SECTION 4

MECHANISM AND SITE OF IMPAIRMENT OF OXIDATIVE PHOSPHORYLATION IN LIVER OF IRRADIATED RAT

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INTRODUCTION

The mechanisms of conservation and transduction of respiratory energy for the synthesis of adenosine triphosphate in mitochondria are now reasonably well understood (1 - 4). Such studies have been greatly helped by the use of a variety of chemicals, notably 2, 4 dinitrophenol, arsenite, alkyl biguanides, oligomycin and others that interfere with oxidative phosphorylation. These agents exert their influence on the overall process of energy coupling and transfer, at different sites (Fig. 1). This difference in their loci of action is also reflected in dissimilarities in their effects on electron transfer, although all of them impair the ability of the mitochondria to synthesise ATP.

On the basis of their action, these chemicals can be categorised either as an uncoupler or as an inhibitor of oxidative phosphorylation. The uncouplers interact with the process of oxidative phosphorylation at a site closer to electron transfer than do the inhibitors; whereas the action of the former class of compounds is associated with an increase in the rate of electron transfer, the latter either inhibit respiration or do not significantly influence it. It is easy to visualise site specificity of action where the interference occurs at a point prior to the convergence of the three independent pathways into a common one (Fig. 1). Both dinitrophenol (5) and some of the alkyl biguanides (6) have been shown to exert

Fig. 1.- Schematic representation of the energy-transfer system linked to the respiratory chain.

Legend: C_1, C_2, C_3 denote electron carrier specific to the three energy-coupling sites of the respiratory chain; I and X stand for hypothetic energy transfer carriers. The arrows indicate the site of action of various chemicals that interfere with the processes of either electron flow or energy transfer.

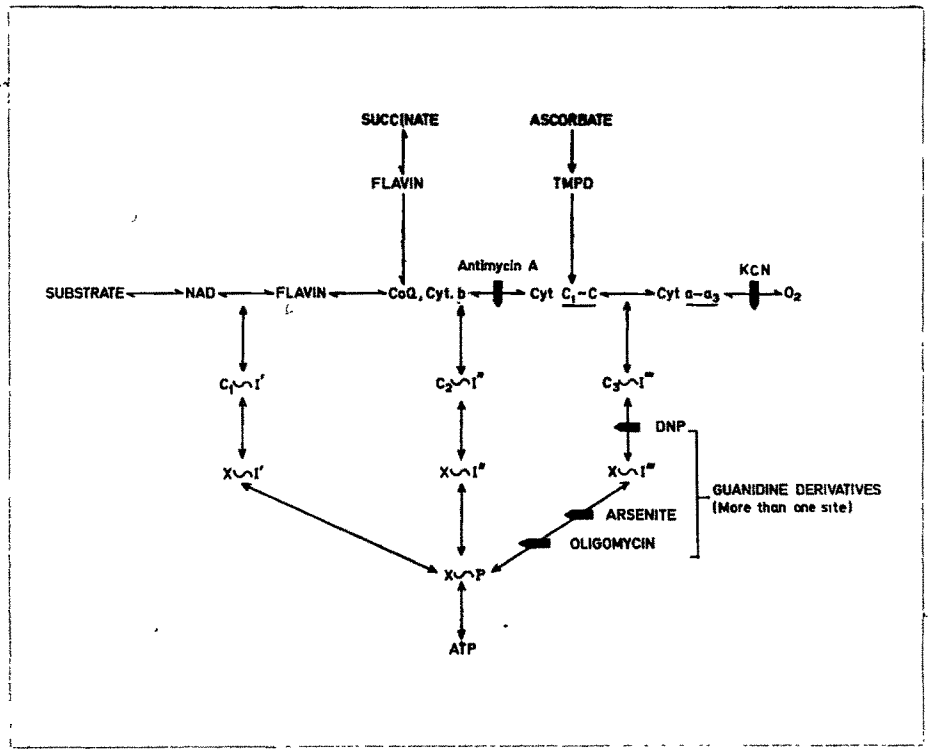

SUCCINATE
ASCORBATE
FLAVIN
TMPD
Antimycin A
KCN
SUBSTRATE
NAD
FLAVIN
CoQ, Cyt. b
Cyt C1-C
Cyt a-a3
O2
C1∼I'
C2∼I''
C3∼I'''
DNP
X∼I'
X∼I''
X∼I'''
GUANIDINE DERIVATIVES
(More than one site)
ARSENITE
OLIGOMYCIN
X∼P
ATP

Figure 1

differential effects on the three sites of coupled phosphorylation, whereas no such specificity of action has as yet been reported for oligomycin. The guanidine derivatives are believed to exert their effects at more than one site (9).

