ENVIRONMENTAL RESEARCH 21, 235–253 (1980)

Nonneoplastic Effects of Vinyl Chloride in Mouse Lung[1]

YASUNOSUKE SUZUKI

Environmental Sciences Laboratory, Department of Community Medicine, and Department of Pathology, Mount Sinai School of Medicine of the City University of New York, One Gustave Levy Place, New York, New York 10029

Received September 10, 1979

In a previous study, a high incidence of pulmonary tumors (alveologenic neoplasia in mouse lung exposed to vinyl chloride at heavy dose (2500 and 6000 ppm) for long durations (5 and 6 months) was reported (Y. Suzuki, 1978, *Environ. Res.* **16**, 285–301). In the present study, nonneoplastic effects in mouse lung were investigated by light and electron microscopy. As major light microscopic alterations, proliferation and hypertrophy of the terminal bronchiolar cells, consisting of ciliated and Clara cells, hypersecretion of the epithelial mucin in the goblet cells of both the bronchial and the proximal bronchiolar epithelium, hyperplasia of alveolar epithelium, mobilization of alveolar macrophages, and occasional presence of peribronchial or bronchiolar chronic inflammation, were observed. Electron microscopically, Clara cells of the terminal bronchiolar epithelium showed proliferation of the rough and smooth surfaced endoplasmic reticulum and appearance of large and abnormally shaped mitochondria. Similar alterations were found in the ciliated cells. Submicroscopic changes of pulmonary alveoli were represented by focal thickening of the basement membrane, multiple foci of hyperplastic type II cell (the precondition of the alveologenic tumor), active discharge of osmiophilic lamellar bodies from the type II cell and phagocytosis of the bodies by macrophages, appearance of cholesterol crystalloids in the macrophages, degeneration of alveolar septal cells and occasional appearance of a large nucleus with swelling of the capillary endothelium.

INTRODUCTION

Although the occurrence of the nonneoplastic pulmonary abnormalities among vinyl chloride polymerization workers has been reported on the basis of chest X ray (14), pulmonary function (18), and smear cytology (16) of the worker's sputum, no histopathological evaluation of the abnormalities has been reported.

It is believed that a relationship between lung cancer and vinyl chloride exposure exists from epidemiological studies (6, 19, 26, 31). A high incidence of pulmonary tumors in mice exposed to vinyl chloride has been reported by several investigators (10, 12, 13, 15, 16, 25). However, the nonneoplastic pulmonary effects of the chemical has not been completely explored.

In our previous study (25), we reported that the alveologenic tumors in the mouse originated in type II alveolar epithelium and that the hyperplastic type II cell was the precursor of the neoplastic cell. It led to an assumption that prior to appearance of the alveologenic tumor certain pulmonary changes, including preneoplastic alterations, occur in mouse lung exposed to vinyl chloride.

We have, therefore, undertaken detailed light and electron microscopy of the mouse lung used in the previous study, to characterize the nonneoplastic pulmonary effects of vinyl chloride.

[1] Supported by Research Grant OH 00681 from the National Institute for Occupational Safety and Health, U.S. Department of Health, Education and Welfare.

0013-9351/80/010235-19$02.00/0

MATERIALS AND METHODS

Twenty-seven CD1 Charles River white strain male mice, 4 to 5 weeks old at first exposure, were used. These animals were divided into three groups. Group I consisted of six animals exposed to vinyl chloride at 2500 (three mice) and 6000 (three mice) ppm/hr, 5 hr/day, 5 days/week, for 5 months. They were then kept for 6 days without exposure before sacrifice. Group II included 13 mice exposed at 2500 (seven mice) and 6000 (six mice) ppm for 6 months and were kept for an additional 2 days for recovery before sacrifice. Group III including eight animals (seven at 2500 ppm and one at 6000 ppm) were exposed to vinyl chloride monomer for 6 months followed by a 37-day recovery period. The inhalation exposures were accomplished at the Industrial Bio-Test Laboratory, Northbrook, Illinois.

In addition to the experimental animals, 16 mice (four for group I, four for group II, three for group III, and five which were 12 months old) were used as controls.

Under anesthesia, the mice of both experimental and control groups were sacrificed by decapitation. Lungs were examined under a dissecting microscope after the organs were removed, to determine whether tumors were present.

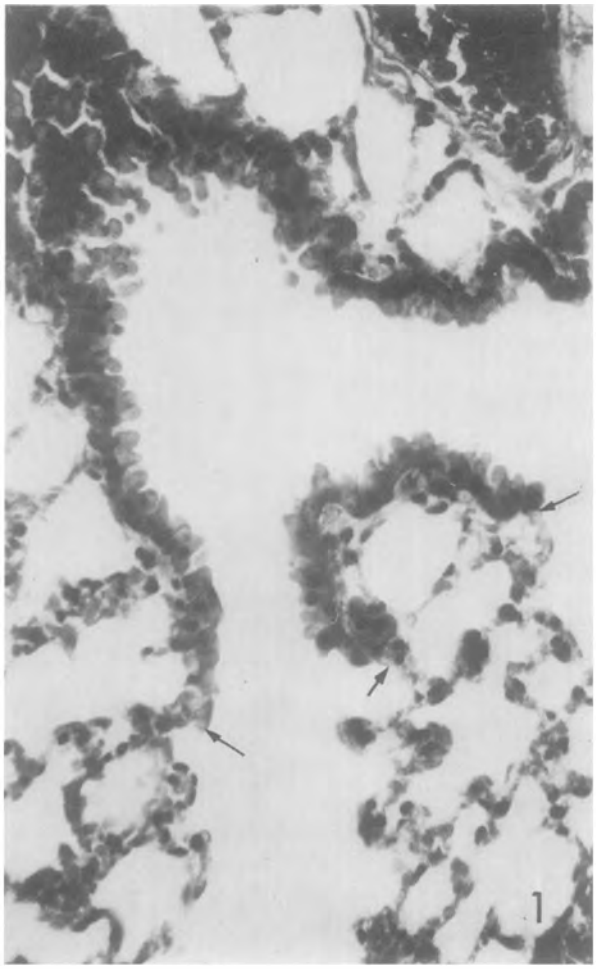
For light microscopy, the organs were fixed in 10% neutral buffered formalin and embedded in paraffin after dehydration in alcohol. Five- to six-micrometer sections were made and stained with hematoxylin–eosin, Masson's trichrome, Weigert's silver, periodic acid–Schiff's (PAS) with and without digestion by diastase, elastin, and Van Gieson's picrofuchsin technique. For electron microscopy small pieces, smaller than 1 mm^3, were taken from both pulmonary tumors and nonneoplastic pulmonary tissues and were fixed in 1% phosphate-buffered osmic acid a pH 7.2–7.4 for 2 hr. After alcohol dehydration, the blocks were embedded in epoxy resin. Ultrathin sections were obtained with a LKB microtome. The sections were stained with uranyl acetate and lead. A Siemens 101 electron microscope was used for ultrastructural observations.

