CHAPTER V

EFFECTS OF CADMIUM TOXICITY ON THE OXIDATIVE ENERGY METABOLISM IN RAT LIVER MITOCHONDRIA

INTRODUCTION

Mitochondria have been shown to be prime targets of Cd-induced hepatotoxicity [1,2]. This was further confirmed by the fact that when rats are pre-treated with either Zn, Cd or Se (all of which induce the synthesis of metallothionein) the concentration of Cd in mitochondria is much lower as compared to the untreated rats in which the concentration is almost 15% of the total injected Cd [3-6]. In the pre-treated rats the Cd accumulates in cytoplasm where it is the least deleterious.

As detailed in the introductory section, (Chapter I) reports are available which describe in details the damage to various subcellular organelles including the mitochondria following the Cd exposure. The studies on Cd-insult to mitochondria pertain to the structural damage as well as impairment of the activity of certain enzymes. However, the Cd-induced changes in the mitochondrial oxidative phosphorylation and energy metabolism have received scant attention and most of the studies report in vitro effects or effects of overdose of Cd (Chapter I).

In addition to the above, most of the work till date is carried out on the adult rats and except for some work on the distribution of Cd in the different tissues in the newborn and adult rats [7-9] no data are available regarding effect of Cd on the liver or any other tissues of the young rats. The distribution studies have shown that the adult livers accumulate larger amounts of Cd, while the lethal dose is lesser in the newborn as compared to the adults. The

distribution and urinary excretion of Cd also was different in the newborn rats and these changes showed significant age-dependent and maturational effects.

The exposure to Cd can occur at any stage of life and since the oxidative phosphorylation and other enzymes of the energy transduction show developmental changes ([10] and Chapter II and III), it was of interest to check what effect Cd treatment has on the oxidative phosphorylation in the young and the adult rats. Early et.al. [6] reported a complete destruction of the mitochondrial membrane structure within 48 h after a single Cd injection. Experimenrs were hence planned to study the mitochondrial oxidative phosphorylation 48 h after the Cd injection. Additionally since Cd has a long half-life in the body (Chapter I), it was of interest to study the mitochondrial functions at a longer period after the Cd dose. Thus experiments were carried out 48 h and one month aftr injecting young and adult rats with Cd.

In parallel studies efects on the -SH content in the mitochondria were also examined.

MATERIALS AND METHODS :

Chemicals :

Details of reagents and chemicals used are as given in Chapters II and III.

Animals and Cd Injections :

Male albino rats of the Charles-Foster strain, young(3 week old) or adults (8-10 weeks) were used. The animals were injected intraperitonealy (i.p.) with 0.84 mg Cd as Cd acetate per Kg body weight [6]. The controls received equivalent amounts of sodium acetate (1.23 mg/ kg body wt.). The animals were killed either 48 h.(Cd-treated-48h. group) or one month (Cd-treated-one month group) after the single Cd injection; corresponding age matched controls were also kept.

Mitochondria were isolated from liver and the assay of primary dehydrogenases (GDH,MDH and SDR) and ATPase activities was as described in Chapter II while the oxidative phosphorylation studies were as described in Chapter III.

The Cd-treated young rats when kept for an additional one month, had almost reached the adult stage (7 - 8 weeks). For the corresponding age matched controls it was found that their oxidative phosphorylation rates and other parameters matched with those of the controls in the adult 48 h and adult one month groups. Hence, the values for the three control groups: young - one month, adult - 48 h. and adult - one month, were pooled, and the averages (pooled controls) were used for comparison with the results obtained from the young Cd-treated - one month group as well as the adult Cd - treated - 48 h. and Cd-treated-one month groups.

Measurement of -SH content :

Estimation of the soluble and total -SH content were carried out on the freshly isolated mitochondria essentially according to the procedure described by Ellman [11], with certain modifications..

