Bull. Environ. Contam. Toxicol. (1986) 37:62–69

Bulletin of Environmental Contamination and Toxicology

Changes in the Adrenals in Lead Treated Rats

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Lead has long been recognised to have deleterious effects on different systems in many species (Bornschein et al,1980). That the endorcrine functions of testes, ovary (Roychoudhury et al,1984), thyroid, and adrenals (Sandstead et al,1970) were affected by lead are known from observations on either man or laboratory animals. In one study adrenal steroid excretion was first found to increase and then to decrease considerably during advanced stages of lead intoxication in exposed workers (Kehoe,1980). No comprehensive studies on this aspect of lead poisoning seem to have been carried out. The present investigation was undertaken to contribute to a better understanding of the adrenal functions in rats treated with different dosages of lead.

MATERIALS AND METHODS

Forty adult male albino rats, Charles-Foster strain, raised at the Institute's animal house, maintained on basal diet,weighing 150±5g, were divided into four experimental groups, including a control group of equal size. Rats in the first,second,third and fourth groups daily received intraperitoneally 1mg/kg, 2mg/kg, 4mg/kg and 6mg/kg lead acetate,respectively, over a period of 30 days. The aqueous solution was slightly acidified with a few drops of acetic acid to enhance the solubility of lead acetate. The control group of animals received the same volume(0.5ml) of the acidified distilled water intraperitoneally,over the same period.

The rats were weighted biweekly and were observed for any overt signs of lead toxicity over a period of 30 days. 24hr. urine was collected from animals which were kept individually in metabolic cages, on the initial day, the 15th and 30th days for estimation of δ-aminolevulinic acid(ALA-U) (Grabecki,1967). On the day prior to sacrifice, 5ml of urine was collected from

each animals in all groups for the estimation of 17-keto steroids (Wooton,1964). Blood was collected from retroorbital venous plexus of the anaesthetised animals for estimation of blood lead by atomic absorption spectrophotometry (Delves,1970) and 4.0ml of blood was used for the estimation of δ-aminolevulinic acid dehydratase (ALA-D) activity (Nakagawa et al,1980).

Following this, the rats were killed by decapitation and adrenals were cleanly dissected out and weighed. An equal number of adrenals from each group were collected and separately digested with concentrated nitric acid for the determination of adrenal lead by atomic absorption spectrophotometry (Mylroe et al,1977). Four adrenals from each group were fixed in Bouin's fluid for histological examinations. 5um thick paraffin sections were stained with hematoxylin and eosin. The remaining adrenals from the different groups were processed for the estimation of ascorbic acid (Roe and Kuether,1943), cholesterol (Hanel and Dam,1955) and catecholamines (Aaron and David,1962). Quantitative histometric analyses of adrenals were performed at x 640 magnification.

Statistical significance of differences between the collected data was calculated by the Student 't' test.

RESULTS AND DISCUSSION

Increasing the dose of lead produced a gradual decrease in body weight gain (Table1). There was significant decrease in the adrenal weights in the Groups III and IV, although the reverse was observed in the animals of Group II (Table1). Blood and adrenal lead levels were significantly higher in animals receiving the higher dosages of lead acetate (Table2). As expected,the elevation of ALA-U was also observed with increased doses of lead acetate in all the experimental groups throughout experimental period (Fig.1). Significant inhibition of ALA-D occurred with doses of 2mg/kg lead acetate and higher (Table2).

Significant decrease in adrenal cholesterol and ascorbic acid levels were observed in Groups I and II, but significant increases were seen to occur in other Groups i.e.III and IV (Table3). Significant increase in the adrenal catecholamines were observed in Groups I and II; a decrease in the Groups III and IV. Urinary 17-keto Steroids (17-K.S.) were significantly increased in Group II and decreased in Groups III and IV as compared to controls.

Cytometric findings indicated (Table4) that the widths of each of the three adrenal cortical zones decreased

Table 1. Changes in body and adrenal weight under different dosages of lead acetate. Number of observations are in parentheses; Mean±S.E.