OBSERVATIONS

Light Microscopy

Bronchi and Bronchioles

As a common finding in the treated animals, proliferation and hypertrophy of the bronchiolar epithelium were noted. As shown in Fig. 1, the terminal and respiratory bronchioles of the control mice were relatively simple in structure and the transitional point (arrows) of the repiratory bronchiole into the alveolus was easily distinguished. To differentiate the two areas, we found that Masson's trichrome was a useful stain since the cytoplasm of the bronchiolar cells was stained an intense brown red. The bronchioles of all the animals treated with vinyl chloride monomer showed proliferation, and cellular hypertrophy, though the degree varied among the animals. (Figs. 2 and 3). The proliferated cells were irregular in arrangement (Figs. 2 and 3). Frequently, hypersecretion of epithelial mucin in goblet cells of the bronchi as well as the proximal bronchioles was observed (Fig. 4, arrows). It was noteworthy that those alterations were still found in the animals of group III, which had a recovery time of 37 days after vinyl chloride exposure. Chronic inflammatory changes represented by marked lymphocyte infiltration into the perivascular and peribronchiolar connective tissue were seen, particularly in the group III.



FIGS. 1 to 8. Light micrographs.

FIG. 1. Showing light microscopy of a bronchiolo-alveolar area. Arrows indicate the site of transition between the respiratory bronchiole and the alveolus. A control mouse. Masson's trichrome staining; ×420.

Pulmonary Alveolus

The most significant finding was a high incidence of alveologenic tumors in the treated mice (25). Structural characteristics of the tumor and its histogenesis have been reported (25). In addition to the neoplasm, multiple foci of hyperplastic alveolar epithelium (Fig. 5), which were strongly suggestive of being preneoplastic for alveologenic tumors (24), were frequently observed. Mobilization of alveolar macrophages in the alveolar space was fairly commonly seen in the three groups of animals. (Fig. 6). In several cases, foam cells were markedly accumulated in alveolar spaces (Fig. 7). Two animals in group III showed bronchopneumonia-like changes. As shown in Fig. 8, coexistence of the proliferated bronchioles with alveologenic tumors (arrow) was frequently observed.

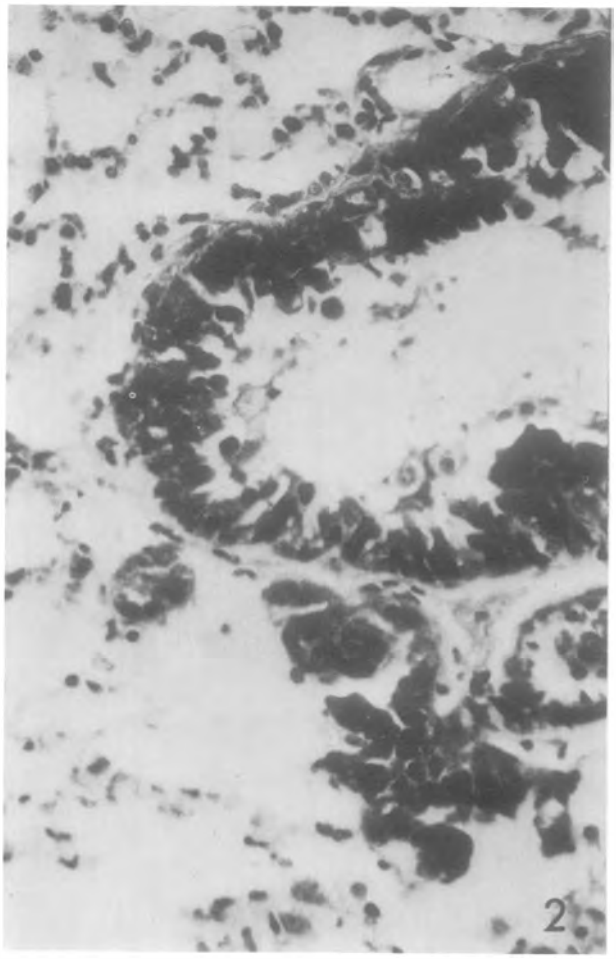


FIG. 2. Proliferated and hypertrophic bronchioles. Desquamated bronchochiolar epithelial areas are illustrated. Masson's trichrome staining; Group I; 2500 ppm; ×420.