For the estimation of soluble -SH groups, 3 - 4 mg of mitochondrial proteins (0.5 ml) were extracted using 3.0 ml of 5% (w/v0 chilled (0 - 4°C) trichloroacetic acid (TCA). The samples were kept on ice and then filtered using a Whatmann No 1 filter paper. Aliquots of the clear supernatant were used for assaying the soluble -SH content.

For this 0.5 ml of the filterate was added to 0.5 ml of 0.1 M phosphate buffer pH 7.8 to which 15 μl of DTNB was added (39.6 mg of DTNB and 5.0 mg of Na_2CO_3 in 10 ml of 0.1 M phosphate buffer pH 7.0)

The absorbance was read immediately at 412 nm in a Shimadzu UV 160 A spectrophotemeter and the content calculated as

A x D / E x protein = nmoles / mg protein

where A = absorbance at 412 nm

D = dilution factor

E = mM extinction coeffecient; 13.6/mM/cm

For determining the total -SH content, the assay was carried out as follows :

To 0.8 ml of 0.1 M phosphate buffer pH 7.8, 1 mg of mitochondrial protein (0.1 ml) was added. The mitochondrial protein is solubilized by adding 0.1 ml of 10 % Triton X-100. This is vigorously vortexed and to this 15 µl DTNB is added. After incubation at room temperature for 30 minutes, absorbance is read at 412 nm.

RESULTS

Table 1 - 3 show the results on effects of Cd exposure on oxidative phosphorylation with glutamate, succinate and ascorbate + TMPD respectively, as the substrates in liver mitochondria from young (3 week old) and adult rats; 48 h and one month after the Cd injection.

Thus in the young rats 48 h after the Cd exposure,state 3 respiration with glutamate showed a stimulation of about 71 % while the ADP-phosphorylation rate also increased by 60 % (Table 1). This pattern repeated when succinate and ascorbate + TMPD were used as the substrates. With succinate, the state 3 and ADP-phosphorylation rates more than doubled while the the state 4 rates increased by 70 % (Table2). When ascorbate + TMPD was used as the electron donor system, state 3 rate increased by 50 % and the ADP - phosphorylation rate by 40 % . The ADP/O ratios were unchanged in all the cases (Table 3).

In the Cd-treated young animals kept for 1 month, however, a totally different pattern was observed. With glutamate, the ADP/O ratio, state 3 and state 4 respiration rates and the ADP

Table 1

Effect of cadmium treatment on the oxidative phosphorylation in rat liver mitochondria with glutamate as the substrate.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate		ADP-phosphorylation rate
			State 3	State 4	
3 Weeks	Control (20)	2.22 ± 0.04	31.2 ± 1.73	11.8 ± 1.16	139.8 ± 10.10
	Cd-treated -48 h (26)	2.13 ± 0.13	53.0 ± 3.10[c]	14.3 ± 1.23	222.8 ± 1.35[c]
	Pooled control (24)	3.04 ± 0.08	41.0 ± 3.12	5.1 ± 0.83	246.3 ± 4.90
	Cd-treated -1 Month (20)	2.18 ± 0.16[c]	6.0 ± 0.36[c]	0.5 ± 0.05[c]	17.6 ± 0.78[c]
Adult	Pooled control (24)	3.04 ± 0.08	41.0 ± 3.12	5.1 ± 0.83	246.3 ± 4.90
	Cd-treated -48 h (16)	3.23 ± 0.22	17.0 ± 1.33[c]	7.4 ± 0.37[a]	108.9 ± 10.10[c]
	Cd-treated -1 Month (22)	3.23 ± 0.25	8.5 ± 0.38[c]	3.7 ± 0.44	56.1 ± 5.20[c]

Results are expressed as mean ± S.E.M of the number of observations indicated in the parentheses.

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Control - age matched control group as described in the text

Pooled control - as described in the text.

