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Pneumoconiosis in Animals Exposed to Poly(vinyl Chloride) Dust

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Rats, guinea pigs and monkeys were exposed by inhalation (6 hr/day, 5 days/week) for up to 22 months to a 13 mg/m^3 concentration of PVC dust. Autopsies on rats and guinea pigs were performed after 12 months of exposure and on monkeys after 22 months of exposure. Lung function tests were performed on monkeys after 9, 14 and 22 months of exposure. Aggregates of alveolar macrophages containing PVC particles were found in the lungs of all animals. These aggregates were more numerous in the monkey lungs. No fibrosis or significant cellular infiltrates were present in or near these cellular aggregates. No significant effects on pulmonary function could be demonstrated in the monkeys exposed to PVC. Under the conditions of this experiment, inhaled PVC produced a benign pneumoconiosis.

Introduction

Approximately seven billion pounds of poly(vinyl chloride) (PVC) were produced in the United States in 1979. There are two major types of PVC production processes, suspension polymerization and emulsion polymerization. The latter process produces particles which are respirable and much smaller than those in the former process. These polymers of vinyl chloride are usually compounded with a variety of ingredients (e.g., plasticizers, light and heat stabilizers, pigments and fillers) and processed in several different ways to produce thousands of end products in common use in our society. About 40% of PVC is used to make sewage pipes, water pipes and conduits. It is also used to make construction siding, window sashes, electrical wire and cable insulation, packaging films (for meat, etc.), vinyl floor tile, wall coverings, phonograph records, shower curtains, bottles, fabrics for clothes, furniture, automotive parts and many other products.

Chest x-ray abnormalities, respiratory dysfunction and pulmonary granulomas have been reported in workers exposed to PVC dust during PVC manufacturing and fabrication (*1–6*). Consequently, experimental studies were initiated in 1975 to study the effects of inhaled PVC dust in animals.

Materials and Methods

Test Material

Ten pounds of PVC (trade name Geon 121) were obtained from Goodrich Chemical Company and used in this experiment. The manufacturer's specifications stated a particle size range of 0.5–1.5 μm. Electron microscopic examinations in this laboratory confirmed that at least 90% of the particles were less than 1.5 μm in diameter. The PVC was stored in its covered, fiberboard shipping container throughout the duration of the study.

Dust Generation and Measurements

PVC powder was packed daily or every other day into a Wright dust feeder at a pressure of 3000

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lb/in.^{2}. The generated dust was fed into the mainstream of the inhalation exposure chamber intake air supply and through a static eliminator before entering the 160 ft^3 chamber. Airflow through the chamber was maintained at 40 ft^3/min with a negative chamber pressure of 0.2 in. water. Animals were exposed to PVC dust 6 hr/day, 5 days/week for up to 22½ months.

Four chamber dust samples were collected each day by drawing chamber environment air, at a rate of 10 liters/min for 20 min, through DM 5 μm pore size Metricel filters with a vacuum pump. In addition, simultaneous samples consisting of particles with an aerodynamic diameter ≥ 7 μm (respirable fraction) were collected with a 10-plate, horizontal elutriator. All samples were weighed immediately after each sampling period and the chamber dust concentrations calculated. Adjustments in the dust generating system after each sampling period were made when necessary to maintain a concentration approximating 10 mg of respirable dust/m^3 of air. Daily and grand means (derived from averaging the daily means) for the total and respirable dust fractions were calculated.

Midway through the experiment, two 6-hr chamber air samples were collected on charcoal tubes while PVC dust was being generated. Those two samples and the bulk PVC were analyzed for vinyl chloride by gas chromatography. Samples of dust that were allowed to settle on Formvar-coated copper grids placed in the chamber during the PVC dust generation were examined by transmission electron microscopy.

Animals

Animals used in this study were male, cesarean-derived, Sprague-Dawley rats (Laboratory Supply Company, Inc., Indianapolis, Indiana); male, Hartley guinea pigs (Sweetwater Farms, Hillsboro, Ohio); and imported, adult, male Cynomolgus monkeys (Primate Imports Corp., Long Island, New York). The treatment and control groups each contained 80 rats, 40 guinea pigs and 10 monkeys. Because of difficulties in obtaining imported monkeys from the supplier, the control group of monkeys was received several months before the exposed group of monkeys. They were not randomly assigned, because the control group was also used for the control group in another ongoing experiment. Consequently, at time of first exposure, the mean weight of the control monkeys was 4219 and that of the exposed group 3361 g. In the ensuing 22½ months, the controls gained 552 g in weight, whereas, the exposed group gained 2116 g.

The rats and guinea pigs were quarantined for two weeks and the monkeys were quarantined for one month prior to the initiation of the inhalation exposures. Stainless and galvanized steel open wire mesh cages were used as exposure caging to provide adequate distribution of the dust aerosols within the exposure chambers. All of the animals in the study were individually marked by toe-clipping or tattoo. All three species were individually housed during the 6-hr exposures, whereas the rats and guinea pigs were housed two to four animals per cage at all other times. Control animals were housed in similar cages in separate animal quarters and exposed to filtered air 24 hr/day. The exposed animals were also housed in the animal quarters except during the 6-hr inhalation exposure. Rats, guinea pigs, and monkeys were fed standard laboratory pellet diets (Rodent Laboratory Chow, Guinea Pig Chow, and Monkey Chow-Jumbo from Ralston Purina, St. Louis, Missouri). Monkeys were given fresh fruit (oranges, bananas or apples) twice a week. Tap water was available *ad libitum* except during the exposure period. Food was available to the rodents at all times except during exposure. The monkeys were fed once daily at the cessation of the exposure period.