In spite of the large body of evidence on the damage to mitochondrial oxidative phosphorylation following whole body irradiation of rat, a detailed analysis of the mechanisms underlying this effect has not been carried out. Studies reported in Section 2 have established a site specific influence of whole body irradiation; also, irradiation exerts an inhibitory rather than an uncoupling effect.

The objective of the studies reported in this section relate to a definition of the possible site and mechanism of impairment of oxidative phosphorylation in liver mitochondria of irradiated rats. The experimental approaches have involved:

i) an assessment of the effect of whole body irradiation on the functional integrity of the mitochondrial coupling factors;

ii) determination of the levels of endogeneous magnesium in the liver mitochondria of irradiated rats;

iii) capacity of submitochondrial particles from livers of irradiated rats to reduce NAD^+ by reversal of electron flow; and

iv) the effect of oligomycin on the ability of exogeneous ATP to support reversed electron flow.

MATERIALS AND METHODS

Chemicals:

In addition to the various chemicals that were obtained from sources as indicated in the earlier sections, diethyl amino-ethyl (DEAE)-cellulose, glycine, nicotinamide adenine dinucleotide (NAD^+), oligomycin and protamine sulphate were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A., hydroxylapatite (Bio-Gel HT) from Bio-Rad Laboratories, Richmond, Calif., U.S.A. and tetramethyl-p-phenylene diamine (TMPD) from British Drug House Ltd., Poole, U.K.

Animals, whole-body irradiation and isolation of liver mitochondria:

Male albino rats of Wistar strain, weighing about 125 g and fed a nutritionally balanced stock diet were used. Whole-body irradiation of the animals and the preparation of mitochondria were carried out as described in Section 2.

Mitochondrial Mg^{++} content:

Liver mitochondria (≡ 4 g tissue) were lyophilized and extracted with glacial acetic acid (10 ml) with mild heating. The acid extract was separated by centrifugation and aliquots of the supernatant taken for the estimation of Mg^{++}. Standard solution of magnesium (0.25 - 1.00 ppm/ml) was also prepared in the same manner in acetic acid. The samples, standard as well as acetic acid blank were diluted with one per cent solution of $LaCl_3$ to

minimise the effect of sodium and potassium (7) and the magnesium content determined in a Perkin-Elmer Model 303 atomic absorption spectrophotometer. The instrument was used under the following operational conditions; wavelength, 2852.1°A; slit, 5; spectral band width, 20°A; burner type, premix air-acetylene; flametype, air-acetylene, oxidizing. Sensitivity for magnesium under the experimental conditions was 0.01 ppm (µg/ml).

Preparation of sub-mitochondrial electron transport particles:

Submitochondrial electron transport particles devoid of any significant phosphorylating capacity, viz., the ammonia particles, were prepared by the method of Fessenden and Racker (9) as described for beef heart mitochondria. Mitochondria corresponding to 50 g of liver were suspended in a total volume of 120 ml containing 22 ml of 0.25 M sucrose and 0.15 ml of 0.5 M EDTA. After adjusting the pH to 9.2 with freshly prepared 1 N ammonium hydroxide solution, the suspension was sonicated in a Raytheon sonic oscillator (250 watts, 10 KC). The sonicated submitochondrial particles were centrifuged at 26000 x g and the ammonia particles were separated from the supernatant by centrifugation at 104000 x g for 60 min. The particles were washed once in 0.25 M sucrose-1-mM EDTA (pH 7.5), and then in 0.25 M sucrose and preserved in 0.25 M sucrose at -55°C.

Isolation and purification of coupling factors:

The various coupling factors were isolated from rat liver

mitochondria essentially by the methods described by Racker and his associates (8 - 10) for their preparations from beef heart mitochondria.