Cd-treated

-48h group- experimental group, killed 48h after a single i.p. Cd injection

-1 Month group- experimental group, killed 1 month after a single i.p. Cd injection

ADP/O ratio - n moles of ADP phosphorylated/ n atoms of O_2 consumed

Respiration rate - nmoles of O_2 consumed/min/mg mitochondrial protein

ADP-phosphorylation rate - nmoles of ATP formed /min/mg mitochondrial protein

$^{a}P < 0.02$; $^{b}P < 0.002$; $^{c}P < 0.001$ compared to the corresponding controls.

Table 2

Effect of cadmium treatment on the oxidative phosphorylation in rat liver mitochondria with succinate as the substrate.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate		ADP-phosphorylation rate
			State 3	State 4	
3 Weeks	Control (16)	0.99 ± 0.08	53.2 ± 3.12	30.7 ± 3.03	102.5 ± 8.8[e]
	Cd-treated -48 h (20)	0.92 ± 0.08	125.9 ± 12.90[e]	52.2 ± 7.08[b]	231.7 ± 22.3
	Pooled control (24)	1.22 ± 0.08	72.6 ± 5.59	28.3 ± 4.21	171.9 ± 14.8
	Cd-treated -1 Month (20)	0.97 ± 0.06[a]	14.5 ± 0.91[e]	5.0 ± 0.36[e]	30.0 ± 1.0[e]
Adult	Pooled control (24)	1.22 ± 0.08	72.6 ± 5.59	28.3 ± 4.21	171.9 ± 14.8
	Cd-treated -48 h (24)	1.30 ± 0.09	46.5 ± 3.62[e]	25.8 ± 1.75	116.1 ± 10.7[c]
	Cd-treated -1 Month (24)	1.23 ± 0.08	19.1 ± 1.13[e]	13.4 ± 0.79[d]	40.1 ± 4.5[e]

Results are expressed as mean ± S.E.M of the number of observations indicated in the parentheses.

Other details are as given in Table 1

[a] $P < 0.02$; [b] $P < 0.01$; [c] $P < 0.005$; [d] $P < 0.002$; [e] $P < 0.001$ compared to the corresponding controls.

Table 3

Effect of cadmium treatment on the oxidative phosphorylation in rat liver mitochondria with ascorbate + TMPD as the electron donor system.

Age	Treatment/ Duration	ADP/O ratio	Respiration rate		ADP-phosphorylation rate
			State 3	State 4	
3 Weeks	Control (12)	0.39 ± 0.06	126.8 ± 11.60	79.6 ± 9.21	98.6 ± 8.9
	Cd-treated -48 h (10)	0.37 ± 0.04	194.3 ± 15.50[c]	84.1 ± 10.10	139.0 ± 12.6[b]
	Pooled control (14)	0.43 ± 0.07	121.8 ± 18.7	71.5 ± 10.50	102.0 ± 30.3
	Cd-treated -1 Month (20)	0.35 ± 0.07	15.4 ± 1.22[d]	9.1 ± 0.78[d]	10.9 ± 0.3[d]
Adult	Pooled control (24)	0.43 ± 0.07	121.8 ± 18.70	71.5 ± 10.50	102.0 ± 13.3
	Cd-treated -48 h (15)	0.40 ± 0.10	12.1 ± 2.70[d]	6.7 ± 1.62[d]	28.6 ± 6.3[d]
	Cd-treated -1 Month (24)	0.47 ± 0.09	16.8 ± 1.55[d]	7.2 ± 1.02[d]	34.8 ± 2.3[d]

Results are expressed as mean ± S.E.M of the number of observations indicated in the parentheses.

Other details are as given in Table 1.

[a]P < 0.05 ; [b]P < 0.02 ; [c]P < 0.002 ; [d]P < 0.001 compared to the corresponding control as described in the text.

Table 4

Effect of cadmium treatment on rat liver mitochondrial dehydrogenases.

