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Metabolic Alterations in Liver and Testes of Adult and Newborn Rats Following Cadmium Administration

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Cadmium is a well known toxic heavy metal responsible for many toxic manifestations. The potential harm to industrial workers and general public generated a great deal of interest to understand the effect of cadmium on general metabolism in different tissues of the body and also to assess the efficacy of the protective agents against the toxicity. A large number of studies have been conducted to understand the effect of cadmium on cellular intermediary metabolism. Although, most of the metal is stored in liver and kidney, the organ affected most in acute toxicity is testis (Parizek and Zahor, 1956; Parizek, 1957). Increased lipid peroxidation and decreased mitochondrial respiration along with other cellular enzyme activities have been reported to take place due to cadmium administration (Klimczak et al, 1984, Rao, 1983, Muller, 1986). Cadmium has been shown to have high affinity to sulfhydryl groups (Dalvi and Robins, 1978). Reducing equivalents such as NADH or NADPH are important for keeping the sulfhydryl groups at a reduced state and maintaining the cellular integrity.

The present experiment was designed to study the effect of acute cadmium administration on the activities of some of the tissue enzyme systems that provide the reducing equivalent NADPH. The levels of NADH and NADPH were also measured. All the measurements were conducted in two tissues: liver and testes. The effect of simultaneous administration of zinc on cadmium induced changes was also determined. Newborn animals have been found to be resistant to many effects of cadmium (Wong and Klassen, 1980). The present studies were also conducted in new born rat liver and testes. It was the intention of this study to compare the effects of cadmium on adult and newborn rats.

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MATERIALS AND METHODS

Male Sprague-Dawley rats (Taconic Farms, Germantown NY) weighing 200-225g were maintained in central animal facilities under a 12hr dark and light cycle. The animals were given cadmium chloride 1mg/kg in normal saline intraperitoneally twice a day for three days. Groups of animals also received twice the equimolar amount of zinc as zinc sulfate simultaneously with cadmium. Controls received normal saline in equal volume. The animals were sacrificed on day 4.

Male newborn rats from different mothers were separated after birth, randomly mixed and again placed with a mother in order to obtain random population. Female newborns were removed. Newborn animals received 5mg/kg cadmium chloride subcutaneously on day 2 of the birth. The injections were repeated every two days for 14 days and the animals were sacrificed on day 15. Controls received normal saline alone.

Liver or testes were removed, washed and homogenized in ice cold 0.25M sucrose in a Potter-Elvejhem homogenizer with a teflon pestle. The homogenates were centrifuged in cold at 700g for 5 min and the supernatants obtained were used for the enzyme measurements. Glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase levels were determined according to Marks (1966) by measuring increase in absorbance at 340nm in a UV-VIS spectrophotometer. A change of 0.001 absorbance/min was considered as one unit of the enzyme activity. NADH and NADPH levels were measured in KOH/ethanol homogenized tissue samples by spectrophotometric method described by Klinberg (1975). Protein was measured according to Lowry et al (1951).

Analysis of variance and student's t test were used to evaluate significance of differences between groups at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Adult rats receiving cadmium showed accumulation of intraperitoneal fluid. The testes were very hard and purple red in color. Animals of the other groups did not show any accumulation of intraperitoneal fluid. Cadmium plus zinc group testes were slightly red.

Cadmium chloride administration decreased the levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase in testes (Fig. 1). The decrease was observed to some extent in liver but also it was not significant. The decreased activities of these enzymes in acute toxicity may be involved in impairing