Pathology

Within one day after the last exposure day, all the surviving rats and guinea pigs were autopsied. Their lungs were inflated with 10% buffered formalin and sections of liver, spleen, heart, kidney, pancreas, adrenals, thyroid, testis and urinary bladder from each animal were fixed in 10% buffered

Table 1. Results of pulmonary function tests in monkeys after 14 months exposure to PVC dust.[a]

Pulmonary function test	Groups	
	Control	Exposed
RL, cm H_2O/l./sec	12.1 ± 4.0	13.8 ± 3.9
CL, ml/cm H_2O	22.9 ± 10	17.5 ± 7.5
TCL, ml	357 ± 39	360 ± 75
VC, ml	334 ± 36	339 ± 73
IC, ml	185 ± 19	210 ± 58
RV, ml	40 ± 10	36 ± 11
RV/TCL, %	11.3 ± 2	9.9 ± 2
FeV 0.5/FVC, %	87.7 ± 5.3	86.5 ± 8.1
PF, ml/sec	943 ± 91	960 ± 139
FEF 50%, ml/sec	920 ± 91	905 ± 138
FEF, 25%, ml/sec	648 ± 173	655 ± 235
FEF 10%, ml/sec	262 ± 114	269 ± 134
CV, ml	21 ± 9	21 ± 11
ΔN_2/100 ml	1.05 ± 0.3	0.67 ± 0.4
Viso V, ml	28.7 ± 19	19.9 ± 14.3

[a]Data are presented as means ± one standard deviation for each of the parameters.

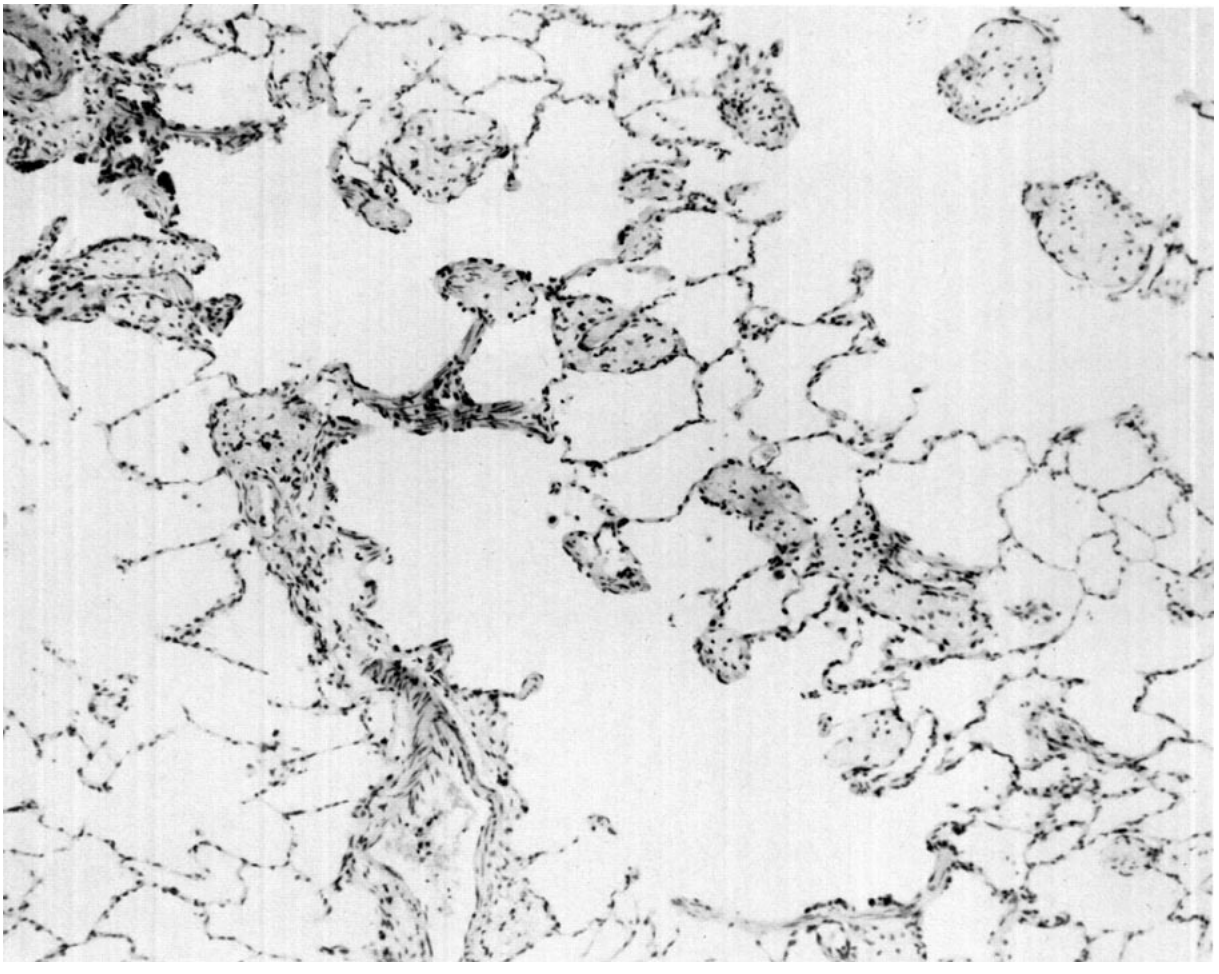

FIGURE 1. Macrophage aggregates in lung of monkey exposed to PVC dust for 22 months. Aggregates are primarily in respiratory bronchioles and alveolar ducts. (Original magnification 40×).

