	3.85 ± 0.28	0.62 ± 0.06	68	33
168	6.40 ± 0.52	0.0	0.0	100	70

Rats were exposed to 800 R x-rays and killed at different intervals as shown. Succinate was used as the substrate for oxidation by liver mitochondria.

The values represent averages of four independent determinations ± S.E.M.

The mortality data are based on observations with 12-20 animals for each of the time periods following irradiation.

On the other hand, when the rats were subjected to a dose of 800 R, a significant lowering of liver mitochondrial P/O ratio was observable by 16 h after exposure. The degree of damage to coupled phosphorylation increased progressively thereafter and there was no detectable ability to conduct oxidative phosphorylation in liver mitochondria obtained from rats surviving one week after irradiation. Oxidative phosphorylation was severely impaired by a dose of 800 R, but oxygen uptake by liver mitochondria did not show major fluctuations, although in general it appeared to be slightly elevated as a result of whole body irradiation. While there was no significant mortality up to 2 days following radioexposure, it rose steeply on subsequent days and seventy per cent of the rats had succumbed by one week post-irradiation.

The ability of the liver mitochondria to oxidise succinate in the absence of exogenous ADP (or an ADP generating system such as ATP + glucose + hexokinase) has also been ascertained and from the data presented in Table 3, it may be seen that whole body irradiation results in inconsistent alterations. There is, in general, a decrease in succinoxidase activity up to the third day but a significant increase by the seventh day.

The ability of liver mitochondria to carry out oxidative phosphorylation has also been investigated with other oxidisable substrates which utilise different segments of the electron transport chain. In view of the significant impairment that was observed with succinate as substrate at 72 h following the exposure

Table 3

SUCCINOXIDASE ACTIVITY IN LIVER MITOCHONDRIA FROM WHOLE-BODY X-IRRADIATED RATS

Post-irradiation period (h)	Oxygen consumption (μ litre of O_2/min/mg protein) x 10^2	Change (%)
0	1.26 ± 0.10	-
4	1.10 ± 0.38	- 13
16	1.20 ± 0.09	- 5
24	0.79 ± 0.09	- 37
48	1.35 ± 0.12	+ 7
72	0.90 ± 0.05	- 28
96	1.38 ± 0.14	+ 9
168	2.3 ± 0.12	+ 82

The rats were subjected to 800 R of whole body irradiation.

The values represent averages of four independent determinations ± S.E.M.

of rats to 800 R of x-rays, these studies were also carried out under the same experimental conditions relating to radiation dose and time. Glutamate, succinate and ascorbate have been used to permit the entry of electrons at different points in the respiratory chain. These findings are summarised in Table 4.

The liver mitochondria from irradiated rats did not show any alteration in their ability to oxidise the various substrates. The overall decrease in coupled phosphorylation at 72 h post-irradiation with electrons flowing through the entire electron transport chain (glutamate as substrate without any inhibitors or artificial electron acceptors) is about 50%, whereas with the second and third sites only (succinate as substrate) it is 63%; the terminal step, utilising ascorbate as the oxidisable substrate shows total lack of coupled phosphorylation. With glutamate being oxidised in the presence of antimycin A and NaCN, and the electrons being diverted via phenazine methosulphate, only the first site of coupling is operative and whole body irradiation is observed to inactivate this site only to the extent of 20%.

The difference between the P/O ratios obtained with ascorbate and succinate may be taken to represent, specifically, the ratio at the second site. The values of P/O ratio at the second site, so computed, for control and irradiated rats are 1.07 (1.94 - 0.87), and 0.71 (0.71 - 0.00), respectively. It may be roughly assessed from these data that the second site of phosphorylation loses its efficiency to the extent of about 30%, as a result of irradiation of the animal.

Table 4

OXIDATIVE PHOSPHORYLATION IN LIVER MITOCHONDRIA FROM WHOLE-BODY X-IRRADIATED RATS

Oxidisable substrate	Other additions	Phospho-rylating sites utilised	Control rats O_2 uptake (μ atom/min/mg prot.)x10^2	Control rats Pi esterified (μ atom/min/mg prot.)x10^2	Control rats P/O ratio	Irradiated rats O_2 uptake (μ atom/min/mg prot.)x10^2	Irradiated rats Pi esterified (μ atom/min/mg prot.)x10^2	Irradiated rats P/O ratio	Inhibition (%)
Glutamate	-	1, 2 & 3	3.10 ± 0.07	9.50 ± 0.43	3.1 ± 0.08	2.96 ± 0.18	4.49 ± 0.33	1.55 ± 0.12	50
Succinate	-	2 & 3	5.26 ± 0.24	9.99 ± 0.78	1.94 ± 0.07	5.68 ± 0.23	3.98 ± 0.35	0.71 ± 0.04	63
Ascorbate	-	3	9.00 ± 0.51	7.83 ± 0.86	0.87 ± 0.09	8.86 ± 0.33	0	0	100
Glutamate	Antimycin A + NaCN + Phenazine methosulfate	1	2.95 ± 0.07	2.94 ± 0.11	0.98 ± 0.02	3.23 ± 0.19	2.58 ± 0.15	0.80 ± 0.01	20

Rats were subjected to 800 R of whole-body x-irradiation and the animals killed 72 h later.