Electron Microscopy

Bronchiolar Epithelium

The epithelium of the terminal bronchiole consists of the Clara cell (nonciliated) and ciliated cells; it lacks mucous producing cells in the mice. Both cell types are illustrated in Fig. 9 obtained from a control mouse. Clara cells lack typical microvilli and their apical portion present a dome-like shape (Fig. 9). The smooth-surfaced endoplasmic reticulum and Golgi complex were well developed. Mitochondria were generally round in shape and their cristae were few in number (Fig. 9). Two distinct granules, a bead-like structure (electron dense; the long axis was 1.6−0.2 μm) and a round phagolysosomal granule (electron dense or opaque; 0.6 × 0.6 μm in size) were observed in the cells. It is noteworthy that the ultrastructure of the cell is not identical among animal species and, further different fixation methods result in different structural appearances of the endoplasmic reticulum in the cell.

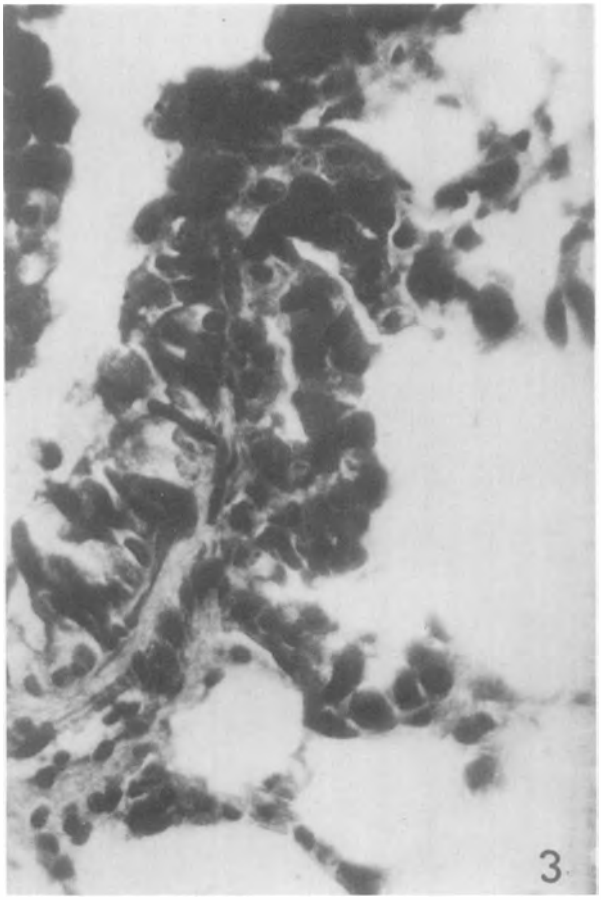


FIG. 3. Higher magnification of the terminal bronchioles, showing hypertrophy and proliferation. Masson's trichrome staining: Group I: 6000 ppm: ×670.

The cytoplasm of the ciliated cell was relatively clear and it contained rod-shaped mitochondria with cristae (Fig. 9). Cilia and microvilli were seen in the cell surface (Fig. 9). The two distinct granules were also occasionally seen in the cell.

Ultrastructural changes seen in those cell types of the treated mice is described below.

Clara cells. A low-power view of the proliferated bronchiole is illustrated in Fig. 10. Hypertrophic Clara cells frequently included dark cells rich in rough-surfaced endoplasmic reticulum and free ribosomes. The cell was usually large and its shape was occasionally irregular. Deep interdigitation of the lateral cell membranes of two adjacent cells was occasionally observed. Golgi complexes were well developed, and the two distinct granules described above were increased in number in the cytoplasm. The dark round granules are shown in Fig. 10. Although smooth-surfaced endoplasmic reticulum of the normal Clara cell was generally vesicular (with a single osmic acid, fixation as used in this study, though this cell organoid is cisternal with double fixation by glutar aldehyde and osmic acid),

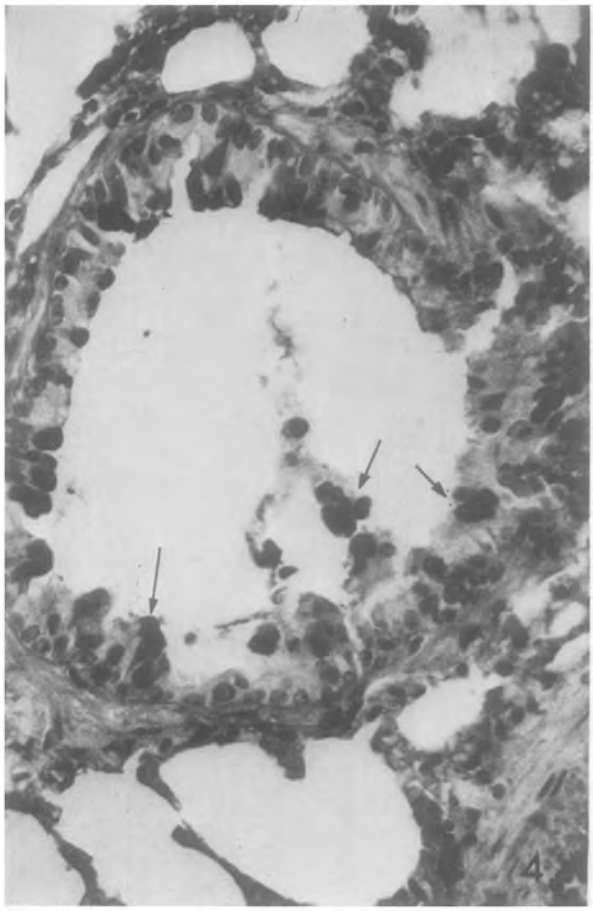


FIG. 4. Hypersecretion of epithelial mucin in bronchial epithelium. Arrows indicate mucinous substance stained with PAS. Group III; 6000 ppm; ×420.

the hypertrophic cells contained various forms of the organoid and the transformation of the rough surfaced endoplasmic reticulum into smooth-surfaced endoplasmic reticulum was easily observed (Fig. 10A). Mitochondria of abnormal shapes and large size occasionally appeared. Cristae were rather clearly shown in such abnormal mitochondria (Fig. 10B).