formalin. Hematoxylin and eosin stained sections of each lobe of each lung and of each of the above tissues were prepared for and examined by light microscopy. Within 48 hr of the last exposure day, all surviving monkeys were autopsied and sections of the same aforementioned tissues plus tracheobronchial and mesenteric lymph nodes, prostate, stomach, duodenum and skin were fixed and processed in the same manner as those for the rodents with the exceptions that two sections from each lobe of the lungs were examined, and some of the lung tissue was processed for examination by transmission electron microscopy.

Pulmonary Function

Before onset of exposures and after 9, 14 and 22½ months of exposure to PVC dust, the following pulmonary function tests were performed on fasted, anesthetized (pentobarbital anesthetic) exposed and control monkeys: total lung capacity (TLC), vital capacity (VC), inspiratory capacity (IC), residual volume (RV), forced expiratory volume in 0.5 sec ($FeV^{0.5}$), peak expiratory flow (PF), maximum expiratory flow volume (FEF) at 50%, 25% and 10% of vital capacity, resistance (RL), compliance (CL), closing volume (CV), N_2 washout (ΔN_2/100 ml) and volume of isoflow (VisoV). These tests were performed with a variable pressure, whole-body plethysmograph. Means and standard deviations for all parameters in each group were calculated at each interval and comparisons made between groups using parametric and nonparametric statistical analyses.

