PULMONARY LESIONS IN EXPERIMENTAL CHRONIC CADMIUM POISONING OF SQUIRRELS: HISTOPATHOLOGICAL AND ENZYMOLOGICAL STUDIES

R. K. MITHAL

*Department of Zoology, D.N. Postgraduate College,
Meerut 250 002, U.P., India*

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Morphological alterations in lungs have been noticed in squirrels with repeated intratracheal injections of cadmium at a dose of 1.0 mg Cd/Kg of body weight. Haemorrhages in alveoli and bronchioles, congestion in blood vessels, emphysema and peribronchiolar infiltration by mononuclear cells along with thickening of interalveolar septa were observed after different exposure intervals. Control and Cd-treated groups increased in body weight by 1.13 and 1.03 folds after 7 weeks, respectively ($P < 0.05$). Maximum rise was also found in lung weight after 7 weeks. Both alkaline and acid phosphatases revealed inhibition in their activities after 4 weeks. But, during 5-7 weeks, enzymatic activities were slightly elevated.

There is no doubt that a prolonged occupational exposure of cadmium compounds can give rise to pulmonary¹⁻²⁾ and hepatic³⁾ dysfunctions. Inhalation studies have been conducted to study toxic effects and body retention of both soluble and insoluble cadmium particles. Harrison *et al*⁴⁾ reported that high doses of cadmium chloride induced pulmonary oedema in dogs. Chronic bronchitis and emphysema have been described from several countries in cadmium-exposed industrial workers^{3,5,6)} after several years of exposure to concentrations as low as 0.1 mg/m³ of cadmium oxide fumes. The author has recently reported about short-term cadmium exposure in lungs of squirrel.⁷⁾

The purpose of this study is to understand the effect of chronic exposure of cadmium acetate on lung of common Indian ground squirrel (*Funambulus pennanti*, Wroughton 1905).

MATERIALS AND METHODS

Thirty two laboratory-bred squirrels of both sexes with body weight of 100 ± 10 g were kept in steel-wired cages (60×48×40 cms) and allowed to feed on wheat flour bread and tap water *ad libitum*. The animals were divided into two equal groups: group-A of 16 squirrels was injected intratracheally with a daily dose of cadmium acetate (1.0 mg Cd/Kg of body weight), and group-B of 16 squirrels served as control.

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The control group received distilled water by the same route. Four squirrels from each of the groups were sacrificed after different intervals viz., 4, 5, 6 and 7 weeks by decapitation after light ether anesthesia. Lungs were removed from the animals of both groups as quickly as possible and weighed. Small pieces (5 mm in thickness) were cut and fixed and 10% formol-saline. Paraffin sections of lungs of treated and control groups were cut at 3–4 μ and stained with haematoxylin-eosin. Body weights of the animals of both groups were recorded weekly.

The activity of acid and alkaline phosphatases was determined from homogenate of lung. The homogenate was prepared in ice-cold 0.25 M sucrose solution by using a Potter-Elvehjem homogenizer and was centrifuged for 20 min at 1000 g in ice-cold centrifuge. The activity of the two enzymes was determined by the method of Bodansky.⁸⁾ Substrate solution for alkaline phosphatase was prepared at pH 9.3 as follows:

Sodium β -glycerophosphate	: 0.5 g
Sodium diethylbarbiturate	: 0.424 g
Distilled water	: 100.00 ml

Small amount (9 ml) of the substrate solution was preincubated at 37°C for 20 min. 1 ml enzyme extrated was mixed with this solution. The mixture of substrate and enzyme was incubated at 37°C for 1 h. 2 ml of 30% trichloroacetic acid (TCA) was added to stop enzyme activity after incubation. After filtering the solution, dilution of mixture was obtained as follows:

Acidic ammonium molybdate solution	: 1.00 ml
Aminonaphthosulfonic acid solution	: 0.4 ml
Filtrate	: 8.0 ml
Distilled water	: 10.0 ml

Colour intensity appeared after 5 min and was read at 660 nm. A similar method was observed for acid phosphatase except the pH was adjusted to 5 with 1 N acetic acid. Statistical analysis of the results was calculated by the method of Fisher⁹⁾. The experiment was repeated twice to confirm the findings.

