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Some Biochemical and Histopathological Changes Induced by Polyvinyl Chloride Dust in Rat Lung

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Enzymatic and pathomorphologic alterations in rat lungs were studied at different time intervals up to 180 days after a single intratracheal administration of 25 mg of polyvinyl chloride dust. The activities of two energy-linked enzymes, succinic dehydrogenase (SDH) and adenosine triphosphatase (ATPase), and three lysosomal enzymes, acid phosphatase, β-glucuronidase, and ribonuclease, were significantly increased in the early period and then started to decline. The activities of SDH and ATPase reached control values at 150 days, while those of the lysosomal enzymes remained significantly higher up to this period. Histopathologically, the pulmonary response was in the form of acute inflammatory changes during the early stages of dust burden, followed by the development of granulomatous lesions containing small amounts of stromal elements.

INTRODUCTION

Polyvinyl chloride (PVC) is widely used for containers, wrapping films, electrical insulation, pipes, conduits, biomedical devices, and a variety of other industrial and consumer products. Due to its inertness to a large variety of chemicals and poor absorption through the gastrointestinal tract and skin, this polymer is generally considered to be nontoxic. However, during the last few years, dermatitis and allergic responses (Weichardt, 1970), bronchial asthma (Dernehl, 1963; Vanhouten and Cudworth, 1974), acro-osteolysis with scleroderma, and Raynaud's phenomenon (Wilson *et al.*, 1967) have been reported in workers handling PVC. Recent reports of hepatic disorders, including angiosarcoma of the liver due to the monomer, vinyl chloride (Marsteller *et al.*, 1973; Creech and Johnson, 1974; Falk *et al.*, 1974; Makk *et al.*, 1974) and cases of still births, miscarriages, and malformations among the workers engaged in the polymerization of vinyl chloride (Selikoff, 1974; Infante *et al.*, 1976), have aroused great interest in the toxicological properties of PVC.

Reports of the occurrence of pulmonary dysfunction and a new form of pneumoconiosis in PVC workers (Szende *et al.*, 1970; Miller *et al.*, 1975) and dust-exposed animals (Bogdan, 1972; Fronzia *et al.*, 1974) point to the need for further studies on the toxicological properties of the polymer.

MATERIALS AND METHODS

Animals and plan of experiment. Female hooded rats (200 ± 10 g) from the Industrial Toxicology Research Centre colony maintained on an *ad libitum* pellet diet and under standard husbandry conditions were divided into two groups. The animals of group I were injected intratracheally with 25 mg of PVC dust suspended in 1.0 ml of normal saline and those of group II received an equal volume of saline to serve as control. The particle size of the dust, prepared as described by Zaidi

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(1969), was below 5 μm. Six treated and four control rats were sacrificed at the desired time intervals, and the lungs were removed immediately and processed for enzyme estimations and histopathological study. The lungs of the animals that died spontaneously during the course of the experiment were processed only for histopathological studies.

Biochemical studies. Suitable portions of the right lung were minced and homogenized in chilled isotonic sucrose (0.25 M) or sucrose (0.25 M)–EDTA (0.001 M) with a Potter–Elvehjem-type homogenizer to yield 10% (w/v) or 4% (w/v) homogenates, respectively, for the enzyme estimations. In some experiments, a portion of 4% (w/v) homogenate was treated with Triton X-100 (final concentration, 0.1%) to release the latent activity of the lysosomal enzymes (de Duve, 1964).

The activities of succinic dehydrogenase (SDH, succinate (acceptor) oxidoreductase; EC 1.3.99.1) and adenosine triphosphatase (ATPase, ATP phosphohydrolase; EC 3.6.1.3) were measured by the methods of Slater and Bonner (1952) and Seth and Tangari (1966), respectively. Estimation of acid phosphatase (orthophosphoric monoester phosphohydrolase; EC 3.1.3.2) was carried out according to Oser (1965), of ribonuclease (RNase, polyribonucleotide-2-oligonucleotide transferase (cyclizing); EC 2.7.7.16) according to McDonald (1955), and of β-glucuronidase (β-D-glucuronide glucuronohydrolase; EC 3.2.1.31) according to Fishman (1967).

Histopathological studies. The left lung was fixed in 10% formol–saline by injecting the fixative through a small raminent of the major airway. It was then dehydrated and embedded in paraffin, and 5 μm sections were prepared. The sections were stained with hematoxylin–eosin or impregnated with silver (Gordon and Sweets, 1936).