Ciliated cells. These cells were also involved in the proliferated alteration. The shape of the cells was occasionally irregular. Golgi complexes were sometimes well developed and phagolysosomal granules as well as round dense granules frequently appeared in the cytoplasm (Fig. 11). Large round mitochondria in which cristae were fewer in number were seen. In some ciliated cells, the rough and smooth surfaced endoplasmic reticulum were markedly developed (Fig. 11).

Pulmonary Alveolus

Although light microscopic observations failed to detect details of damages in the pulmonary alveolus, various ultrastructural alterations of the alveolar cells were revealed by electron microscopy.

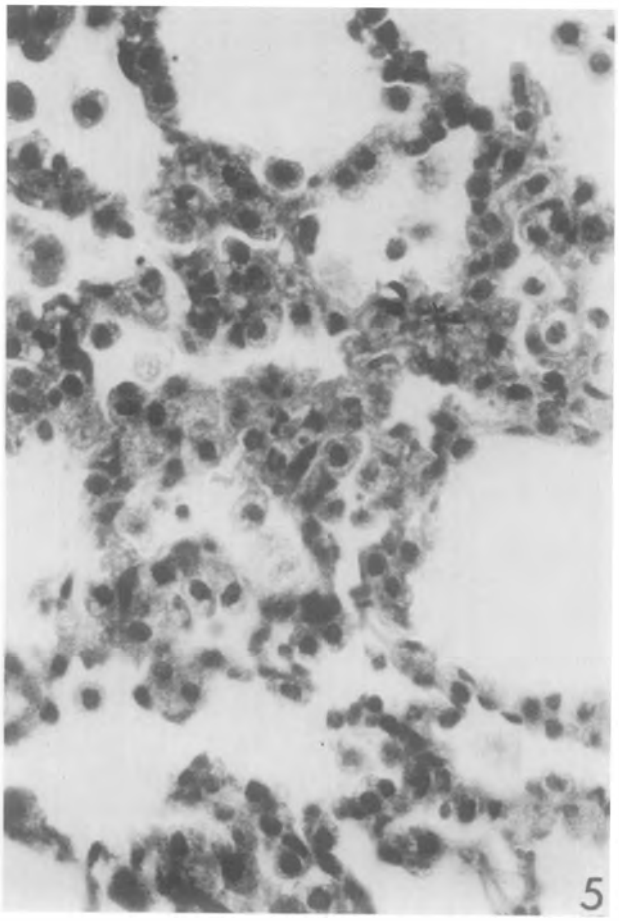


FIG. 5. Hyperplasia of alveolar epithelium is shown. Hematoxylin–eosin; Group III: 2500 ppm; ×670.

Alveolar macrophages. Mobilized alveolar macrophages were rich in phagolysosomal granules, as shown in Fig. 12. Other cell organellae were also well developed. Basophilic macrophages contained a large number of free ribosomes as well as the rough-surfaced endoplasmic reticulum, while clear macrophages were represented by intracytoplasmic osmiophilic lamellar bodies (Fig. 12) which seemed to be phagocytosed from the alveolar space. Occasionally, cholesterol crystalloids were found in the macrophages (Fig. 13, arrows).

Type II cells. As shown in Fig. 14, hyperplastic alveolar epithelium consisted of type II cells, precursors of alveolar epithelium (25). The evolutional process of the neoplastic transformation has been reported (25). Deformation of the cell shape, appearance of giant mitochondria with abnormal cristae, retention of large osmiophilic lamellar bodies, early intracytoplasmic "sequestration," and occasional huge lipid granules were observed. Microvilli of the cell surface were frequently decreased in number. These alterations were observed in almost all mice exposed to vinyl chloride, regardless of difference in dose and duration of recovery time.

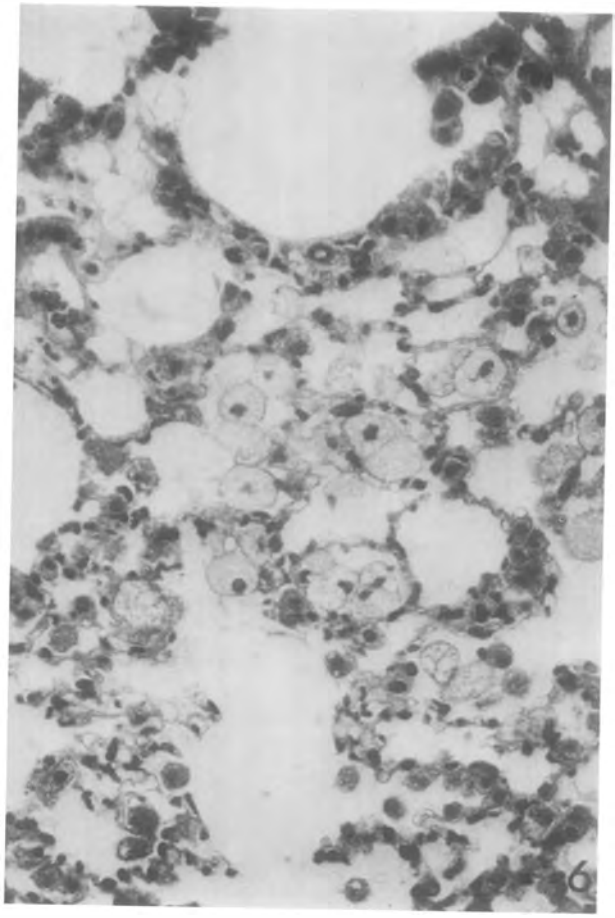


FIG. 6. Large clear macrophages (foam cells) as well as small basophilic macrophages are seen in the alveolar air spaces. Hematoxylin–eosin; Group III; 2500 ppm; ×420.

