A Qualitative and Quantitative Study of Few Enzymes in the Liver of Cadmium Fed Rats

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Abstract: Cadmium when administered to animals is known to accumulate in the liver and kidney, causing identifiable toxic effects. These effects on enzyme systems have been described in the present investigation selecting few of them viz. alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and cholinesterase in the liver of rat, *Rattus rattus* albino. Topographical study on dysenzymia thus made have been found helpful in manifesting further the mechanism of Cd toxicity. Variations in the level of enzyme protein, state of cellular organelle together with processes of phosphorylation, adenylation and oxidative phosphorylation have been discussed explaining the cause and significance of these changes in enzyme activity.

Key words: Enzymes—Liver—Cadmium—Rat

INTRODUCTION

The retention of Cd in the liver is related to its selective storage in a protein-metallothionein, however, as accumulation increases there is spillage of Cd to other proteins and toxicity appears¹⁾. A sailent feature of Cd metabolism is its powerful interaction with other divalent metal ions particularly in the liver and kidney. *In vivo* and *in vitro* studies of its effects on brush border enzymes²⁾, hepatic detoxication enzymes³⁾ and nine other enzymes⁴⁾ have already been made. However, visual evidences on dysenzymia are not available. Present attempt describes the qualitative and quantitative modification of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase and cholinesterase thus caused by Cd in the liver of rats. Enzymological changes have been discussed in terms of changes in the micro-environment of the cell, formation of apoenzymes, metal protein complexes and oxidative phosphorylation etc.

MATERIALS AND METHODS

Forty adult male rats (*Rattus rattus* albino), two months old and weighing 100 ± 10 gm were selected from the laboratory stock. At random the groups were

formed, each containing twenty rats. They were housed individually in suitable cages, fed on laboratory diet (Hindustan Lever Ltd., Bombay) and tap water ad libitum. Rats of group A were administered cadmium sulphate (BDH mol. wt. 769.51), at 0.5 gm/kg body weight by gavage on each day for thirty days. Rats of group B served laboratory diet alone were used as controls.

On 31st day, all the rats were starved for 24 hours and then sacrificed by decapitation. For histochemical study small pieces of liver from each rat were fixed in chilled absolute acetone, frozen for few hours and cut on a freezing microtome. Paraffin sections (whenever applicable), were also processed for alkaline phosphatase⁵⁾, acid phosphatase⁶⁾, glucose-6-phosphatase⁷⁾ and cholinesterase⁸⁾. Rest of the liver lobes were quickly excised and immediately

Fig. 1. A uniform reaction for alkaline phosphatase is exhibited by the entire hepatic parenchyma of control rat. $\times 320$

Fig. 2. After cadmium treatment only the perilobular region could show the presence of alkaline phosphatase. $\times 125$

Fig. 3. A strong reaction for acid phosphatase was observed in liver of control rat. $\times 320$

Fig. 4. Liver from a Cd fed rat shows a diffused reaction throughout the lobule. $\times 500$

frozen at -4°C . 10% (w/v) homogenate was prepared in 0.25 M ice-cold sucrose solution at 0°C . The homogenate was centrifuged for 20 min at 1500 g and the clear supernatant fluid was used as the source of enzymes. Alkaline and acid phosphatases were determined using sodium- β -glycerophosphate as substrate⁹⁾. For the estimation of glucose-6-phosphatase, 0.01 M glucose-6-phosphate solution was incubated with homogenates for 15 min at pH 6.5¹⁰⁾. Cholinesterase activity was determined by the method of Rappaport *et al.*¹¹⁾ The protein contents in the homogenates were determined using bovine serum albumin as standard¹²⁾. For each enzyme, triplicate samples were analysed and the incubations were repeated three times. The Fisher 't' test¹³⁾ was employed for statistical significance.