For preparing coupling factor F_1, mitochondria corresponding to 400 g liver were suspended in sucrose solution (0.25 M) containing 2 mM EDTA (pH 7.4) and 2 mM ATP and were disrupted by sonication (20 k cycles, 2 min). The sonicate was warmed to room temperature, centrifuged and the supernatant allowed to stand overnight. This crude extract was purified by pH fractionation at pH 5.4 using 1N acetic acid, and protamine sulphate and ammonium sulphate precipitations as detailed by Racker et al. (9, 10). The temperature fractionation step as described by these authors was, however, omitted as this resulted in some loss of activity, the extent of inactivation being different for control and irradiated samples. The purified enzyme was stored at $4^{\circ}C$ as a suspension in ammonium sulfate. In addition to its capacity to specifically restore oxidative phosphorylation in ammonia particles, in the presence of an excess of the other coupling factors, F_1 was also identified by its ability to catalyse the hydrolysis of ATP. In terms of protein content, the yields of F_1 from liver mitochondria of control and irradiated rats were approximately 75 mg and 50 mg, respectively.

Coupling factor F_2 was purified from mitochondria corresponding to 200 g of rat liver (8). The mitochondrial acetone powder was extracted with 0.1 M potassium phosphate buffer

and the residue homogenized in 0.06 M glycine buffer (pH 10.5). The glycine buffer homogenate was treated with calcium phosphate gel and the coupling factor F_2 was extracted in 0.3 M potassium phosphate buffer, pH 7.4. The yield from both control and irradiated rats was about 45 mg protein and the coupling factor was stored at -55°C.

For the isolation of coupling factor F_3, mitochondria corresponding to 200 g liver were suspended in 0.25 M sucrose, sonicated (250 watts, 10 KC) and purified by repeated pH adjustment (pH 8) and centrifugation at 104000 x g for 45 min. The supernatant was subjected to freezing and thawing and purified by DEAE cellulose chromatography and ammonium sulfate fractionation (9). The preparation ($\pm$ 90 mg protein)was suspended in 50 mM Tris $SO_4^{=}$ buffer (pH 8) and stored at -55°C.

Coupling factor 4 (F_4) was isolated by the procedure described by Conover et al. (10) with a slight modification. Mitochondria corresponding to 200 g liver, suspended in 0.3 M KCl and 1.5 mM EDTA, were extracted with 1.2 M ammonium hydroxide and centrifuged at 105000 x g for 60 min. The supernatant was adjusted to 5-6 mg protein/ml and purified by ammoniacal ammonium sulphate fractionation. The precipitate was dissolved in 0.25 M sucrose-0.02 M Tris (pH 7.4), and sonicated for 2 min to give a stable suspension which does not sediment upon storage. Traces of ammonium sulfate were removed by dialysis against 0.2 M KCl-0.02 M Tris (pH 7.4) and 1 mM EDTA and the preparation was finally stored

at -55°C. The yield of coupling factor F_4 was approximately 80 mg protein.

The ammonia particles showed a specific requirement for each of the coupling factors (with the exception that F_3 and F_4 were interchangeable) and the full complement of factors was needed for obtaining maximal P/O ratios.

Reconstitution of the phosphorylating electron transport assembly:

Ammonia particles, obtained from livers of unirradiated rats, were reconstituted with the coupling factors in the usual manner (8) and P/O ratios were measured in a Warburg respirometer using succinate as substrate. Each flask contained in a total of 1.2 ml: (a) main compartment - 0.4 ml of a solution made up of glucose (56 µmole), sucrose (72 µmole), $MgSO_4$ (20 µmole), ATP (3 µmole), ADP (2 µmole), hexokinase (54 units; 3 mg) and bovine serum albumin (2 mg); 0.2 ml of a solution of F_1 (3 mg); 0.1 ml of F_2 (0.5 mg); 0.2 ml of F_3 (3 mg) or 0.2 ml of F_4 (4 mg); and 0.2 ml of ammonia particles (4 mg). (Since F_2 was in 0.3 M potassium phosphate buffer (pH 7.5), no additional buffer was added to the incubation medium) (b) side arm - 0.1 ml of sodium succinate (50 µ mole) (c) central well - 0.2 ml of KOH (10%). Oxygen uptake and phosphorus esterification were measured during an incubation period of 30 min at 25°C.

Preparation of phosphorylating sub-mitochondrial particles:

Liver mitochondria, obtained by differential centrifugation

were suspended in 0.08 M sucrose and subjected to sonication (20 KC; 2 min). The sonicate was centrifuged at 12000 x g for 10 min and the supernatant containing the phosphorylating species of sub-mitochondrial particles was used as such.