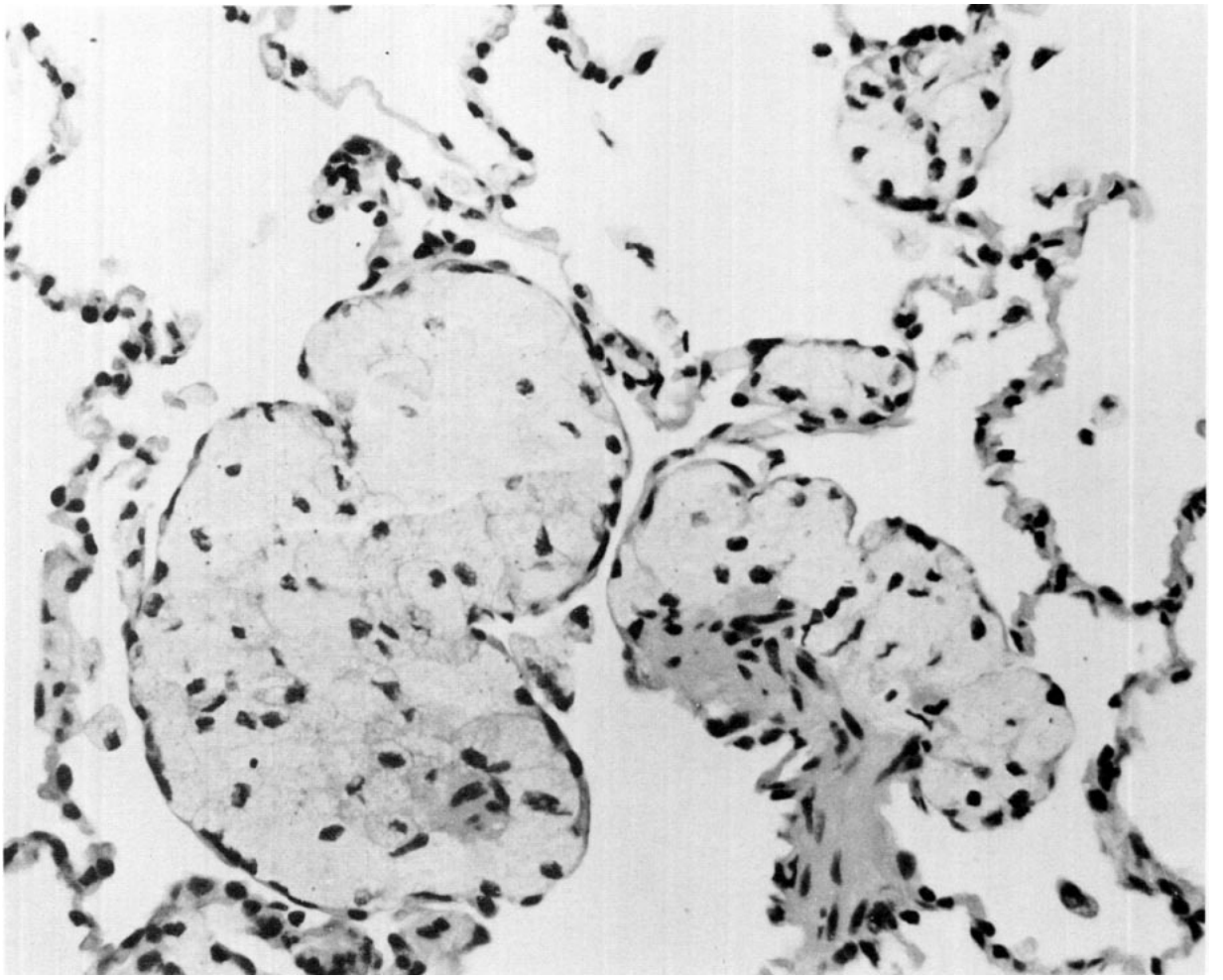

FIGURE 2. Macrophage aggregates in lung of monkey exposed to PVC dust for 22 months. (Magnification 385×).

Results

The mean total dust concentration in the chambers for the rodents was 13.0 mg/m^3 (SD = 2.34) and for the monkeys 12.9 mg/m^3 (SD = 4.69). The mean respirable dust concentration was 10.4 mg/m^3 for both the rodents and the monkeys. The monkeys were exposed on 464 days for a total of 2818 hr, while the rodents were exposed on 245 days for a total of 1426 hr.

The vinyl chloride concentrations in the chambers at the two sampling periods were 0.01 and 0.02 ppm. A small nonquantified amount of vinyl chloride was detected in the bulk PVC.

Electron micrographs of the settled chamber dust showed individual particles ranging from 0.13 to 1.68 μm in diameter and agglomerates measuring up to 12.8 μm in diameter. Although the agglomerates measuring greater than 7 μm in diameter accounted for only 2.3% (5/222) of all the particles counted, they were estimated to account for more than 20% of the mass.

Pulmonary Function

A summary of the extensive pulmonary function evaluations indicated some signs of loss of lung recoil pressure, probably a result of the animals' aging process. In most cases, differences were noted during the second and third testing periods (exposure months 9 and 14) and were indicative of some small airway obstruction. At this time, however, these differences were not statistically significant (see Table 1). At the last evaluation (month 22) compared with baseline (preexposure) data, there were no significant differences for any parameter tested.