Type I cell. Swelling of the cytoplasm and appearance of lysosomal granules were occasionally observed in these cells. Transformation of the type I cells into the type II cells was suggested since intermediate cell types between the two were found on the alveolar lining.

Basement membrane. Focal thickening of the basement membrane was commonly seen. (Fig. 15). Sometimes, the thickened basement membrane showed a fibrillar appearance ("f": Fig. 16) and contained cell debris (arrows: Fig. 16) which seemed to be derived from alveolar septal cells.

Alveolar capillary endothelium. In addition to swelling of the cytoplasm, lysosomal granules and a large nucleus (Fig. 17), segmented or nonsegmented, were sometimes observed in the alveolar endothelium.

Alveolar septal cells. Hypertrophy ("h": Fig. 18) and degeneration (arrows: Fig. 18) of cells were frequent. In some cases, focal reticulosis was observed in the alveolar septum.

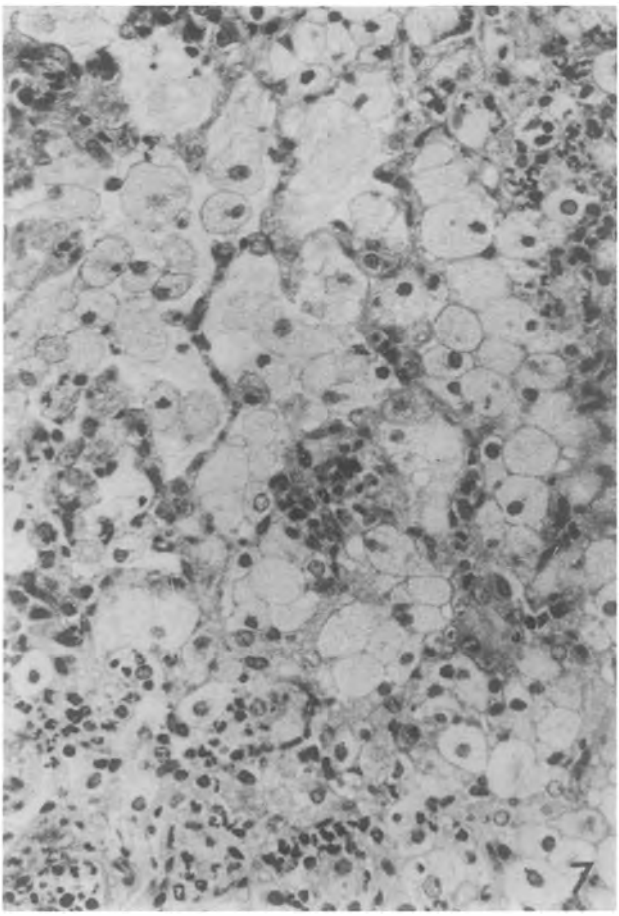


FIG. 7. Striking accumulation of a large number of foam cells. Hematoxylin–eosin; Group III; 6000 ppm; ×420.

From these light and electron microscopic studies, mouse lungs exposed to vinyl chloride at 2500 and 6000 ppm for 5 and 6 months clearly showed nonneoplastic pulmonary changes in both bronchiolar and alveolar cells. Since these findings were not detected in control mice, they are considered related to vinyl chloride.

DISCUSSION

The pulmonary effects of vinyl chloride may be considered from two distinct aspects, neoplastic and nonneoplastic. The neoplastic pulmonary effect has been postulated from epidemiological (19, 26, 31) and animal (10, 12, 13, 15, 16, 25) studies. It is noteworthy, however, that the experimental production of pulmonary tumors by vinyl chloride has only been successful in mice. Unlike human bronchogenic carcinomas, the pulmonary tumor was derived from alveolar epithelium (type II alveolar cell, following hyperplasia of the cell). The malignant

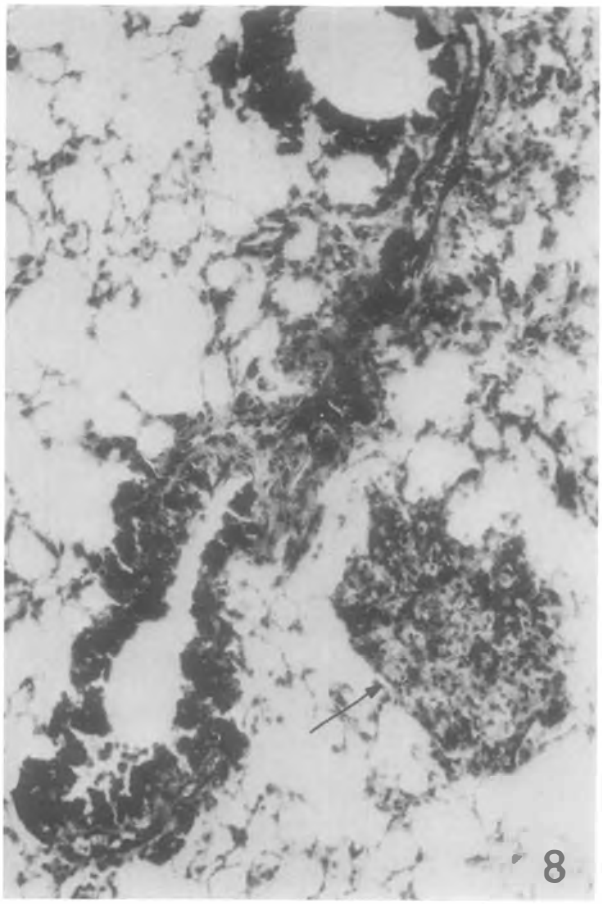


FIG. 8. A part of lung tissue represents co-existence of the alveologenic tumor (arrow) with the proliferated bronchiole. Masson's trichrome staining: Group I; 6000 ppm; ×170.