Age	Treatment/ Duration	Glutamate Dehydragenase	Malate dehydrogenase	Succinate-DCIP Reductase
3 Weeks	Control (12)	45.8 ± 3.45	9564.5 ± 482.8	25.0 ± 1.01
	Cd-treated -48 h (10)	39.4 ± 1.55	8602.5 ± 362.5	39.3 ± 1.80[d]
	Pooled control (12)	80.6 ± 2.90	7465.5 ± 573.1	60.7 ± 4.36
	Cd-treated -1 Month (10)	67.3 ± 4.73[a]	6808.6 ± 591.3	13.9 ± 0.97[d]
Adult	Pooled control (12)	80.6 ± 2.90	7465.5 ± 573.1	60.7 ± 4.36
	Cd-treated -48 h (10)	44.1 ± 2.49[d]	2327.9 ± 132.9[d]	47.6 ± 2.67[b]
	Cd-treated -1 Month (16)	64.0 ± 4.23[c]	5927.3 ± 353.3[a]	19.2 ± 0.63[d]

Result are expressed as mean ± S.E.M of the number of observations indicated in the parantheses.

Enzyme activity = nmoles/min/mg protein

$^{a}P < 0.05$; $^{b}P < 0.02$; $^{c}P < 0.01$; $^{d}P < 0.001$; compared to the corresponding controls.

Table 5

Effect of cadmium treatment on rat liver mitochondrial ATPase activity.

Age	Treatment/ Duration	ATPase activity			
		Basal	Mg^{2+}	DNP	Mg^{2+} + DNP
3 Weeks	Control (12)	10.3 ± 0.52	22.6 ± 0.70	30.8 ± 1.26	39.4 ± 1.56
	Cd-treated -48 h (12)	15.3 ± 1.10[b]	29.2 ± 1.34[b]	39.8 ± 2.91[a]	49.3 ± 2.90[a]
	Pooled control (13)	12.3 ± 0.71	25.5 ± 1.31	56.1 ± 1.2	64.4 ± 1.29
	Cd-treated -1 Month (09)	8.1 ± 0.58[b]	16.0 ± 0.82[b]	14.0 ± 1.48[b]	18.1 ± 2.31[b]
Adult	Pooled control (13)	12.3 ± 0.71	25.5 ± 1.31	56.1 ± 1.26	64.4 ± 1.29
	Cd-treated -48 h (12)	5.1 ± 0.36[b]	12.0 ± 0.85[b]	27.9 ± 1.24[b]	29.6 ± 2.06[b]
	Cd-treated -1 Month (12)	7.7 ± 0.29[b]	24.0 ± 0.69	28.6 ± 1.72[b]	30.1 ± 0.99[b]

Results are expressed as mean ± S.E.M of the number of observations indicated in the parentheses.

Enzyme activity = μ moles Pi liberated /h/mg mitochondrial protein

[a] $P < 0.01$; [b] $P < 0.001$ compared to the corresponding controls.

Table 6

Effect of cadmium treatment on the soluble and total -SH content in rat liver mitochondria.

Age	Treatment/ Duration	- SH content	
		Soluble	Total
3 Weeks	Control (16)	2.60 ± 0.13	15.5 ± 1.53
	Cd-treated -48 h (16)	0.96 ± 0.06[c]	9.1 ± 1.32[b]
	Pooled Control (16)	———	33.0 ± 0.69
	Cd-treated -1 Month (16)	2.09 ± 0.27[c]	16.0 ± 0.86
Adult	Pooled Control (16)	———	33.0 ± 0.69
	Cd-treated - 48 h (16)	0.56 ± 0.01[c]	40.0 ± 0.36[b]
	Cd-treated -1 Month (16)	———	30.0 ± 0.91[a]

Results are expressed as mean ± S.E.M. of the number of observations indicated in the parentheses.

-SH content - n moles/ mg mitochondrial protein.

[a]P < 0.02 ; [b]P < 0.005 ; [c]P < 0.001 compared to the corresponding controls.