Because of the differences in animal sizes be-

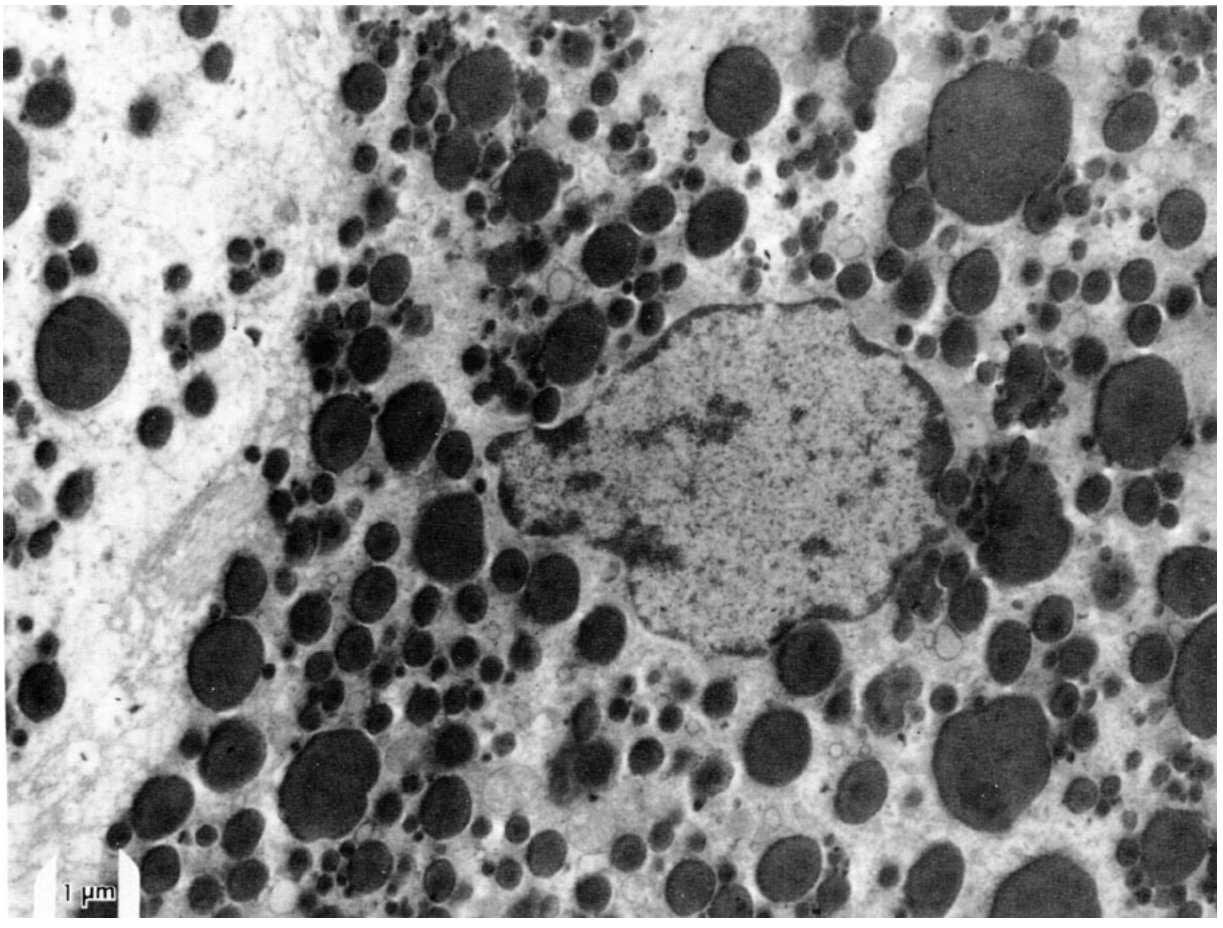

FIGURE 3. Transmission electron micrograph of a macrophage from the lung of a monkey exposed to PVC dust for 22 months. Note the numerous round and oval, electron dense particles comprising most of the cytoplasmic space. These are the inhaled PVC particles.

tween the control and exposed groups for all except the 14-month interval, meaningful comparisons could be made only at that time period. At that time, the mean weight of the controls was 4470 g and that of the exposed animals was 4190 g. The results appear in Table 1. No statistically significant differences existed. Impairment of respiratory function does not appear to be indicated under the conditions of this study from exposure to respirable PVC dust.

Pathology

Ten exposed and ten control monkeys were autopsied 22½ months after initiating exposures. No gross abnormalities were seen in the lungs or other organs that could be related to the exposures. Light microscopic examination of the lung sections of exposed animals revealed macrophage aggregates (Fig. 1 and 2) in the alveolar walls, alveoli, alveolar ducts, respiratory bronchioles and around veins. The majority of the aggregates were 100-200 μm in diameter and some were as large as 475 μm in diameter. Their concentration varied from three to seven per microscopic field at 100 × magnification. The tightly packed, large, spherical macrophages contained colorless cytoplasm which appeared light blue under phase contrast microscopy. Only a rare aggregate contained any other type of infiltrating cells, which usually consisted of a few polymorphonuclear leukocytes. No other lesions that could be related to experimental treatment were present, and no metaplasia, interstitial fibrosis, nodular fibrosis or pneumonitis were present. The tracheobronchial lymph nodes contained aggregates of the same type of macrophages described above in the lungs. Transmission electron microscopic examination of the macrophage aggregates revealed numerous round particles that do not normally occur in macrophages