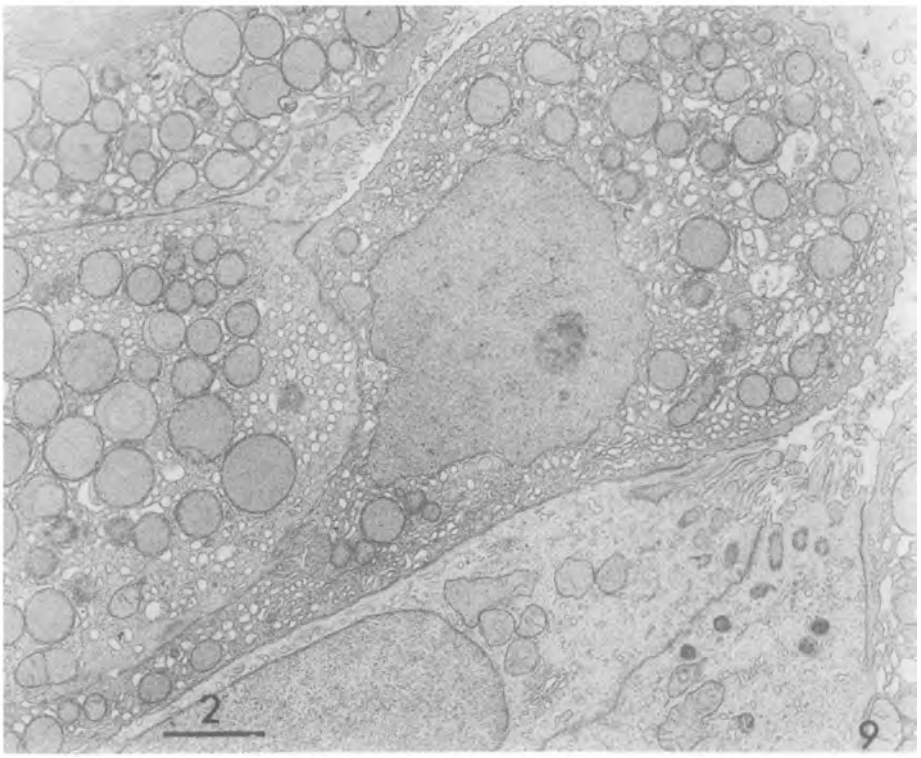
nature of the mouse pulmonary tumor (alveologenic tumor) has been disputed, although production of this tumor has been widely used as a parameter for evaluation on the carcinogenicity of chemical substances (9, 24). A high incidence with vinyl chloride has been confirmed by some investigators (10, 12, 13, 15, 16, 25) and may be used as evidence that the chemical is a carcinogen, although other clear evidence (3, 6, 7, 20, 27) had simultaneously been obtained.

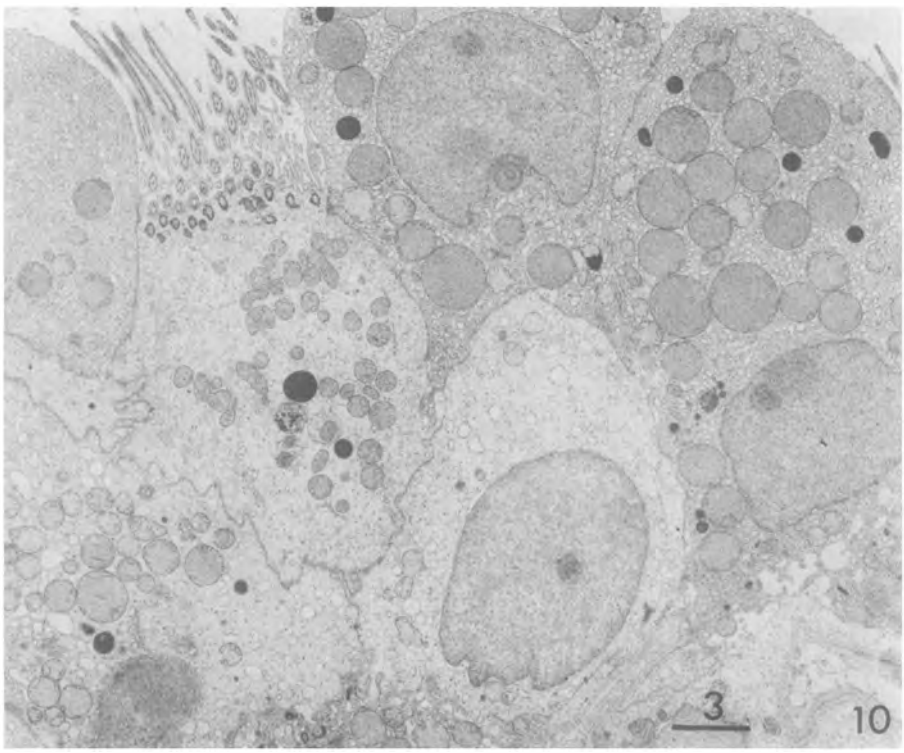
Nonneoplastic pulmonary effects of vinyl chloride has been suggested by observations among workers in vinyl chloride polymerization plants. This suggestion was based on data obtained by chest X-ray examinations, pulmonary function tests, and sputum cytology studies among workers. (i) Lilis and her associates (14) have found radiologic pulmonary changes, such as linear, reticular, and nodular

FIGS. 9 to 18. Electron micrographs.