Reduction of NAD^+ through reversal of electron transport:

The reduction of NAD^+ by reversal of electron transport (11) was energised either by high energy intermediates formed by oxidation of substrates in a portion of the respiratory chain or by exogenous ATP. The system (3 ml) contained sonicated submito-chondrial phosphorylating particles (4.5 mg) in Tris-HCl buffer (pH 8.0; 165 μmole), $MgCl_2$ (18 μmole), sucrose (750 μmole), succinate (21 μmole), antimycin A (1.8 μg), ascorbate (120 μmole) and TMPD (0.96 μmole) at 25°C. Where exogenous ATP served as the source of energy, ATP (18 μmole) and NaCN (1.62 μg) were included and ascorbate and TMPD were omitted from the system. Reaction was initiated by addition of NAD^+ (3 μmole) and increase in absorbance at 340 nm was measured in a Perkin-Elmer double-beam spectrophotometer. In experiments using oligomycin, it was added at a concentration of 0.2 μg/mg particle protein.

The reduction of NAD^+ through energy generated by substrate oxidation was found to be inhibited by 2,4-dinitrophenol whereas oligomycin showed similar inhibition when the reaction was driven by ATP.

Protein estimation:

The protein content of mitochondria, the submitochondrial particles and the coupling factors was determined by the method as described in Section 2.

RESULTS

To elucidate the nature of radiation-induced impairment of oxidative phosphorylation observed earlier, especially of third site, the reduction of NAD^+ by reversal of electron flow, with succinate as electron donor and with the oxidation of ascorbate furnishing the needed energy has been investigated.

In the absence of oligomycin, the kinetics of NAD^+ reduction, in both control and irradiated, showed three distinct phases - a rapid reduction during the first min, a considerably slower rate thereafter and no further reduction after some time (Fig. 2). During the first min, the control phosphorylating submitochondrial particles form NADH at almost twice the rate as the irradiated ones, whereas the two showed identical rates thereafter. There is no further reduction in control beyond 10 min, whereas it is still observable up to 20 min in the irradiated; the total amounts of NADH formed, however, are similar.

The inclusion of oligomycin in the system almost totally abolishes the initial rapid rate of NAD^+ reduction in both control and irradiated particles (Fig. 3). The subsequent steady-state

Fig. 2. Reduction of NAD^+ by reversal of electron flow energized by oxidation of ascorbate.

Legend: o———o control; ●———● irradiated
Sonicated submitochondrial phosphorylating particles were incubated at 25°C with Tris-HCl buffer (pH 8.0), $MgCl_2$, sucrose, succinate, antimycin A, ascorbate and TMPD at the concentrations mentioned in the text. Reaction was initiated by addition of NAD^+ and increase in absorbance at 340 nm was measured in a Perkin-Elmer double-beam spectrophotometer. The total volume was 3 ml. Reduction of NAD^+ is shown in terms of μmole of reduced NAD^+ formed per 5 mg of particle protein.