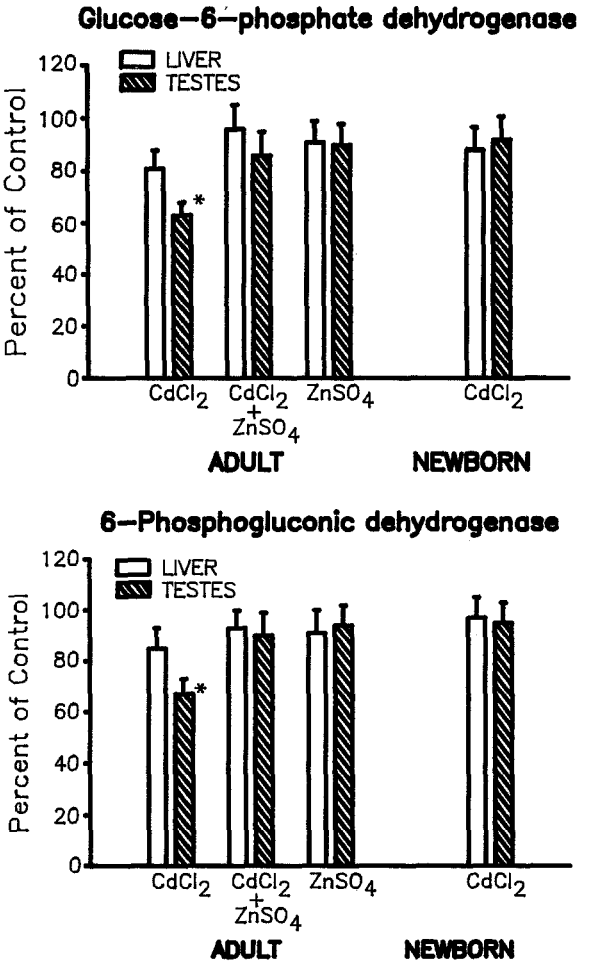

Fig. 1: Glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase activties in adult and newborn rats. Livers and testes from animals treated with cadmium, cadmium plus zinc, zinc alone or normal saline were removed, homogenized in 0.25 M sucrose and centrifuged at 700g for 5 min. The supernatants obtained were used to determine the enzyme activties by spectrophotmetric method. Change in absorbance of 0.001/min at 340 n m was considered to be one unit. The results are expressed as perecent of normal saline controls and are mean ± SEM of 4-5 samples. Glucose-6-phosphate dehydrogenase activities (units/mg protein) in control adult and newborn rat livers were 76.2 ± 9.3 and 88.4 ± 7.6 respectively whereas in testes they were 65.8 ± 5.4 and 49.7 ± 4.3 respectively. 6-phospho-gluconic dehydrogenenase values (units/mg protein) in control adult and newborn livers were 131 ± 12.7 and 94.5 ± 11.4 respectively whereas in testes the values were 76.5 ± 7.2 and 53.6 ± 6.4 respectively. Asterisks denote the significance of difference at $p < 0.05$ from controls.