FIG. 9. Showing a part of the terminal bronchiole. Clara cells and ciliated cells are illustrated. A control mouse. ×6700.

FIG. 10. Proliferated bronchiolar epithelium. Group I; 6000 ppm; ×3300.


2
9


3
10

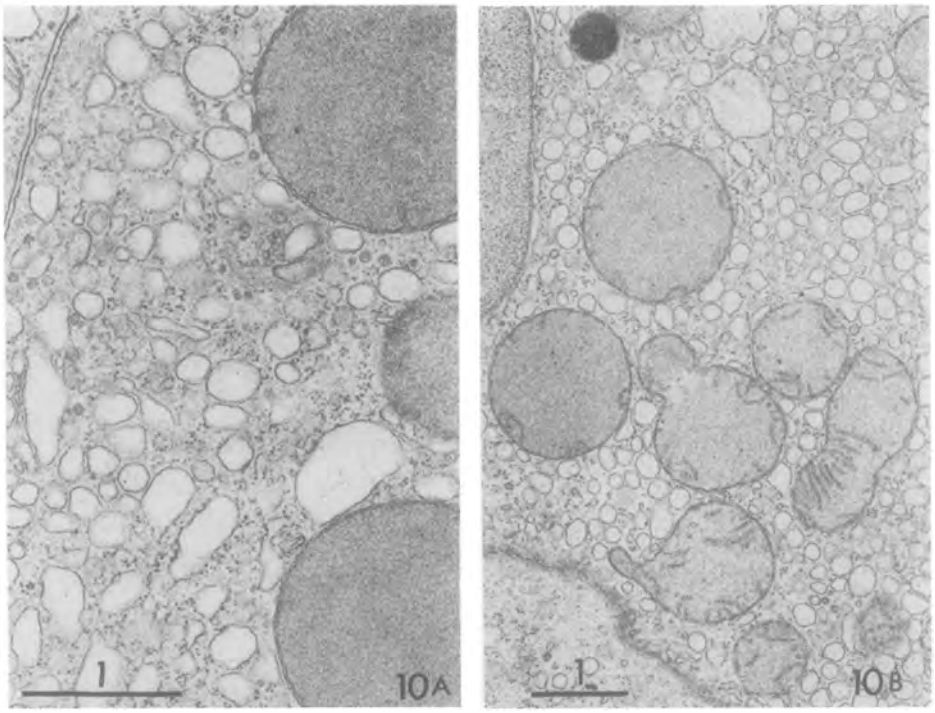
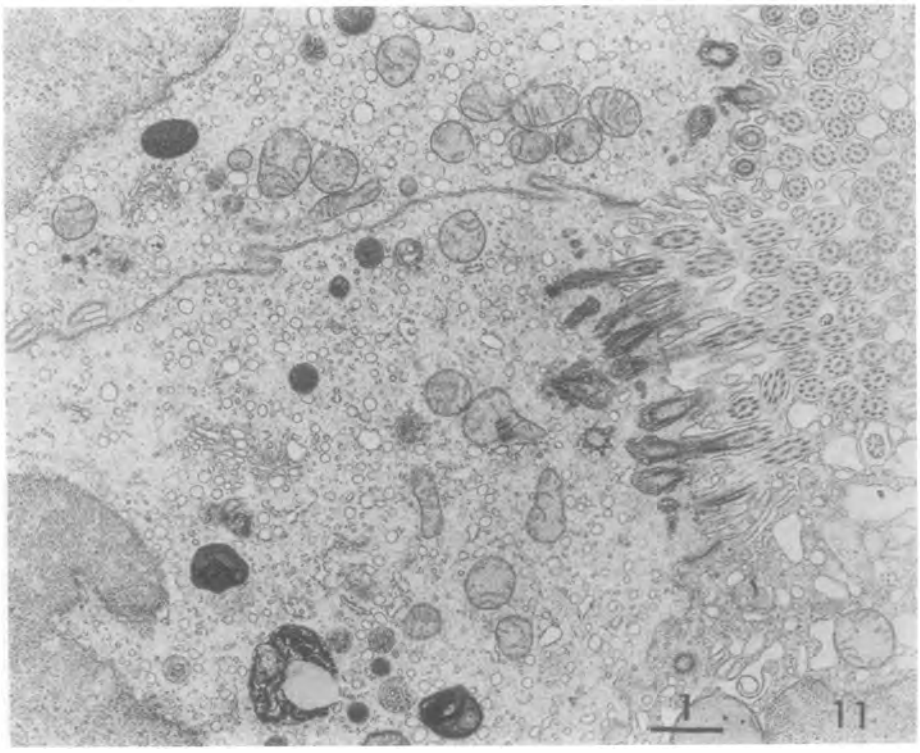


FIG. 10A. Part of the cytoplasm of a Clara cell. Various profiles of the endoplasmic reticulum are seen. Group I; 2500 ppm; ×21,000.

FIG. 10B. Irregular shaped mitochondria with cristae in the cytoplasm of a Clara cell. Group I: 6000 ppm; ×12,600.