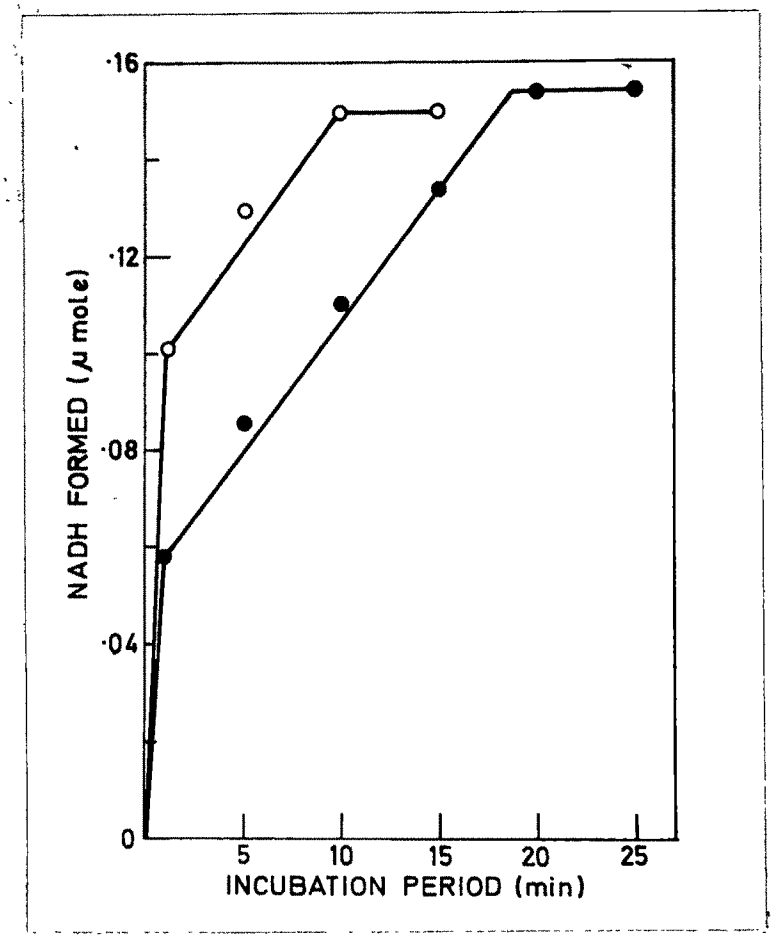
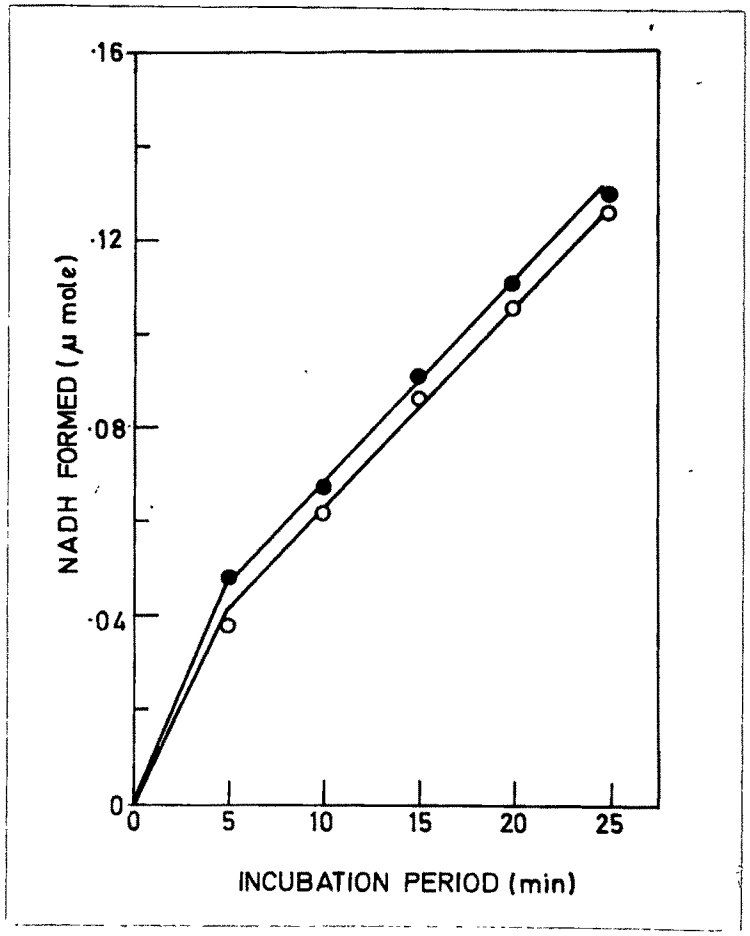

·16
·12
·08
·04
0
NADH FORMED (μ mole)
5
10
15
20
25
INCUBATION PERIOD (min)

Figure 2

Fig. 3. Reduction of NAD^+ by reversal of electron flow energized by oxidation of ascorbate, in presence of oligomycin.

Legend: o———o control; ●———● irradiated

Sonicated submitochondrial phosphorylating particles were incubated at 25°C with Tris-HCl buffer (pH 8.0), $MgCl_2$, sucrose, succinate, antimycin A, ascorbate TMPD and oligomycin, at the concentrations mentioned in the text. Reaction was initiated by addition of NAD^+ and increase in absorbance at 340 nm was measured in a Perkin-Elmer double-beam spectrophotometer. The total volume was 3 ml. Reduction of NAD^+ is shown in terms of μmole of reduced NAD^+ formed per 5 mg of particle protein.


·16
·12
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0
NADH FORMED (μ mole)
0
5
10
15
20
25
INCUBATION PERIOD (min)

Figure 3

rate is, however, not influenced by the presence of oligomycin. There are no significant differences in the rates of NAD^+ reduction by the phosphorylating particles from control or irradiated rats, in the presence of oligomycin. In both cases, a linear rate of reduction is sustained for up to 25 min.