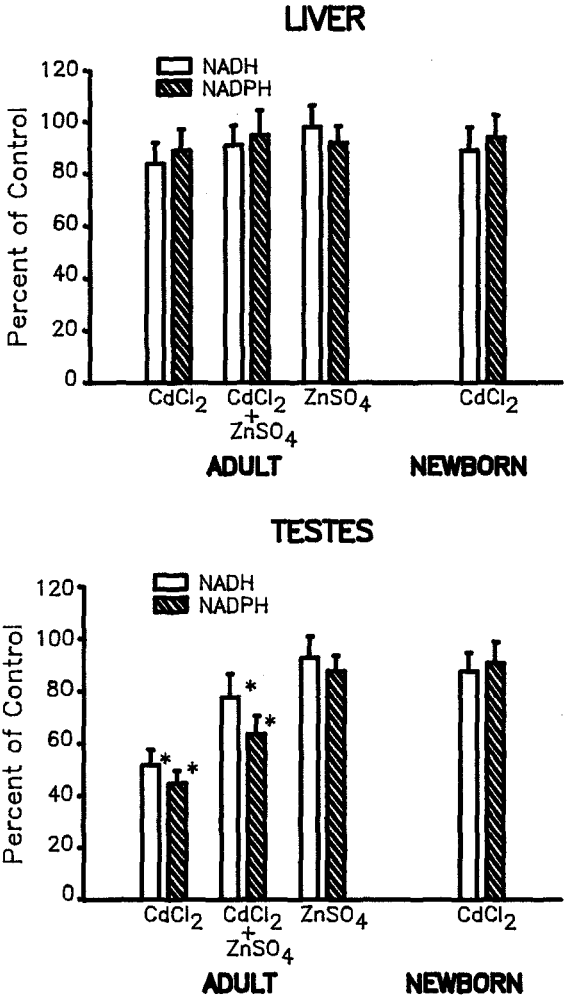

Fig. 2 NADH and NADPH levels in liver and testes of adult and newborn rats. Liver and testes were quickly homogenized in KOH/ethanol, centrifuged and supernatant was used for measuring the levels of NADH and NADPH. The results expressed as nmoles/mg protein are given as percent of normal saline controls. The results are mean ± S.E.M. of 4 to 5 samples. Levels of NADH and NADPH in control adult rat livers were 1.08 ± 0.09 and 1.47 ± 0.23 respectively and in control newborn rat livers were 2.74 ± 0.33 and 3.14 ± 0.51 respectively. NADH and NADPH in control adult testes were 0.87 ± 0.19 and 1.03 ± 0.11 respectively and in control newborn testes were 1.98 ± 0.27 and 2.75 ± 0.38 respectively. Asterisks denote the significance of difference at $p < 0.05$ from controls.

normal levels of reducing equivalents NADH and NADPH. Reduced pyridine nucleotide levels decreased to 50% of the control level in testis (Fig. 2). Whether there is a concomitant increase in oxidized form needs to be further evaluated. Changes in pyridine nucleotide levels might affect the redox state of the cell and thereby damage the ion transport mechanisms and cellular function. Decreased levels of glucose-6-phosphate have been demonstrated in testes following acute cadmium exposure (Harkonen and Kormano, 1970). Since the effect of cadmium on testes is associated with its effect on the membranes (Lee and Dixon, 1973, Muller, 1986), it is possible that lower availability of the reducing equivalents as observed in the present study might augment cadmium induced membrane damage. Increased activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase i.e. increased formation of NADPH from NADP via pentose phosphate pathway has been observed in a variety of tissue injury, inflammation and repair processes (Braasch et al, 1968; Vorne and Arvela, 1971). Although NADP reduction may occur via many other enzymes, the reactions catalyzed by glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase have frequently been used as markers for the occurence of reparative processes in the injured tissue (Buckly and Basset, 1987; Grose et al, 1987).

Simultaneous administration of zinc with cadmium completely protects many cadmium induced changes (Gunn et al, 1961; Webb, 1972; Goering and Klaasen, 1984). In the present experiment no significant changes in enzyme activities or in pyridine nucleotides levels were observed in cadmium plus zinc group (Fig. 1 and 2). Non-availability of free cadmium due to increased metallothionein (Webb, 1972, Goering and Klaasen, 1984) might be responsible for this effect.

Newborn animals did not show any changes in enzyme activities or in the levels of NADH or NADPH (Fig. 2) after cadmium administration which is consistent with their resistance to cadmium induced damage. Due to low excretion of cadmium in these animals (Kello and Kostial, 1974), there might be a higher retention. Although newborn animals have been reported to have very high levels of metallothionein in their liver, it may not contribute to resistance to cadmium induced damage (Wong and Klaasen, 1980). It has been suggested that altered subcellular distribution of cadmium might be responsible for this resistance (Wong and Klaasen, 1980, Goering and Klaasen, 1984a). The dose of 5mg/kg produces lethality in adult rats but repeated administration of this dose in newborns did not produce any effect on pyridine nucleotides. Cell membranes in

newborn rats might be resistant to the damaging effect of cadmium.

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