The values represent averages of four independent determinations ± S.E.M.

Substrate oxidation and coupled phosphorylation pertaining exclusively to the third site has been investigated in greater detail, since it showed the maximum radiosensitivity and these findings are presented in Table 5. The oxidation of ascorbate was not significantly influenced at any of the time intervals up to 96 h; the P/O ratio shows a significant decrease even at 4 h after the rats were exposed to radiation and declined further progressively with time. Although the liver mitochondria from irradiated rats were still capable of oxidising ascorbate at 72 h, there was no coupled ATP synthesis at this period.

The effect of whole body irradiation on mitochondria of two other tissues, viz., brain and spleen as representing radioresistant and radiosensitive tissues, respectively, have been assessed and the data are shown in Table 6.

A comparison of these findings with the earlier data on liver mitochondria reveals that the damage suffered by brain mitochondria is considerably less whereas spleen shows a far greater influence of whole body irradiation. The maximum decrease in P/O ratio observable with brain mitochondria is about 20%, on the fourth day of irradiation. With spleen mitochondria, on the other hand, the P/O ratio decreases by over 60% by 24 h after irradiation and becomes zero on the fourth day. As with liver, the mitochondria from brain and spleen do not show any marked fluctuation in the oxygen uptake with succinate as the substrate.

Table 5

OXIDATIVE PHOSPHORYLATION AT TERMINAL SITE IN LIVER MITOCHONDRIA FROM WHOLE-BODY X-IRRADIATED RATS

Post-irradiation period (h)	O_2 uptake (μ atom/min/mg protein)	Pi esterified x 10^2	P/O ratio	Inhibition (%)
0	9.00 ± 0.51	7.83 ± 0.86	0.87 ± 0.09	-
4	9.35 ± 0.71	5.49 ± 0.30	0.59 ± 0.02	32
24	9.25 ± 1.03	2.63 ± 0.44	0.28 ± 0.02	67
48	7.05 ± 0.21	1.90 ± 0.07	0.27 ± 0.01	69
72	8.86 ± 0.33	0.0	0.0	100

Rats were subjected to 800 R of whole body x-irradiation and killed at different intervals as shown. Ascorbate was used as the oxidisable substrate.

The values represent averages based on four independent determinations ± S.E.M.

Table 6

OXIDATIVE PHOSPHORYLATION IN BRAIN AND SPLEEN MITOCHONDRIA FROM WHOLE-BODY X-IRRADIATED RATS

Post-irradiation period (h)	Brain				Spleen			
	O_2 uptake (μ atom/min/mg prot.)x10^2	Pi esterified	P/O ratio	Inhibition (%)	O_2 uptake (μ atom/min/mg prot.)x10^2	Pi esterified	P/O ratio	Inhibition (%)
0	6.5 ± 0.62	11.7 ± 0.44	1.8 ± 0.14	-	3.9 ± 1.80	5.85 ± 0.62	1.5 ± 0.10	-
24	5.8 ± 1.2	8.5 ± 0.56	1.6 ± 0.33	11	4.2 ± 0.83	2.31 ± 0.31	0.55 ± 0.05	63
48	7.6 ± 0.69	12.16 ± 0.93	1.6 ± 0.09	11	4.1 ± 0.34	1.48 ± 0.12	0.36 ± 0.01	75
72	6.6 ± 0.83	9.9 ± 0.71	1.5 ± 0.09	17	3.5 ± 0.18	1.26 ± 0.11	0.36 ± 0.01	75
96	7.8 ± 0.33	10.92 ± 0.55	1.4 ± 0.08	22	3.3 ± 0.14	0.0	-	100

Rats were exposed to 800 R of whole body irradiation and killed at different periods as shown. Succinate was used as the substrate. Values represent averages based on four independent determinations ± S.E.M.