Fig. 5. Fresh frozen section of liver from a control rat shows glucose-6-phosphatase rich parenchyma. $\times 320$

Fig. 6. In Cd fed rats only the nuclei show a dull reaction for glucose-6-phosphatase. $\times 320$

Fig. 7. A moderate reaction for cholinesterase was visualized in the entire lobule of control rat. $\times 320$

Fig. 8. Liver of Cd fed rat shows a dull but uniform reaction for cholinesterase. $\times 80$

Table 1. Enzyme activities in liver of cadmium fed and control rats

S. No.	Enzymes	Treated liver				Control liver			
		I	II	III	IV	I	II	III	IV
1.	Alkaline phosphatase	—	—	—	+	+	—	+	+
2.	Acid phosphatase	±	—	±	±	+	—	±	±
3.	Glucose-6-phosphatase	n±	—	n±	n±	+	—	+	+
4.	Cholinesterase	±	—	±	±	+	—	+	+
I	Hepatic parenchyma	+ Strong							
II	Bile Canaliculi	+ Moderate							
III	Centrolobular region	± Weak							
IV	Perilobular region	n Only in nucleus							

Table 2. Enzyme activity in the liver of control and treated rats

Enzymes	Control	Cadmium treated	Sign. Diff.
Alkaline phosphatase ^b	0.38 ± 0.020 ^a	0.22 ± 0.03	5.7142(+)
Acid phosphatase ^b	0.43 ± 0.012	0.38 ± 0.01	5.0000(+)
Glucose-6-phosphatase ^b	0.72 ± 0.026	0.64 ± 0.02	0.3900(—)
Cholinesterase units	42.00 ± 5.23	18.00 ± 1.53	11.3900(+)

^a Values are mean ± S.E. of 3 observations.

^b Activity is expressed in mg of inorganic phosphate liberated per mg of protein/h at 37°C.
(+) Indicate statistically significant difference from control at 95% confidence interval.

RESULTS

A uniform reaction for alkaline phosphatase was exhibited by hepatic parenchyma of the centrolobular and periportal zones in control rats (Fig. 1). Cadmium largely inhibited its activity, however, perilobular parenchymal cells could show a dull activity (Fig. 2). For acid phosphatase, as usual, a strong reaction was exhibited by the hepatic cells (Fig. 3), however, in the liver of cadmium fed rats, a diffused reaction was observed throughout the liver lobule (Fig. 4). Glucose-6-phosphatase could very well be localized in the entire hepatic parenchyma (Fig. 5). In Cd fed rats, a dull and diffused reaction was exhibited only by the nuclei (Fig. 6). Cholinesterase too was inhibited by cadmium. In normal rats, a moderate reaction was recorded in the entire lobule (Fig. 7), but a dull reaction was observed in Cd fed rats (Fig. 8). Topographical differences on the distribution and intensity of these enzymes have been presented by Table 1.

Quantitative data as presented in Table 2 supported histochemical observations. All the enzymes were inhibited significantly in the liver by Cd whereas an insignificant decline was noticed in the activity of glucose-6-phosphatase only.

DISCUSSION

Accumulation of Cd in the liver is roughly proportional to dosage level¹⁴. The

appearance of lesions depends on liver Cd level. Structural changes have also been observed in the kidney, liver, gastrointestinal tract, heart, testis, pancreas, bones and blood vessels¹⁵⁻¹⁸). However, mechanisms of that injury are not fairly known. Since, enzyme histochemistry is a link between morphology and chemistry, and enzyme proteins are first target of metal ions, few key enzymes were localized in the liver of Cd fed rats. It inhibited all of them without exception. Cadmium might have inhibited these enzymes by: (i) the removal of the essential metal ions leaving the apo-enzyme, (ii) the replacement of some of the protein groups giving mixed enzyme inhibitor metal complex. However, Cd induced injury to membranes through a characteristic change in the micro-environment of the cell needs further mention. Inhibition of alkaline phosphatase indirectly reflects an injury to plasma membrane. How the plasma membrane of perilobular parenchyma remained intact is a subject of further research. However, the fact that histological lesions were also not significant in the perilobular region support this observation. Dull and diffuse reaction for acid phosphatase reflects lysosomal leakage. A change in glucose-6-phosphatase activity probably points to changes in carbohydrate metabolism. The mechanisms behind chemical modification of enzymes might be phosphorylation, adenylation, ADP ribosylation, oxidation of thiol groups or respective reverse reactions¹⁹) nevertheless, all these reactions need to be established for metal ions. A balance between synthesis and degradation of the enzyme protein might also be modified by nutritional variables²⁰). Presumably, both variations in the level of enzyme protein and state of cellular organelle should be implied to interpret these changes in enzyme activity.

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