Group	Body weight (gm)			Adrenal weight (mg)
	Initial	Final	%gain	
CONTROL	146.00 ± 1.25	226.50 ± 8.13	55.14 ±0.34	19.36 ±0.67
I	151.00 ± 2.08	210.00 ± 4.47	39.07 ±0.32**	21.08 ±0.74 NS
II	146.00 ± 1.98	193.50 ± 4.60	32.08 ±0.20**	22.23 ±0.69**
III	150.50 ± 1.38	185.00 ± 5.16	22.92 ±0.12**	15.05 ±0.90**
IV	150.50 ± 1.57	176.50 ± 3.66	17.28 ±0.82**	14.23 ±0.82**

Table 2. Distribution of blood lead, adrenal lead and ALA-D action in the different lead acetate exposed groups (i.p.); Mean±S.E. The number of observations are in parentheses.

Group	Blood-Lead (µg/100ml) (7)	ALA-D (µmol/PBG/hr/lit RBC) (5)	Adrenal Lead (µg/100mg) (8)
CONTROL	4.54± 0.39	176.46± 7.73	4.57±0.86
I	62.51± 5.76**	119.72±27.59 NS	4.75±0.46 NS
II	102.59± 4.74**	81.32±11.89**	6.24±0.47**
III	202.59±15.95**	72.98± 9.56**	7.94±0.50**
IV	332.47±13.63**	38.93± 4.20**	8.72±0.50**

NS, Not Significant ** $p < 0.001$

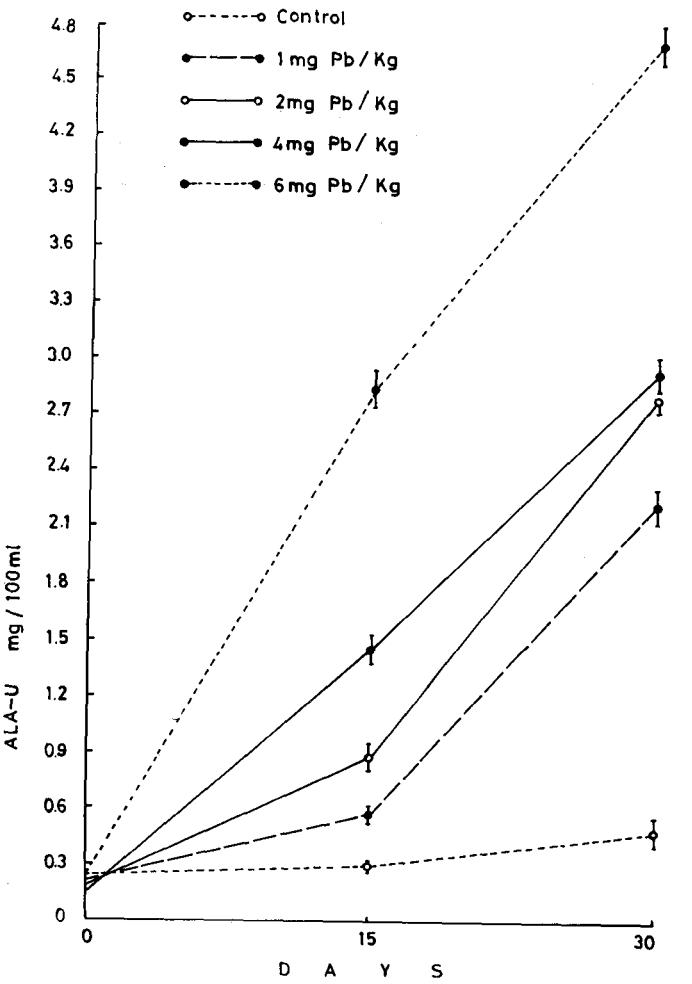

Figure 1. Changes of the urinary δ-aminolevulinic acid (ALA-U) excretion after treatment with different dosages of lead acetate in male rats.

in Groups II,III and IV. However,not all observed changes were statistically significant as shown in Table4. There was a significant lowering of the cortical cells nuclear diameters in Groups III and IV animals.

The corticomedullary cells are metabolically stimulated initially after intraperitoneal treatment with the smaller(1mg/kg and 2mg/kg) dosages of lead acetate. Administration of lead acetate in increasing dosages produced increases in blood lead concentration and urinary ALA. The levels of blood ALA-D (Table1) significantly changed along with the above two parameters in the respective directions noted. These are the principal indicators of lead toxicity. Significant depletion

Table 3. Biochemical changes of adrenals in rats under treatment with lead acetate in different dosages. Number of observations are in parentheses, Mean±S.E.