Chemicals. All the chemicals used in this study were of Analar grade obtained from BDH or E. Merck, except phenolphthalein–β-glucuronide, which was obtained from Schwarz/Mann. Polyvinyl chloride sample grade GEON-121 was gifted by Chemicals and Plastics India Ltd., Madras.

RESULTS

No significant difference was observed in the rate of mortality of control and PVC-administered rats.

Biochemical Studies

Table 1 shows the effects of PVC dust on the activities of SDH and ATPase in lung homogenates. The activities of the two enzymes exhibited a sudden increase at 15 days and a further increase at 30 days; they then started to decline and attained normal levels at 150 days. Although the two enzymes followed a similar pattern of change, SDH showed a greater sensitivity to the PVC dust at all time intervals.

Table 2 shows the effect of PVC dust on acid phosphatase, β-glucuronidase, and ribonuclease. The activities of these enzymes were increased in treated animals and acid phosphatase and β-glucuronidase reached their maxima at 60 days, while ribonuclease reached a maximum at 30 days. Later, the enzymes exhibited decreases, but their activites remained significantly above the control values up to 150 days. The data summarized in Table 3 show that no significant alterations

TABLE 1
EFFECT OF PVC DUST ON SUCCINIC DEHYDROGENASE AND ADENOSINE TRIPHOSPHATASE ACTIVITY OF RAT LUNG[a]

Days	Succinic dehydrogenase (nmole of $K_3Fe(CN)_6$ reduced/min/g of fresh tissue)	Percentage increase	Adenosine triphosphatase (nmole of P_i liberated/min/g of fresh tissue)	Percentage increase
0	207 ± 8 (201 ± 3)	2.9	450 ± 40 (442 ± 6)	1.8
15	253 ± 23**** (131 ± 35)	93.1	331 ± 15** (230 ± 16)	43.9
30	285 ± 9** (131 ± 35)	117.5	354 ± 13* (230 ± 16)	53.9
60	240 ± 10*** (131 ± 35)	83.2	411 ± 7**** (331 ± 26)	24.1
90	236 ± 19**** (158 ± 22)	49.3	433 ± 23**** (362 ± 17)	19.6
120	229 ± 23 (183 ± 5)	25.1	396 ± 25 (350 ± 22)	13.1
150	158 ± 13 (144 ± 18)	9.7	383 ± 66 (355 ± 8)	7.8
180	213 ± 7 (201 ± 3)	5.9	444 ± 13 (442 ± 6)	0.4

[a] All the values are means ± SE for six observations in experimental and four observations in control groups. Values in parentheses indicate their respective controls. Statistical significance was evaluated by Student's t test. * $P < 0.001$; ** $P < 0.01$; *** $P < 0.02$; **** $P < 0.05$.

TABLE 2
EFFECT OF PVC DUST ON LYSOSOMAL ENZYMES OF RAT LUNG[a]

Days	Acid phosphatase (nmole of P_i liberated/min/g of fresh tissue)	Percentage increase	β-Glucuronidase (nmole of phenolphthelein liberated/min/g of fresh tissue)	Percentage increase	Ribonuclease (ΔO.D./min/g of fresh tissue)	Percentage increase
0	542 ± 30 (523 ± 51)	3.6	525 ± 27 (541 ± 82)	—	1.36 ± 0.26 (1.40 ± 0.24)	—
15	937 ± 78** (818 ± 21)	14.5	316 ± 13* (211 ± 14)	49.7	2.54 ± 0.05* (1.15 ± 0.05)	120.8
30	1284 ± 78* (818 ± 21)	56.9	386 ± 11* (211 ± 14)	82.9	2.61 ± 0.06* (1.15 ± 0.05)	126.9
60	1160 ± 30* (597 ± 35)	94.3	577 ± 26* (273 ± 22)	111.3	2.35 ± 0.23** (1.26 ± 0.18)	86.5
90	1140 ± 32* (681 ± 29)	67.4	1038 ± 23* (518 ± 28)	100.3	2.12 ± 0.16*** (1.45 ± 0.15)	46.2
120	1120 ± 30* (681 ± 29)	64.4	1427 ± 22* (716 ± 22)	99.3	2.05 ± 0.05* (1.37 ± 0.07)	49.6
150	538 ± 25** (406 ± 14)	32.5	447 ± 26* (248 ± 11)	80.2	2.22 ± 0.12* (1.55 ± 0.05)	43.2
180	763 ± 52 (597 ± 35)	27.8	840 ± 34 (634 ± 82)	32.4	2.00 ± 0.15 (1.40 ± 0.24)	42.8