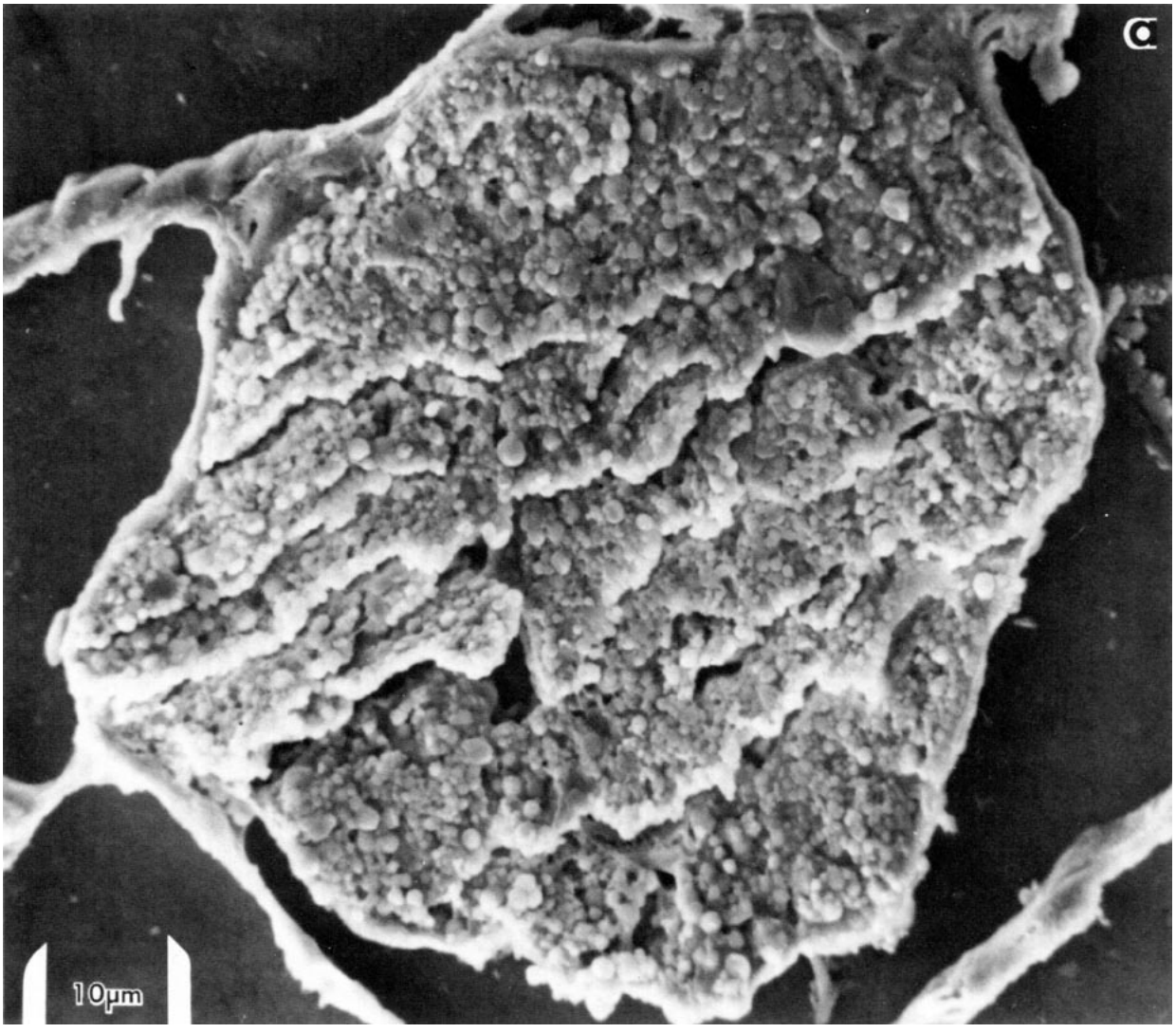

FIGURE 4. Scanning electron micrograph of a 5-μm section of lung of monkey exposed to PVC dust for 22 months. This photo shows the numerous PVC particles which obscure the cellular outlines of the macrophages in which they are residing.

and were of the same size and shape as PVC particles (Fig. 3). Analysis of these particles with the microprobe revealed high concentrations of chlorine (Fig. 4 and 5), thus, further establishing their identity as PVC particles. A few aggregates of birefringent particles appeared in the lungs and high concentrations in the lymph node of both the exposed and control monkeys. These particles were identified as mica, kaolin and quartz by the use of microprobe, electron and x-ray diffraction methods. The bulk PVC did not contain these minerals. No macrophage aggregates were present in the control monkey lungs. No treatment related lesions were seen in sections of the other organs examined.

The 57 exposed and 64 control rats were autopsied 12 months after initiating exposures. Microscopic examination of the lungs of the exposed animals revealed 0–3 macrophage aggregates/microscopic field at 100× magnification (Fig. 6). These aggregates were smaller and much less frequent than those seen in the monkey lungs. Chronic pneumonitis, typical for rats, was present with equal severity and frequency in the control and exposed rats. No treatment related lesions were seen in sections of the other organs examined.

The 36 exposed and 39 control guinea pigs were autopsied 12 months after initiating exposures. Light microscopic examinations of the lungs of exposed guinea pigs revealed fewer collections of giant macrophages with clear foamy cytoplasm than those