Studies have also been carried out on the kinetics of NAD^+ reduction, energised by exogenous ATP. As compared to the earlier experiments, in which the oxidation of ascorbate provided the energy, the reduction of NAD^+ in presence of added ATP is rapid; the control and irradiated particles, however, exhibit significant differences (Fig. 4). During an incubation period of 4 min, the phosphorylating particles show greater NAD^+ reduction in the ATP system, than during (i) 10 min in the absence of oligomycin (Fig. 2); or (ii) 25 min in the presence of oligomycin (Fig. 3), when energy to drive the reaction was derived from oxidation of ascorbate.

The inhibition of mitochondrial oxidative phosphorylation, due to whole-body irradiation, has also been investigated in terms of effects on the catalytic activities of the different coupling factors in a reconstituted phosphorylating system (Table 1). The 'ammonia particles' from control rat livers, on supplementation with coupling factors F_1, F_2 and F_3, also derived from control animals, yield an average P/O ratio of 0.63, with succinate as substrate. When coupling factor F_1 from control rats is replaced by F_1 from irradiated rats, the P/O ratio falls markedly by over

Fig. 4. Reduction of NAD^+ by reversed electron flow energized by exogenous ATP.

Legend: o———o control; ●———● irradiated

Sonicated submitochondrial phosphorylating particles were incubated at 25°C with Tris-HCl buffer (pH 8.0), $MgCl_2$, sucrose, succinate, ATP and NaCN, at the concentrations mentioned in the text. Reaction was initiated by addition of NAD^+ and increase in absorbance at 340 nm was measured in a Perkin-Elmer double-beam spectrophotometer. The total volume was 3 ml. Reduction of NAD^+ is shown in terms of μmole of reduced NAD^+ formed per 5 mg of particle protein.

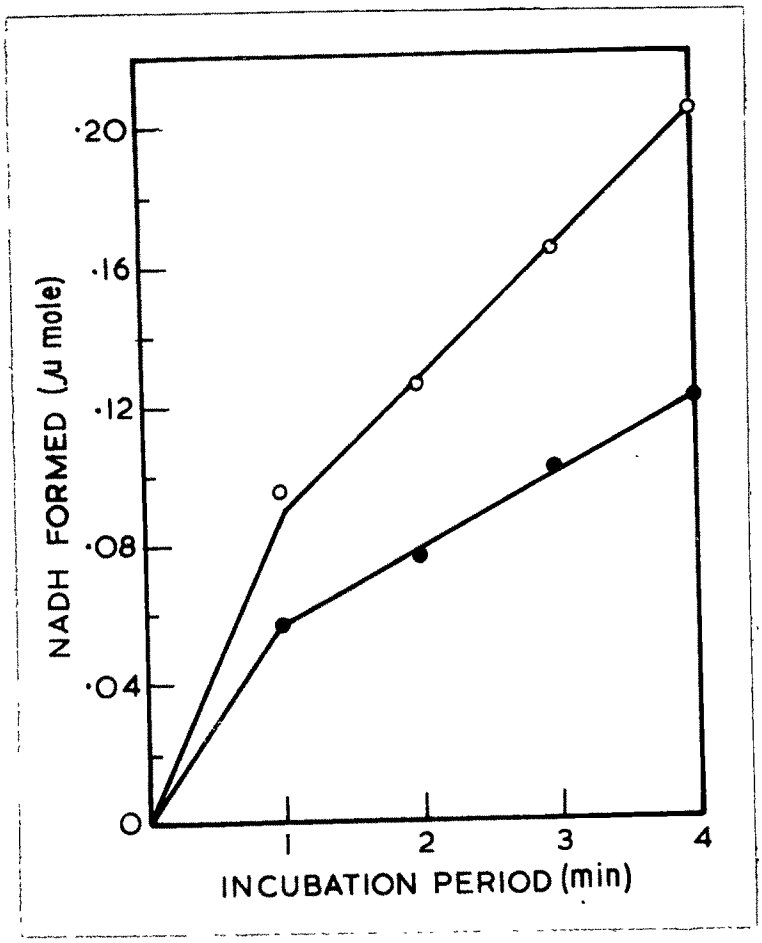

Figure 4

Table 1

ACTIVITY OF COUPLING FACTORS FROM LIVER MITOCHONDRIA OF RATS EXPOSED TO WHOLE BODY IRRADIATION

Coupling factors derived from Control	Irradiated	P/O ratio	Inhibition (%)
F_1, F_2, F_3	-	0.63 ± 0.02	0
F_2, F_3	F_1	0.15 ± 0.01	76
F_1, F_2, F_3	F_1	0.63 ± 0.01	0
F_1, F_3	F_2	0.48 ± 0.01	24
F_1, F_2	F_3	0.45 ± 0.01	29
F_1, F_2, F_4	-	0.65 ± 0.02	0
F_1, F_2	F_4	0.55 ± 0.01	15

The rats were subjected to 800 R of whole body x-irradiation and killed 72 h later. Submitochondrial electron transport particles (ammonia particles) from livers of control rats were reconstituted with coupling factors obtained from livers of either control or irradiated rats as indicated. P/O ratios were determined with succinate as substrate.