RESULTS

Squirrels showed an increase in final body weight after intratracheal administration of chronic cadmium acetate. Control and cadmium treated groups increased in body weight by 1.13 and 1.03 folds after seven weeks, respectively. Final weight gain was significantly different from the control. (Table-1)

Lung weight in the experimental group increased in comparision to control. Maximum rise was observed after 7 weeks. (Table-2)

Alkaline and acid phosphatase activities in lungs of squirrels after cadmium exposure are given in Table-3.

Four weeks, after cadmium administration, revealed intraalveolar haemorrhages,

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Table 1. Body weight of squirrels after chronic cadmium exposure.

Groups	Body weight (g) at weeks								Folds
	0	1	2	3	4	5	9	7	
Control	93.0*	94.3	95.0	95.6	99.3	101.0	104.6	105.6**	1.13
	±8.8	±9.6	±6.1	±7.2	±6.6	±9.9	±9.4	±7.6	
Cadmium	92.6*	93.0	93.5	93.8	94.0	95.3	95.8	96.2**	1.03
	±9.5	±6.4	±8.7	±8.9	±9.2	±8.4	±9.7	±7.8	

± Standard deviation

Initial (*) and final (**) body weight have been taken into consideration to work out the folds.

Table 2. Effect of cadmium on lung weight in squirrels

Exposure time (weeks)	Lung weight (g)	
	Control	Treated
0	1.02* ± 0.0302	1.25* ± 0.0415
4	1.08 ± 0.0352	1.13 ± 0.0513
5	1.15 ± 0.0475	1.19 ± 0.0672
6	1.21 ± 0.0417	1.26 ± 0.0680
7	1.30 ± 0.0506	1.34 ± 0.0428

* mean; ± standard error

Table 3. Activity of alkaline and acid phosphatases in lungs after chronic cadmium exposure.^a

Enzyme	Exposure time of cadmium treatment in weeks				
	0	4	5	6	7
Control	0.0476	0.0452	0.0488	0.0482	0.0472
	±0.0014	±0.0042	±0.0018	±0.0036	±0.0024
Alkaline Phosphatase ^b	0.0452	0.0401	0.0526	0.0510	0.0500
	±0.0028	±0.0020	±0.0004	±0.0018	±0.0024
Difference	—	4.82 ^c	2.10	3.76 ^c	4.80 ^c
Control	0.0564	0.0542	0.0548	0.0527	0.0548
	±0.0018	±0.0021	±0.0027	±0.0025	±0.0014
Acid Phosphatase ^b	0.0524	0.0428	0.0568	0.0580	0.0548
	±0.0000	±0.0032	±0.0028	±0.0030	±0.0014
Difference	—	2.82 ^c	4.38 ^c	6.24 ^c	5.30 ^c

a — values are mean ± SE.

b — activity expressed in mg of inorganic phosphate liberated/mg of protein/h at 37°C.

c — indicates statistically significant difference from control at 95% confidence interval.

haemorrhage of the bronchioles, and emphysematous alveoli (Fig. 2). Whereas, after five weeks of treatment, these changes were significant alongwith congestion in blood vessels (Fig. 1). Six weeks after intoxication, all these changes became well pronounced and haemorrhage in interalveolar septa (Fig. 1) was clearly observed. Peribronchiolar infiltration by mononuclear cells was also found during this time. After seven weeks, cadmium exposure, besides peribronchiolar infiltration by mononuclear cells (Fig. 3) revealed thickening of interalveolar septa (Fig. 1). Haemorrhage in the alveoli and interalveolar septa along with congestion in blood vessels was also observed.

Fig. 1. Mild haemorrhage and thickening in interalveolar septa and congestion of blood vessels after seven weeks of chronic cadmium exposure. $\times 70$, H & E.
(Indicated magnifications are that of original)

Fig. 2. Marked degree of emphysematous change in alveoli after six weeks of chronic cadmium exposure. $\times 70$, H & E.