- phosphorylation rate decreased by 40 - 90 % , while with succinate the decrease in ADP/O ratio was 20 % and in the respiration rates and the ADP - phosphorylation rate it was 80%. On using ascorbate + TMPD, only about 10 - 15 % activities were evident compared to the controls with respect to all the parameters except the ADP/O ratio.

In the adults impaired oxidative phosphorylation was evident in the 48 h group itself. With glutamate the state 3 rate and ADP-phosphorylation rates were only 40 - 45 % of the pooled control values. However, state 4 rates were actually 45 % higher. When succinate was used as the substrate the state 3 rates decreased by about 35 %, while the ADP-phosphorylation rate was only 40 % of the pooled control value. With ascorbate + TMPD, the inhibition in the oxidative phosphorylation was found to be 90 % in state 3 and state 4 rates and 70 % in the ADP-phosphorylation rate.

The inhibition seen in the 48 h Cd-treated group persisted or intensified when the animals were kept for one month. Thus oxidative phosphorylation parameters with glutamate and succinate, further decreased and now only 20 - 25 % of state 3 rate and ADP-phosphorylation rate were evident compared to the controls. With succinate, in addition, the state 4 rate also decreased by 55 %. With ascorbate + TMPD the inhibition seen in the 48 h group persisted.

The effects of Cd treatment on the mitochondrial primary dehydrogenase activities are presented in table 4. Thus the

GDH and MDH activity in the young animals 48 h after Cd treatment decreased marginally (11 - 14 %) which however, was not statistically significant. On the other hand, SDR activity was about 60 % higher. One month after Cd-treatment the GDH activity decreased by about 20 %, while MDH was not affected. SDR activity was inhibited to an even greater extent, by about 80 %.

In the adult rats 48 h after Cd treatment GDH activity was about 45 % lower, while the MDH activity in this group was more drastically impaired, by about 70% and SDR activity decreased by 20 %. When the animals were kept for one month some recovery in the GDH and MDH activities could be seen. However , the values were still 20 % lower than the pooled control. SDR activity in contrast, registered a further decline and now was only 30 %.

The mitochondrial ATPase activities under basal and different stimulatory conditions as affected by Cd are shown in table 5. as is evident from th data in the 3 week group, 48 h after Cd treatment , the ATPase activity under basal and Mg^{2+} and/or DNP stimulation were higher than the controls, (25 - 50 % increase).One month after the Cd treatment however,the activities decreased from 35 - 75 %.

In the adults, 48 h after Cd treatment the activities were inhibited by 50 - 60%. After 1 month, the basal and Mg^{2+} ATPase activities were higher as compared to the 48 h group. The basal activity was able to recover by 40 - 60%. In case

of Mg^{2+} ATPase the activity was similar to the control. The DNP and Mg^{2+} + DNP ATPase activities, however, remained unchanged from the 48 h. group values.

The changes induced by Cd-treatment in the soluble and total -SH content are shown in table 6. It can be noted that the mitochondrial soluble -SH content in the 48 h. treated 3 week group was only 37 % and the total -SH content only 58 % of the controls. However, 1 month later, although, the age matched controls did not have any soluble -SH content, the Cd treated group showed a recovery and had about the same content as that in the young 3 week controls. The total -SH content was about 50% of the controls.

The 48 h Cd-treated group had about 0.56 nmoles/mg protein soluble -SH content, although the controls did not have any. The total -SH content was about 20 % of the controls. One month after Cd treatment, the soluble -SH content was not-detectable while the total -SH content was 10 % lower than the controls.

DISCUSSION

As is apparent from the data,the liver mitochondria from young rats did not show any adverse affects on Cd treatment in terms of the oxidative phosphorylation parameters within 48 h. In fact, the respiration and ADP-phosphorylation rates actually increased. Early et. al. [6] and Meiss et. al. [12]

have shown that in the adult, liver mitochondria are maximally damaged within this period. The results of the present studies on the adults further substantiate these observations. However, the phenomenon does not seem to apply to th young animals.