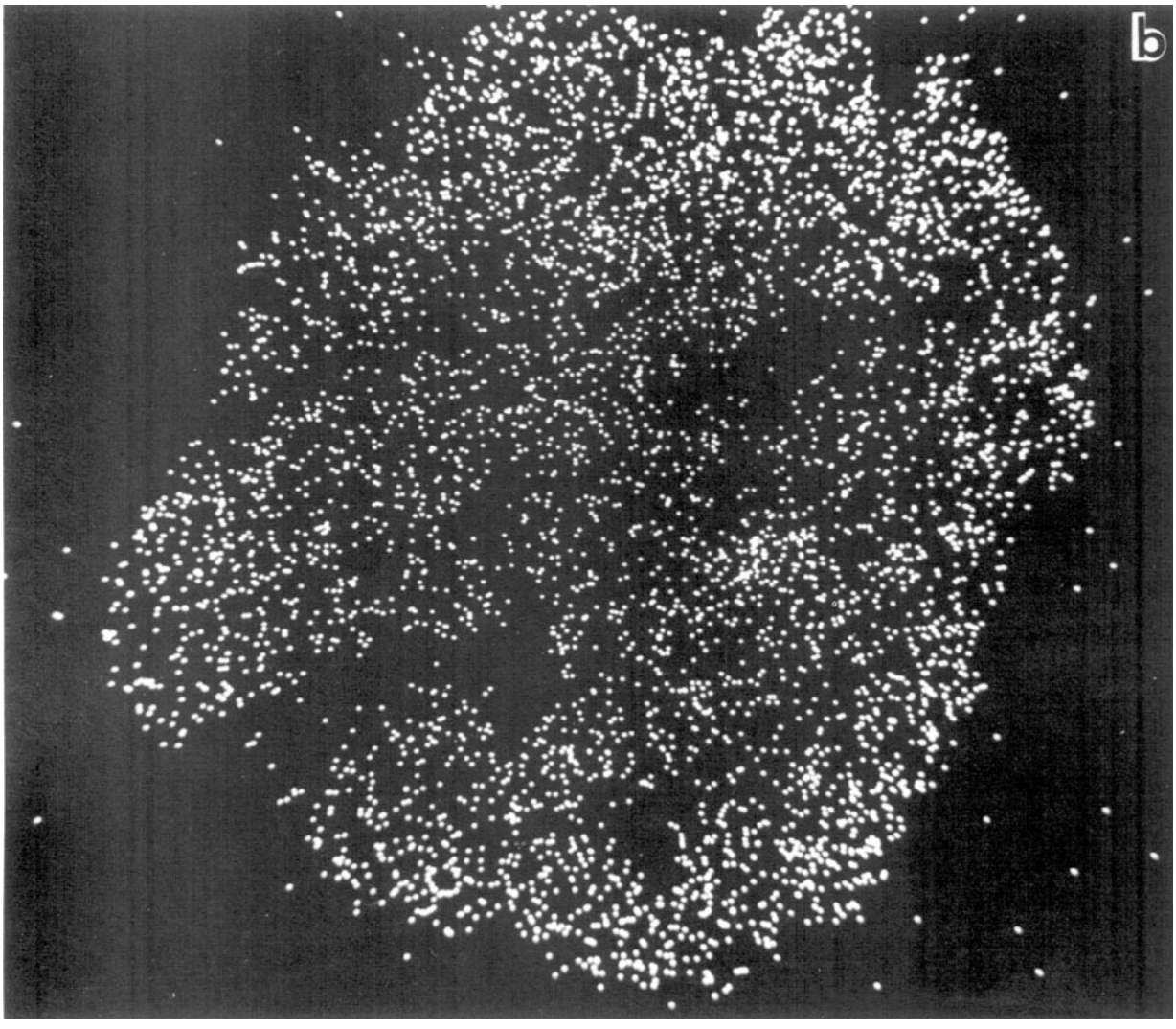

FIGURE 5. Chlorine x-ray map of the same area and magnification depicted in Fig. 4. The white dots indicate the presence of the element chlorine. Note that the dots are clustered in the same area as the PVC particles, thus confirming the identification of the PVC particles.

seen in the exposed monkeys or rats. Interstitial lymphocytic pneumonia was a constant finding in most of the exposed and control animals, and the severity and incidence were not related to treatment. Few macrophage aggregates similar to those seen in the lungs were present in the tracheobronchial lymph nodes of the exposed guinea pigs. No treatment-related lesions were seen in sections of the other organs examined.

Discussion

The monkeys in this study developed a benign, simple pneumoconiosis resulting from the inhalation of PVC dust at a mean concentration of 13 mg/m^3 for 464 days (2818 hr). The cellular response visible at 22 months of exposure consisted of PVC-laden macrophages aggregated into clusters in the lungs. On the other hand, the lungs of the rats and guinea pigs contained very few macrophage aggregates and in a much lower concentration than in the monkey lungs. This difference in degree of cellular response could possibly be explained on the basis of fewer PVC particles being deposited in the lungs of these rodents.

Rodents at rest in the chambers during the day breathe exclusively through their noses. Monkeys under the same conditions do not. The rodents in this experiment had extensive bronchitis and