[a] All the values are means ± SE for six observations in experimental and four observations in control groups. Values in parentheses indicate respective controls. Statistical significance was evaluated by Student's t test. * P < 0.001; ** P < 0.01; *** P < 0.02

TABLE 3
ACTIVATION OF LYSOSOMAL ENZYMES IN THE NORMAL AND PVC-TREATED RAT LUNG[a]

Day	Acid phosphatase	β-Glucuronidase	Ribonuclease
0	2.36 ± 0.43 (2.39 ± 0.39)	1.36 ± 0.09 (1.18 ± 0.07)	1.26 ± 0.12 (1.25 ± 0.08)
15	1.53 ± 0.03 (1.59 ± 0.20)	2.60 ± 0.05 (2.51 ± 0.08)	1.07 ± 0.01 (1.25 ± 0.08)
30	1.36 ± 0.09 (1.59 ± 0.20)	2.44 ± 0.09 (2.51 ± 0.08)	1.16 ± 0.01 (1.25 ± 0.08)
60	1.15 ± 0.02 (1.23 ± 0.07)	2.10 ± 0.09 (1.87 ± 0.09)	1.20 ± 0.02 (1.23 ± 0.04)
90	1.16 ± 0.01 (1.19 ± 0.08)	1.25 ± 0.01 (1.17 ± 0.06)	1.16 ± 0.01 (1.44 ± 0.13)
120	1.70 ± 0.07 (1.57 ± 0.08)	1.08 ± 0.02 (1.04 ± 0.02)	1.27 ± 0.03 (1.28 ± 0.05)
150	1.38 ± 0.10 (1.43 ± 0.09)	1.11 ± 0.02 (1.19 ± 0.09)	1.38 ± 0.04 (1.14 ± 0.09)
180	1.89 ± 0.05 (1.59 ± 0.20)	1.16 ± 0.01 (1.18 ± 0.02)	1.25 ± 0.03 (1.25 ± 0.08)

[a] Values are means ± SE for six observations in experimental and four observations in control groups, indicating the ratio of enzyme activity before and after Triton X-100 treatment. Figures in parentheses indicate corresponding controls.

were observed in the latent activities of the enzymes in experimental and control rats upon treatment of the homogenates with Triton X-100.

Histopathological Studies

The microscopic study of the tissue sections of the lungs of dead animals or those sacrificed at various time intervals revealed that 3 days after the inoculation of dust suspension, the dust deposits were situated in various segments of the airways, predominantly in the terminal and respiratory bronchioles (Fig. 1). Besides these locations, the dust was discernible in the alveolar ducts and attached to the membranes of the alveoli bulging from the latter structures. Congestion of blood vessels in the alveolar septa, leakage of erythrocytes, presence of edematous fluid in the alveolar walls or alveolar space, and hemosiderosis were also seen.

At 15 days after the inoculation, vascular and inflammatory changes persisted, as at the earlier period. However, the dust deposits seen as translucent foamy material were situated predominantly in the alveolar portion of the pulmonary tissue drained by the alveolar ducts. The cellular response to the presence of dust in the lungs was in the form of cellular hyperplasia at the periphery of the dust masses. Alveoli contained macrophages laden with dust (Fig. 2). In the neighborhood of dust loci there were focal areas of interstitial fibrosis due to the proliferation of histiocytes and reticulin fibers. Transport of the dust by lymphatics was made evident by the presence of dust within tissue spaces, situated on the periphery of bronchioles (Fig. 3).

Encroachment of the dust masses by multinucleated cells was evident at 30 days. Many of the cells were heavily laden with PVC dust in their cytoplasm (Fig. 4). At 60 days, the granulomatous lesions containing the dust-filled multinucleated

FIG. 1. Section of the lung of a rat which died spontaneously 3 days after intratracheal inoculation of PVC dust. The dust deposits are seen predominantly in the proximal alveoli budding from the respiratory bronchiole. Hematoxylin and eosin. ×135.

cells, lymphocytes, and a few macrophages were surrounded by a zone of fibroblasts. The lesions were diffusely distributed and the stroma were composed of reticulin and collagen fibers. The lumens of the airways still contained erythrocytes and the epithelia of these structures were detached from the underlying

FIG. 2. Section of the lung of a rat sacrificed 15 days after intratracheal inoculation of PVC dust showing compaction of alveolar lumen with dust-laden macrophages. Hematoxylin and eosin. ×540.