The values represent averages of four determinations using pooled preparations of the ammonia particles and the various coupling factors.

Table 2

MAGNESIUM CONTENT OF LIVER MITOCHONDRIA OF RATS EXPOSED TO WHOLE BODY IRRADIATION

Post-irradiation period (h)	Endogenous Mg^{++} content (µg/mg protein) x 10	Decrease (%)
0	6.38 ± 0.70	-
2	4.78 ± 0.10	25
24	5.24 ± 0.20	18
48	2.22 ± 0.20	65
72	2.65 ± 0.10	58
96	0.78 ± 0.20	88

The rats were subjected to 800 R of whole body x-irradiation and killed at various intervals thereafter. The values represent averages of four independent determinations ± S.E.M.

75%; when F_1 from irradiated rats was added in addition to, and not as a replacement for, F_1 from control animals, the P/O ratio remains unaltered. Similar experiments, in which one of the other coupling factors at a time was derived from the irradiated animals show that whole-body irradiation causes a far less loss of activity in any of these as compared to the effect on F_1. It is also seen that similar P/O ratios are obtained when the 'ammonia particles' are supplemented with F_1, F_2 and either one of F_3 or F_4.

In addition to the coupling factors, magnesium plays an important role in mitochondrial energy metabolism. Data on the endogenous magnesium content of liver mitochondria of rats sacrificed at different time intervals subsequent to whole body radio exposure are given in Table 2. Even at 2 h post-irradiation, there appears to be a significant efflux of magnesium from mitochondria as indicated by the drop in the mitochondrial content of this cation. The mitochondrial magnesium content decreases further with time and at the end of 96 h is about a tenth of the normal level of magnesium.

DISCUSSION

In the studies reported in earlier sections, a significant impairment of oxidative phosphorylation has been observed in rat liver mitochondria 72 h following whole-body exposure of animals to 800 rad of x-rays.

In an attempt to locate the site of radiation interaction in terms of the partial reactions involved, recourse has been taken to studies on NAD^+ reduction by reversal of electron flow mediated by an energy source. A schematic representation of reversal of electron flow is shown in figure 1. It is now well established that this reaction can be driven not only by ATP but also by the high energy intermediates, which have been implicated in its synthesis (12). With energy originating from ascorbate-TMPD oxidation, it was generally observed that the kinetics of NAD^+ reduction was rather slow. Such a low rate has also been reported by other investigators (13 - 15) in washed submitochondrial particles and is believed to be due to the lack of a soluble stimulating factor of either mitochondrial origin (13) or from the supernatant (14, 15).

In the absence of oligomycin (Fig. 2) the initial rate of formation of NADH is very high and is followed by a slower reaction persisting for several minutes. A likely explanation for the early spurt in both control and irradiated particles is that this represents the reduction that is energised by the endogenous ATP present in the preparations; the irradiated particles presumably having a lower amount of ATP show a somewhat lesser initial spurt as compared to the control particles. While the presence of endogenous ATP in these submitochondrial particles has not been established in the present studies, it has been ascertained that the initial rapid increase in absorption does not represent an artifact arising from turbidity changes. The

subsequent slower rate of NAD^+ reduction very likely reflects the rate at which high-energy intermediates are being generated by the oxidation of ascorbate. It may further be inferred from the equal rates of NAD^+ reduction obtaining beyond the first minute in the submitochondrial particles from livers of control and irradiated rats that the formation of high-energy intermediate(s) proceeds normally in the latter.

The absence of the initial rapid rate of reduction when oligomycin is included in the system (Fig. 3), affords additional support to the inference of ATP involvement in this effect. The control and irradiated samples show almost identical activities in the presence of oligomycin; however, the amounts of NAD^+ reduced during 20-25 min are significantly lower in both control and irradiated particles as compared to the amounts formed in the absence of oligomycin. In the presence of oligomycin, high-energy intermediate(s) alone can serve as the energy source and the finding that the rate of reduction of NAD^+ in the mitochondrial particles from irradiated rats is unaffected provides conclusive evidence of radiation not interfering with this intermediary step in the utilisation of oxidative energy for ATP synthesis.