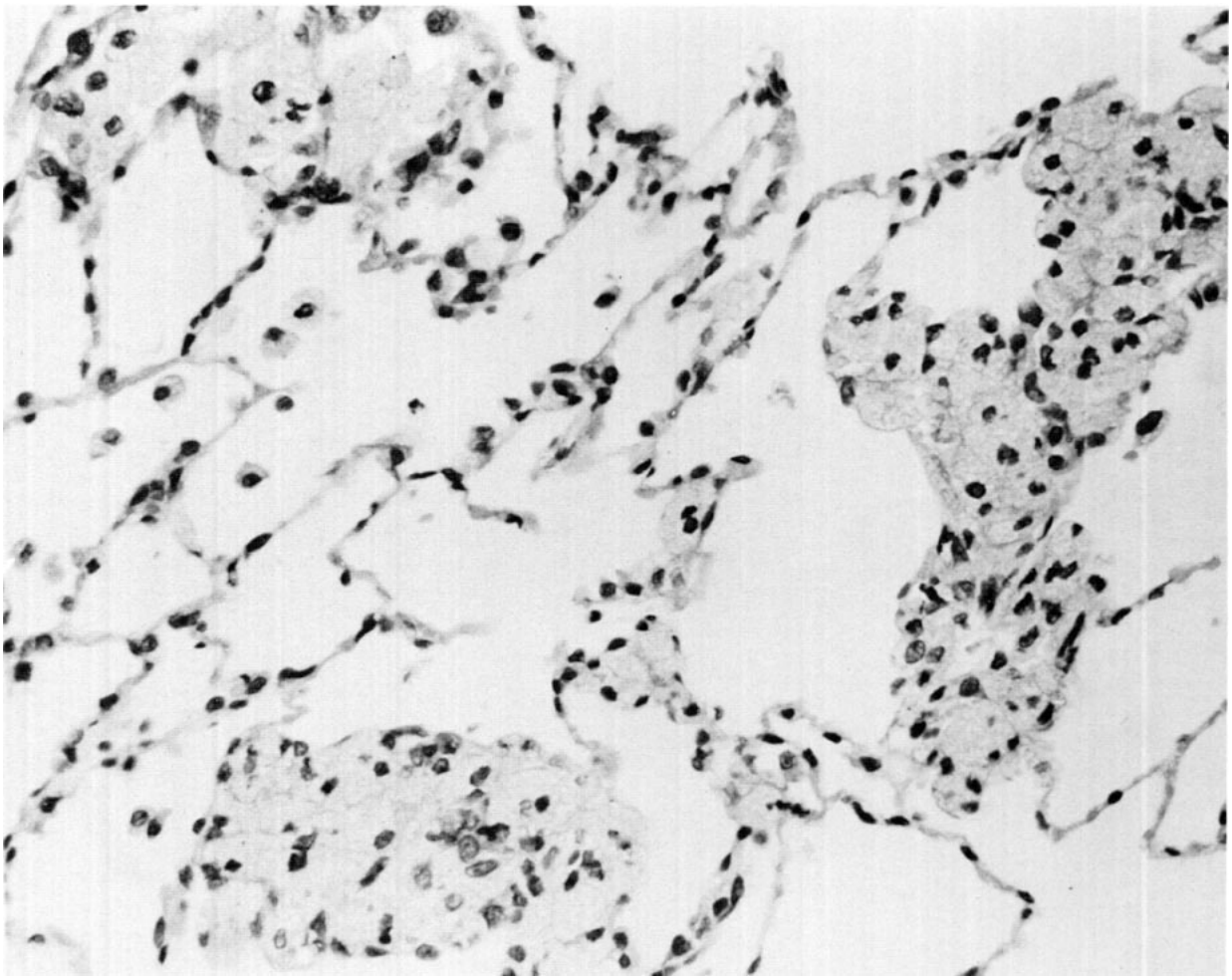

FIGURE 6. Macrophage aggregates in the lung of a rat exposed to PVC dust for 12 months. Note vacuolated appearance of cells. (Magnification 385×).

pneumonitis, whereas the monkeys did not. Both nose breathing and bronchitis are factors which reduce the percentage deposition of inhaled particles in the alveolar regions of the lungs.

Two studies have been published on the pathological effects of inhaled PVC in rats (*7*, *8*). In one study (*7*) rats and guinea pigs were placed in a PVC bagging facility. Two guinea pigs and an unstated number of rats were autopsied 2, 4 and 7 months after their continuous residence in the facility. Although the animals developed pulmonary diseases, the small number of animals studied and the lack of adequate controls makes it impossible to assess the significance of the exposure to PVC. In the other study (*8*) rats exposed to high concentrations (97 gm/m^3) of PVC dust in a static air chamber for 1 hr/day for up to 12 months developed bronchiectasis, emphysema, purulent pneumonia, lung abscesses and squamous metaplasia in bronchial epithelium. Although the control rats were free of these diseases, there were only 10 controls examined. In view of the propensity for rodents (controls) to contract acute and chronic pulmonary diseases, the experimental conditions under which control animals are kept is extremely important, and should be stated in the protocol. No mention of this was made in that experiment.

Two cases reports showing granulomas in lungs of two PVC workers have been reported (*3*, *5*). Except for the more closely packed macrophages in the lesions shown by Aranaud et al. (*5*), the morphology of the lesion is similar to that seen in our exposed monkey lungs. Part of the compactness of the cells in their lesion could be explained on the basis of artifactual compression caused during the taking of the lung biopsy. No excess collagen was

apparent in the light microscopic pictures of the granulomas they showed in their article, although they did refer to their case as a "case of discrete pulmonary fibrosis." Their electron microscopic pictures of the PVC particles in *in vitro* cell systems were very similar to what we saw in the macrophages in the monkey lungs. The PVC particles they showed in a macrophage from the lung biopsy appear to be clustered in lysosomes and the individual particles are not as dense as the ones we found in the monkey lungs. That difference might be related to the different exposure durations and differing intervals since last exposure. Their patient was exposed to PVC for 23 years (our monkeys were exposed for 22½ months) and had no PVC exposure for 6 years (our tissue was sampled within 48 hr of last exposure). It is possible that some of the ingredients in the PVC particles in the human lung were altered and leached out over a period of time.

Szende et al. (*3*) reported that the lung biopsy specimen from a worker who shoveled PVC for one year showed moderate diffuse fibrosis and contained a granuloma with concentrically arranged connective tissue fibers. We did not see this in the monkey lungs.

The apparent difference in response of the lung tissue from two workers exposed to PVC compared to the monkeys exposed to PVC might be related to a variety of factors, including differences in the PVC dusts. Since various types of emulsifiers and initiators are used in making PVC, and since these are not completely removed from the powdered PVC, they might explain the differences observed. It would be prudent in the future to obtain more information on PVC manufacturing processes and content of PVC dusts when studying their health effects.

Mastrangelo et al. (*6*) published health findings on employees who were currently working in a PVC production factory. Twenty cases of pneumoconiosis were found in a population of 1216 workers. Although the data were not given, the authors stated that slight restrictive respiratory function impairments were associated with chest x-ray changes in a small percentage of cases. In our study, no differences in pulmonary function tests could be detected between exposed and control monkeys after 14 months of exposure to 13 mg PVC/m^3. Whether any pulmonary function deficits would have occurred upon further exposure cannot be ascertained from this study. The lack of any significant pulmonary function abnormality in these monkeys, however, does not mean that animals or humans exposed to other types of PVC dust or mixtures of PVC dust with vinyl chloride (or a multitude of other ingredients that might be used in fabricating PVC products) might not exhibit pulmonary function changes.

For the first time, we have been able to show that a PVC dust generated under controlled experimental conditions is respirable and is deposited in cell aggregates in the lungs of monkeys, that PVC particles within macrophages are fairly unique in appearance, and that their identity can be confirmed with microprobe analyses. No pulmonary function deficits were found after 14 months of exposure to PVC concentrations of 13 mg/m^3, thus, confirming the pathological diagnosis of a benign pneumoconiosis.

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