	Adrenal Cholesterol (10) (mg/g fresh tissue)	Adrenal Ascorbic acid (10) (mg/g fresh tissue)	Adrenal Catecholamine (8) (µg/mg fresh tissue)	17 Ketosteroid (8) (µg/24 hr urine)
CONTROL	26.66±1.42	6.46±1.02	1.02±0.18	6.01±0.50
I	20.28±1.73*	5.96±0.14*	2.10±0.12**	6.20±1.28^{NS}
II	13.24±2.14**	3.10±0.74**	2.20±0.19**	8.24±0.32**
III	34.10±0.62**	10.00±1.20**	0.75±0.08**	2.02±0.58**
IV	36.45±1.20**	10.26±0.94**	0.73±0.08**	1.82±0.75**

NS, Not Significant
* $p<0.05$
** $p<0.001$

Table 4. Histometric analysis of adrenals of rats under the different concentrations of lead acetate exposure. Number of observations are in parentheses; Mean±S.E.

Group	Zonal width (μm)x640† (15)				Zonal nuclear diameter (μm)x1600† (20)			
	ZG	ZF	ZR	M	ZG	ZF	ZR	M
CONTROL	91.50 ±4.72	643.00 ±14.00	97.50 ±14.68	587.50 ±16.30	5.70 ±0.18	5.95 ±0.17	4.68 ±0.11	7.03 ±0.20
I	84.00 ±4.65^{NS}	680.00 ±18.41^{NS}	97.00 ±8.60	680.83 ±16.62**	5.85 ±0.17^{NS}	5.45 ±0.20^{NS}	4.28 ±0.14*	7.20 ±0.18^{NS}
II	77.50 ±4.18**	689.00 ±17.68^{NS}	98.60 ±6.78^{NS}	681.67 ±15.96**	5.50 ±0.15^{NS}	5.35 ±0.18*	4.05 ±0.16**	7.23 ±0.18^{NS}
III	76.00 ±4.08**	475.00 ± 7.91**	74.50 ±4.88**	339.17 ±18.54**	4.85 ±0.13**	4.23 ±0.18**	4.00 ±0.18**	5.58 ±0.18**
IV	73.50 ±5.04**	452.54 ± 8.29**	61.00 ±6.25**	305.00 ±12.22**	4.10 ±0.16**	3.00 ±0.15**	4.00 ±0.19**	4.78 ±0.14**

ZG, Zona glomerulosa
ZF, Zona fesiculata
ZR, Zona Reticularis
M, Medulla

† microscopic observation at magnification

* $p<0.05$
** $p<0.001$
NS Not significant.

of cholesterol and ascorbic acid in the adrenals along with high levels of excretion of 17-K.S.after treatment with lead acetate in the lower dosaged indicated a stimulation of cortical functions. The morphological proliferation of zona fasiculata in these groups also provides additional support to the above observation. In contrast,degeneration of the adrenalcortical cells was conspicuous with the higher dosages of lead acetate. This may be due to the over stimulation and subsequent exhaustion of the cortical cells (Roy Chowdhury et al,1984).

The initial adrenal response to any stress increases the catecholamines secretion with hypertrophy of the medullary chromaffin cells (Wurtman et al,1972). Proliferation of chromaffin cells and significant increase of adrenal catecholamines with the lower dosages (1mg/kg and 2mg/kg)of lead acetate were presumably the consequences of medullary activation. Dosages of 4mg/kg and 6mg/kg lead acetate,however,produced degeneration in medullary internal cells and significantly low adrenal catecholamines values. Lead inhibits the synaptic transmission in adrenergic nerve ending (Copper and Steinberg,1977). The adrenal medulla also contains adrenergic synapses and lead may produce the observed changes by similar inhibition of the synaptic transmission in the intramedullary adrenergic nerve endings.

Acknowledgements. The project was supported by the Indian Council of Medical Research. The authors are thankful to Mrs.H.S.Trivedi, Miss S.Padmavathi and Mr.G.M.Shah for their technical assistance. Thanks are due to Mr.P.A.Thomas for his secretarial assistance.

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Received August 10,1985; accepted September 27, 1985.