Wong et. al.. [8] have shown that the livers in the young rats accumulate lower amounts of Cd and since the liver weight in the young is the same as in the adults on a % body weight basis, the amount of Cd in the liver itself would be low and the amount entering into the mitochondria would also be correspondingly lower.

In addition to the above factor the seemingly lower toxicity and the apparent stimulatory effect of Cd on the respiration in the young rat liver mitochondria within 48 h. could be explained as follows : Jani et al [10] have shown a gradual increase in the respiratory activity in liver mitochondria from the 20-day to adult stage. In Chapter II of this thesis the development-induced increase in the activities of some other enzymes involved in the mitochondrial energy metabolism is shown. Thus at around 21 days of age the liver mitochondria have not yet reached their full capacities and are not functioning at full efficiency. The damage that may have been caused by the presence of Cd in the initial periods may have triggered the mitochondrial building machinery of the cell and while trying to recoup the damage, the activity could have overshot. However, the exact mechanism behind this remains obscure. Another fact in favour

of this hypothesis is that in adults where the mitochondria have attained their full working effeciency such recovery is not seen.

The other experimental group in which the injected rats are killed one month after Cd injection show a drastically inhibited respiration as compared to the pooled controls. In the young one month Cd-treated group, the rats injected on the 21st day had attained adult stage at the end of 4 weeks and hence the recovery that the young rats were able to attain in the 48 h period is not possible in these near-adult rats. In addition, Cd has a long half-life and stays in the body for about 15 - 30 years in the humans (Chapter I). A comparable long half-life in the rats would lead to a continuous presence of Cd in the liver and hence in the mitochondria for a considerably longer period of time. This prolonged exposure could lead to damage and subsequent dysfunction of the mitochondria.

In case of adults the damage caused in the 48 h. group did not show any recovery and in most cases the damage became even more acute in the one month group. This is to be expected since even in the young animals the 1 month group showed impairment of function. These results once more emphasize the point that the adult rats have a lower capacity to cope with the Cd-insult.

Vallee and Ulmer [13] reported that large oral doses of Cd caused uncoupling of oxidative phosphorylation in liver mitochondria. Liu and Liun [14] also reported uncoupling

under *in vitro* conditions and this effect was thought to be due to altered membrane permeability and fluidity [14]. In the present study, also uncoupling was detected in the young rats killed one month after the Cd-treatment. However, in the adult rats uncoupling was not seen.

Earlier reports have shown that short-term exposure to Cd leads to altered mitochondrial membrane integrity and loss of membrane potential and cell viability [1]. In addition the inhibition of uncoupler-stimulated respiration with NADH-linked substrates and succinate has been reported by Miccadei and Floridi [2]. Jacobs *et. al.* [15] and Diamond and Kench [16] also reported inhibition of oxidative phosphorylation by Cd in rat liver.

Similar to the results of the membrane - bound enzymes of oxidative phosphorylation the membrane bound SDR also is stimulated in the young 48 h group while in the young one month group and the adult 48 h and one month Cd treated groups the activity is inhibited. GDH and MDH are adversely affected within the 48 h period itself while the other groups show some recovery. Thus in case of SDR the young animals show resistance to Cd-insult. However, in adults this resistance is lost and the activity is totally inhibited while initially the presence of Cd in the matrix adversely affects the soluble dehydrogenases, i.e. GDH and MDH although later this effect seems to be slightly alleviated.

Swarnalatha et.al. [17] reported that on Cd-treatment SDH, NADH - dependent ICDH and cytochrome oxidase in liver and kidney are inhibited and gradually decrease in activity. The site of inhibition on the membrane was thought to be an amino acid present at the active site having a pka of 7.23 [18].

The membrane - bound ATPase, similar to other membrane - bound enzymes was stimulated in the young 48 h group while in all the other groups the activity was drastically inhibited. Webb [19] also reported inhibition of ATPase involved in maintaining ionic balance.