When the reduction of NAD^+ was carried out in the absence of oxidative energy, but with exogenous ATP (Fig. 4), a significant difference is observed between the control and irradiated particles. With ATP as the energy source, the formation of NADH is rapid, the rate being similar to that observed

during the first minute in the experiments with ascorbate as oxidisable substrate, in the absence of oligomycin. The significantly decreased rate of reduction of NAD^+ by the irradiated particles in the presence of ATP can be taken as an indication of an interference with the transphosphorylation reaction.

Studies on the reconstitutability of a phosphorylating system, by the addition of coupling factors to ammonia particles, also point to a loss of function involved in the final step of ATP synthesis in liver mitochondria of irradiated animals. Thus, coupling factor F_1, which has been visualised to play a role in the transphosphorylation reaction resulting in ATP formation (16) shows a marked fall in activity due to irradiation. It may be pointed out that, in addition to this decreased catalytic activity of F_1, the liver from irradiated rats also yielded a smaller amount of this coupling factor. The possibility of an inhibitory effect of F_1 from irradiated animals on the reconstituted system is ruled out by the experiment in which no deleterious effect was observed when F_1 from both control and irradiated rats was added together with the other complements to the ammonia particles.

It is noteworthy that the earliest indication of a significant decrease in mitochondrial magnesium content (2 h) precedes the inhibition of oxidative phosphorylation (4 h). It is also interesting that the efflux of magnesium from mitochondria and impairment of oxidative phosphorylation show a parallel and progressive increase with time. It would thus appear that, in

addition to the effects on coupling factors and other proteins involved in energy transfer, loss of magnesium from mitochondria may also contribute to the inhibition of oxidative phosphorylation in irradiated rats.

The major point of interest arising from these studies relates to the nature of radiation-induced impairment of oxidative phosphorylation. On the basis of our current concept of the partial reactions involved in oxidative phosphorylation coupled to electron transport (17, 18), it is difficult to reconcile the two salient findings of the present studies. These relate to: (i) whole-body irradiation exerts site-specific inhibition, where the terminal phosphorylating assembly associated with the cytochrome c region is far more sensitive than the other two; (ii) irradiation does not interfere with the generation of high energy intermediates but has its locus of action at the transphosphorylase system, which should inhibit equally well phosphorylation coupled to all the three sites.

The present findings may be considered in the light of one of the following possibilities. Contrary to the generally accepted concept, the pathways of energy transfer from the three coupling sites to the final formation of ATP need to be represented as being independent of each other. This may arise either due to the topography of the various components of respiratory chain in the mitochondrial membrane or due to differences in the specificity of interaction of transphosphorylase with the different high

energy phosphates (at the present time, designated as a single entity) arising from the three energy-generating sites. These suggestions although hypothetical, are not altotether without supporting evidence from the work of other investigators. The spatial distribution of the different carriers of the electron transport chain in definite positions on the mitochondrial membrane has been suggested by Racker (19) and, from their studies on the ease of accessibility of arsenite to the three phosphorylating sites, Azzone and Ernster (20) have indicated that the first phosphorylating site may be more superficially situated in the mitochondria than the other two. It has been shown by Beyer (21) that ATP synthetase II, which restores phosphorylation coupled to the oxidation of both NADH and succinate in submitochondrial particles with low phosphorylating ability, has little or no effect on phosphorylation during oxidation of cytochrome c reduced by ascorbate. It is not unlikely that these findings may have a bearing on the site-specific inhibition of mitochondrial oxidative phosphorylation following whole-body irradiation.

SUMMARY

Studies on NAD^+ reduction by reversal of electron flow, with the energy for the process being derived from the oxidation of ascorbate-tetramethyl p-phenylene diamine, suggest that the formation of high energy intermediates is not interfered with

by irradiation. On the other hand, there is 2-fold evidence in favour of an impairment of the transphosphorylation function, the final step in the synthesis of ATP. Thus, by reconstitution studies, the activity of coupling factor F_1 is found to be greatly decreased in irradiated rat livers. With ATP as an energy source, phosphorylating submitochondrial particles from livers of irradiated rats show a lower rate of reduction of NAD^+. Increased efflux of hepatic mitochondrial magnesium may also contribute to the inhibition of coupled phosphorylation in irradiated rats.

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