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Fig. 3. Intense peribronchiolar infiltration by mononuclear cells after seven weeks of chronic cadmium exposure. $\times 70$, H & E.

DISCUSSION

Final body weight gain of cadmium treated squirrels was significantly different ($P < 0.05$) from the controls. Present finding suggests the stunted growth rate after chronic cadmium exposure. Stunted growth rate after cadmium intoxication was also reported in rats^{10,11} and rabbits.^{12,13}

Lung weight was disturbed after cadmium acetate exposure. Increase in organ weight was found from 6-7 weeks and microscopic study revealed infiltration by mononuclear cells in the vicinity of bronchioles. Such a pulmonary lesion could possibly explain the increase in lung weight.

Both alkaline and acid phosphatases revealed inhibition in activity after 4 weeks of cadmium acetate exposure, which suggests an alteration in physiological function of lung. During 5-7 weeks, enzymatic activities were slightly elevated. The decrease in activities may be attributed to histopathological alterations; whereas, the increase after 5-7 weeks could have appeared, possibly, due to greater accumulation of cadmium in lung with the passage of exposure time.

Daily exposure of cadmium resulted in emphysema after 4-6 weeks. Thurlbeck and Foley¹⁴ reported that cadmium chloride is capable to produce pulmonary damage resembling to human emphysema in rats and guinea pigs, either by intratracheal instillation or by inhalation.¹⁵ In the present study, cadmium acetate was administered intratracheally. Prodan¹⁶ also observed emphysema in rats and pointed out that small amounts of cadmium oxide fume and dust were capable of producing scars in the lungs, although the animal was little disturbed by the exposure. Lane and Campbell¹⁷ reported that cadmium emphysema, while wide-spread, shows a narrow zone of normal lung tissue under the pleura and no emphysematous bullae at the periphery after chronic

exposure in humans. Administration of cadmium by intratracheal route might have resulted in accumulation of the metal in alveoli which in turn could produce emphysematous change. Peribronchiolar infiltration by mononuclear cells revealed histopathological alteration. Some of the bronchioles showed the presence of inflammatory cells inside the lumen. As can be anticipated, the inflammatory reaction in the chronic forms of the bronchitis consists of a mixture of mononuclear cells, which include macrophages with a large number of lymphocytes and plasma cells and a few neutrophils. Occasionally, lymphocytes were collected within the subepithelial and deeper submucosal tissues into larger collections. Shabalina¹⁸⁾ reported that cadmium stearate revealed the accumulation of lymphocytes in the bronchial wall, chronic emphysema and haemorrhagic area in rats. Bouley *et al*¹⁹⁾ showed that inflammatory phenomenon was demonstrated by a temporary increase in lung weight and by the presence of numerous microphages. Increase in lung weight was also recorded in the present study and the possible reason of increase in weight may be attributed to inflammatory reaction. Cadmium aerosol has been reported to induce lung injury and the healed lesion showed localized intraalveolar aggregation of alveolar macrophages mainly in alveoli surrounding respiratory bronchioles.¹⁵⁾ The increase in lymphocytes has been more striking. Although, it has been newly generated bone marrow macrophages,²⁰⁾ which might have entered the inflammatory zone from the lung interstitium.²¹⁾ The increased number of lymphocytes noted with cadmium chloride exposure could represent part of the inflammatory reaction²²⁾ or it might reflect a local immune response.²³⁾

Haemorrhages in alveoli and interalveolar septa alongwith congestion in blood vessels were observed after different intervals of cadmium exposure. These two changes might have appeared due to the break in the wall of the vessel or by escape of red blood cells by a process of diapedesis. Cadmium has been proved by Gabbiani, *et al*²⁴⁾ to have degenerative effect on endothelium of blood vessels, and, therefore, it is suggested that the possibility of appearing these changes might have occurred due to this effect. Cadmium intoxication revealed thickening of interalveolar septa after 7 weeks. It has been observed that organization of haemorrhage could have yielded fibrous scars, which might have resulted in thickening of alveolar spaces by organized connective tissue. Christensen and Olsen²⁵⁾ have observed thickening of alveolar septa. Present observation of thickened alveolar septa is in agreement with these authors.

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