The decreased ATPase activity along with the depressed ADP - phosphorylation rates would lead to a decrease in ATP content in the cell. Liu and Liun [14] also have reported a decreased ATP content and concomittant decrease in cell viability. Thus the liver cell necrosis and cell death observed in Cd exposed rats [12,20] could be correlated with this decreased content of `energy currency ' of the cell. Since ATP is required for all biosynthetic process eg. DNA, RNA and protein synthesis, all these will be affected. In fact inhibition of RNA polymerase [21], protein synthesis [22], DNA polymerase [23] and DNA synthesis [24] following Cd exposure have already been reported. This could be either due to direct effect of Cd on these enzymes or due to the decreased ATP content or a combination of both.

A mechanism by which Cd can inhibit DNA synthesis is proposed by Rana et.al. [25]. Cd blocks the formation of thymidine triphosphate (TTP), a pre-requisite for DNA

synthesis. Theocaris et.al. [26] have shown that the enzyme thymidylate kinase (TK), which is required to convert thymidinemonophosphate (dTMP) to thymidinediphosphate (dTDP) is also inhibited in presence of Cd. It is known that this enzyme requires ATP to carry out the above conversion and hence the lower levels of ATP could limit the reaction leading to inhibition of DNA synthesis.

The pathogenicity of Cd has been related to the interaction of Cd with high molecular weight proteins containing -SH groups [13,27]. These -SH groups if present at the active sites of enzymes could lead to the inactivation of these enzymes. As seen in Chapter I a major mechanism of tolerance to Cd toxicity is the synthesis of soluble, low moleculer weigth proteins containing -SH groups, such as metallothionein (MT) or glutathione. These are produced in response to entry of Cd in to the cell.These sequester Cd and prevent its interaction with the -SH groups at vital points [27].

The study of soluble and total -SH groups was done to check the alterations in these as affected by Cd treatment. However the soluble as well as the total -SH content decreased in the 3 week 48 h group. Thus the protection that the soluble -SH groups are supposed to provide to the membrane-bound -SH groups does not seem to be functioning. In fact Klaassen and Wong [9] have stated that MT concentration in the tissue can not really be a true indication of the level of tolerance to Cd. They have shown that even in the presence of

20 times more concentration of MT in the livers of newborn rats, as compared to the adults the toxic response induced by Cd is still evident in the liver of the newborn rats. Data from the present studies can further corroborate this fact since, in the adult group, although there is drastic inhibition of all enzymes, the total -SH content is not seen to be much altered.

Thus it can be inferred from these results that the energy transduction in the rat liver is susceptible to and is damaged by Cd exposure. The effect in the 3 week group is anamolous in that the 48 h group is able to cope with the damage while once the adult stage is reached the mitochondria loose their resistance and become susceptible to Cd-insult. In addition, a single exposure to Cd is sufficient to cause a sustained damage to the mitochondrial oxidative phosphorylation and in fact, after a longer period of time has elapsed following the entry of Cd, the activity further deteriorates.

SUMMARY

Effect of Cd treatment on mitochondrial enzyme activities in the young (3 week) and adult (8 - 10 week) rat liver was checked 48 h and one month after a single i.p. Cd injection.

The oxidative phosphorylation was stimulated in the young 48 h group while in the adult 48 h group the activity was inhibited. However, in the 1 month group the activity in both the age groups was drastically reduced.

The dehydrogenases in the two age groups showed differential response. The soluble GDH and MDH were inhibited in the 48 h period in both the young as well as the adult rats. While in the 1 month group there was no further damage. SDR, a membrane-bound enzyme, on the other hand was stimulated in the young 48 h group, while in all the other experimental groups, the activity progressively decreased.

The ATPase activity under basal and Mg^{2+} and/or DNP stimulated conditions was deranged in all the experimental groups except, as is observed in this study with all the membrane-bound enzymes, a stimulation in the young 48 h. group.

The alterations in the -SH group content however, did not follow any pattern and the data on -SH content could not explain the observed results.

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