FIG. 3. Difference low-power microscopic field of the same section as shown in Fig. 2. The distal alveoli show some degree of interstitial fibrosis, hyperplasia of microphages, and the presence of dust in some tissue spaces, presumably lymphatics. Hematoxylin and eosin. ×135.

lamina propria. The interalveolar septa were thickened and contained a network of argyrophilic fibers. The dust was found to have been encased in cellular envelopes, as at 60 days, and without the penetration of the argyrophilic fibers into its substance (Fig. 5).

At 90 days the multinucleated cells were less frequently seen to invade the substance of the dust masses. The general cellular reaction at this time interval was similar to that observed at 60 days. The subsequent development of the lesions did not progress beyond this stage; instead, there seemed to be a tendency to clear out the entrapped dust. This was evident from the presence of dust within the cytoplasm of macrophages and the lumen of bronchioles. In many cases the absence of dust in the lung sections may have been due to the efficient clearing mechanism of these animals.

DISCUSSION

The present results indicate that a single intratracheal administration of PVC dust leads to marked biochemical and histopathological alterations.

Increases in the activities of oxidative enzymes in the lungs of the experimental animals have been reported in the presence of silica (Kilroe-Smith and Breyer, 1963) and asbestos (Beg *et al.*, 1973). It may be possible that some dusts induce metabolic changes in tissues and to cope with the energy demands, the activities of these enzymes are increased. The increases in the activities of the two energy-linked enzymes, SDH and ATPase, suggest a similar effect during the early phase of the exposure of the lung to PVC dust. The lysosomal enzymes showed a pattern similar to that of the oxidative enzymes in treated rats. The activities of all three

FIG. 4. Section of the lung of a rat sacrificed 30 days after the intratracheal inoculation of PVC dust. The alveolar lumen contains multinucleated cells heavily laden with PVC dust. Hematoxylin and eosin. ×540.

FIG. 5. Section of the lung of a rat sacrificed 60 days after intratracheal inoculation of PVC dust. Highly granulomatous lesion contains within its substance an aggregate of dust mass. Hematoxylin and eosin. ×135

lysosomal enzymes studied in the present experiments increased gradually and then decreased. However, in contrast to SDH and ATPase, the levels of the lysosomal enzymes remained significantly higher than the controls up to 150 days of the experiment. Histopathological changes also exhibited a more or less similar pattern. There were acute inflammatory changes, phagocytosis, and encasement of the dust without significant stromal proliferation. The sequestration of the dust masses and a relatively lesser degree of phagocytosis may be responsible for the lower levels of lysosomal enzymes during the later periods of the experiment.

Certain toxic agents, biochemical stimuli, and inflammation are reported to cause lysosomal fragility (Allison *et al.*, 1966; Allison, 1967). Alterations in the activity of acid phosphatase, β-glucuronidase, and ribonuclease in the present study suggest an action of dust on lysosomes. Cytotoxicity of PVC implants (Pelling *et al.*, 1973), a degenerative effect on peritoneal and alveolar macrophages (Salthouse *et al.*, 1973), and inhibition of cell growth in culture with a clumping of lysosomes (Dehaan, 1971; Grasso *et al.*, 1973) strengthens this assumption. The increases in the activities of lysosomal enzymes may be due to the labilization of lysosomes, as observed with asbestos dust (Viswanathan *et al.*, 1973), or to their increased permeability to the substrates. There were no significant alterations in the ratio of the enzyme activities before and after Triton treatment between control and experimental animals, indicating that PVC dust does not increase the enzyme activity by disrupting the lysosomes. Thus, the increased permeability of lysosomal membranes to their substrates or increases in lysosome content, as observed with aflatoxins (Pokrovsky *et al.*, 1972), or increases in the activity per se may be responsible for the observed change in the activities of acid phosphatase, β-glucuronidase, and ribonuclease in PVC-treated animals. Further studies are needed at the molecular and ultrastructural levels to delineate the *locus operandi* of membrane damage by the polymer.

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