DISCUSSION

In the studies reported here, a significant impairment of oxidative phosphorylation has been observed in rat liver mitochondria following whole-body exposure of the animals to 800 R of x-rays. The severity of this effect increases with the lapse of time after irradiation and on the seventh day there is no detectable synthesis of ATP coupled to oxidation. The mitochondrial damage at this dose does not show any recovery and there is very high mortality among these rats. The reversible nature of the damage at 400 R and the considerably lower mortality at this dose may imply an association between impairment of oxidative phosphorylation and ability of the animal to withstand radiation induced alterations in metabolism. Although the considerable delay in the onset of damage to mitochondrial oxidative metabolism may indicate the indirect nature of the effect, such an impairment may significantly influence the course of radiation injury as well as subsequent repair.

Decreased phosphorylation due to irradiation has been reported by other investigators as well, and a comparison of the present data with the recorded observations reveals some similarities and differences. While Scaife and Hill (2) and Thomson *et al.* (3) have not been able to obtain any effect on P/O ratio of liver mitochondria 4 h after exposure to 1000 rad, significant uncoupling using a variety of substrates that utilise either 2 or 3 sites of phosphorylation has been reported by Hall *et al.* (7)

to occur as early as 3 h after irradiation. A very pronounced decrease in phosphorylating efficiency at the terminal site alone has been noted in our investigations at such an early period. However, whereas Hall et al. (7) have observed a return to normalcy between 24 and 72 h, our data show the deleterious effect to be progressive with time reaching 100 per cent inhibition, in rats that are still alive at 168 h and 72 h post-irradiation with succinate and ascorbate, respectively. A similar trend, as observed in these investigations, has been reported in regenerating liver of irradiated rats (9).

Perhaps the most significant finding emerging from these studies relates to the effects on coupled phosphorylation vis a vis its influence on the rate of oxidation of substrates. While the earlier reports on the subject have invariably referred to the 'uncoupling' effect of whole body irradiation, the lack of any significant spurt in oxygen uptake associated with the lowered ATP synthesis observed in these studies would suggest that whole body irradiation cannot be considered as a true uncoupler such as dinitrophenol. On the other hand, its action is similar to that of oligomycin, and other inhibitors of coupled phosphorylation which are believed to act by interfering with the utilisation of high energy intermediates for ATP synthesis. The inhibitory effect of whole body irradiation, rather than a true uncoupling action, argues against the suggestion of Benjamin and Yost (22) that release from tight coupling and consequent acceleration of metabolism might be to the advantage of the organism to overcome the radiation stress.

Yet another finding of considerable interest is the extreme sensitivity of the terminal phosphorylating site of the electron transfer chain to whole body irradiation as compared to the other two sites. By the use of suitable oxidisable substrates that transfer electrons either through the entire chain or from the flavoprotein stage, Hall et al. (7) have concluded that there are no differences in the extent of inactivation of the first two sites in liver mitochondria due to irradiation of the rat; similar observations have also been made with thymus mitochondria (2). Yost et al. (13) have reported a greater sensitivity of the third site of phosphorylation, as denoted by experiments using ascorbate. However, an analysis of their data reveal that the difference in the extent of inhibition between the second and third sites is not as pronounced as has been observed now. It is relevant to note in this connection that the damage to oxidative phosphorylation following in vitro irradiation of liver mitochondria is more pronounced in the first site (14).

The tissue differences in response to radiation, as between brain, liver and spleen would seem to indicate a relationship between damage to mitochondrial function and the degree of radiosensitivity exhibited by the tissue. On the basis of extensive electron microscopic and biochemical investigations(5)Goldfeder has proposed that radiation resistance of different cell types may be conditioned by the quality and quantity of their mitochondria. The present findings would seem to lend support to this hypothesis.

SUMMARY

Whole-body x-irradiation of rats results in decreased efficiency of liver mitochondrial oxidative phosphorylation. The effects are reversible when the animals are exposed to a sub-lethal dose (400 R), with normalcy being attained after 4 days, whereas at the higher dose (800 R), the degree of damage increases progressively with time till there is total inactivation of coupled phosphorylation on the seventh day.

Although the synthesis of ATP coupled to electron transport in liver mitochondria is severely impaired following whole body irradiation, the oxidation of substrates is not significantly influenced, suggesting that radiation does not act like a true uncoupler.

With the use of different substrates the oxidation of which results in transfer of electrons to the respiratory chain at different points as well as of specific inhibitors of electron transport and artificial electron carriers, the third site of coupled phosphorylation has been shown to be far more sensitive than the other two.

Comparative studies with liver, brain and spleen point to tissue differences in the extent of radiation induced damage to oxidative phosphorylation. The degree of inactivation of coupled phosphorylation parallels the known radiosensitivities of these tissues with spleen mitochondria exhibiting a greater and earlier fall in P/O ratio than liver mitochondria whereas brain mitochondria are more resistant.

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