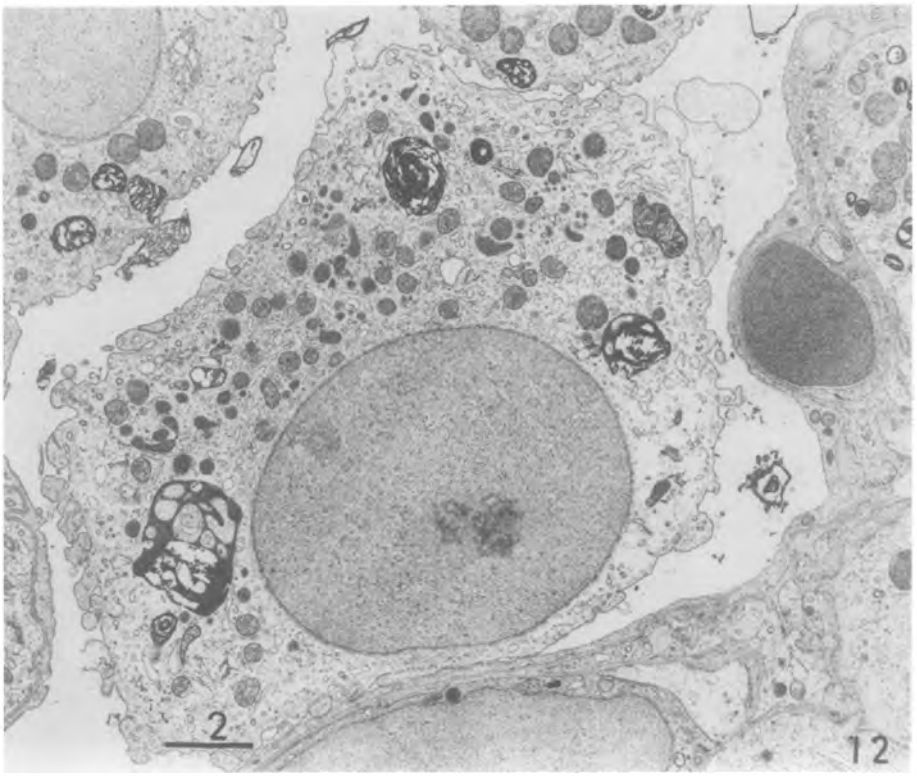


FIG. 12. An alveolar macrophage including phagolysosomes and osmiophilic lamellar bodies. Group I; 2500 ppm; ×5800.

opacities, in the lower and mid lung fields in a proportion of cases. They found that the prevalence of pulmonary changes increased with longer duration of exposure and that there was a significant association with peripheral circulatory abnormalities. However, pathological evaluation of these changes was not available. (ii) Miller *et al.* (18) have examined pulmonary function of 348 workers in a vinyl chloride polymerization plant. The major finding was diminution of air flow in 200 workers (57.7%). Again, no physicopathological relations were established. (iii) Maltoni and Lefemine (16) reported cytological studies of sputum in vinyl chloride and polyvinyl chloride workers. They found a significant increase in cellular changes of the bronchial epithelium; squamous metaplasia and squamous dysplasia were common among workers heavily exposed to vinyl chloride monomer.

Recently, McNamara and McLaughlin (17) have confirmed that a single 1-hr exposure to vinyl chloride in doses of 500 ppm or more induced pneumonitis in ICR mice and that aggravation of latent pulmonary changes, particularly bronchopneumonia, occurred in Fisher 344 rats.

Our present study has shown that CD1 Charles River male mice exposed to vinyl chloride at a heavy dose (2500 and 6000 ppm), over relatively long term (5

FIG. 11. Two ciliated cells of a terminal bronchiole. Irregular shaped mitochondria, a well developed Golgi area, round dense granules and phagolysomes are shown. Group I: 6000 ppm; ×9800.

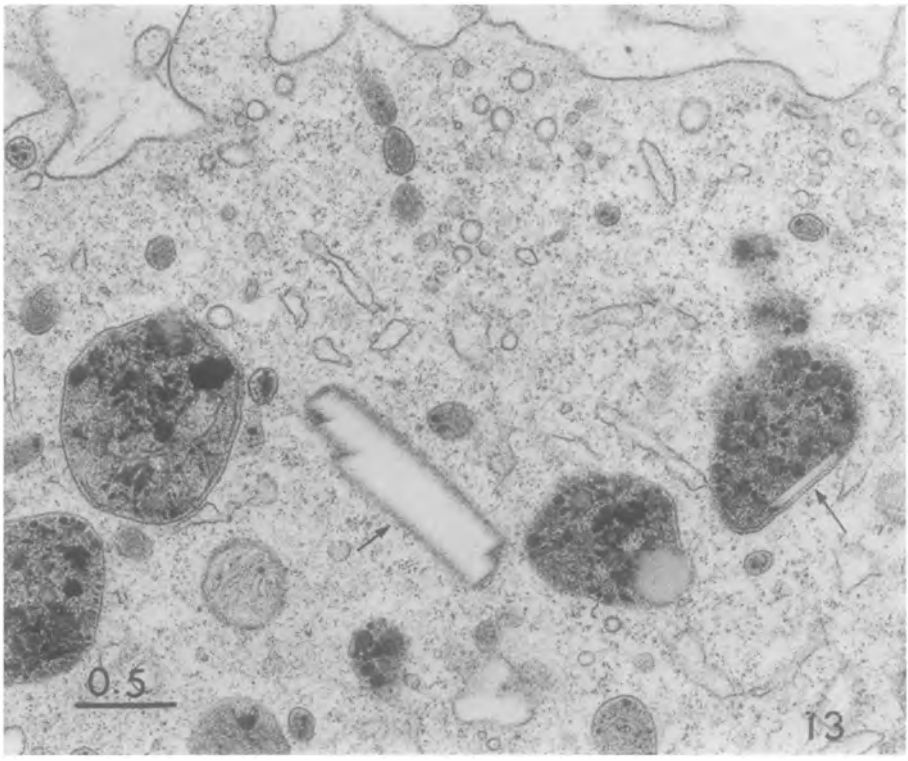
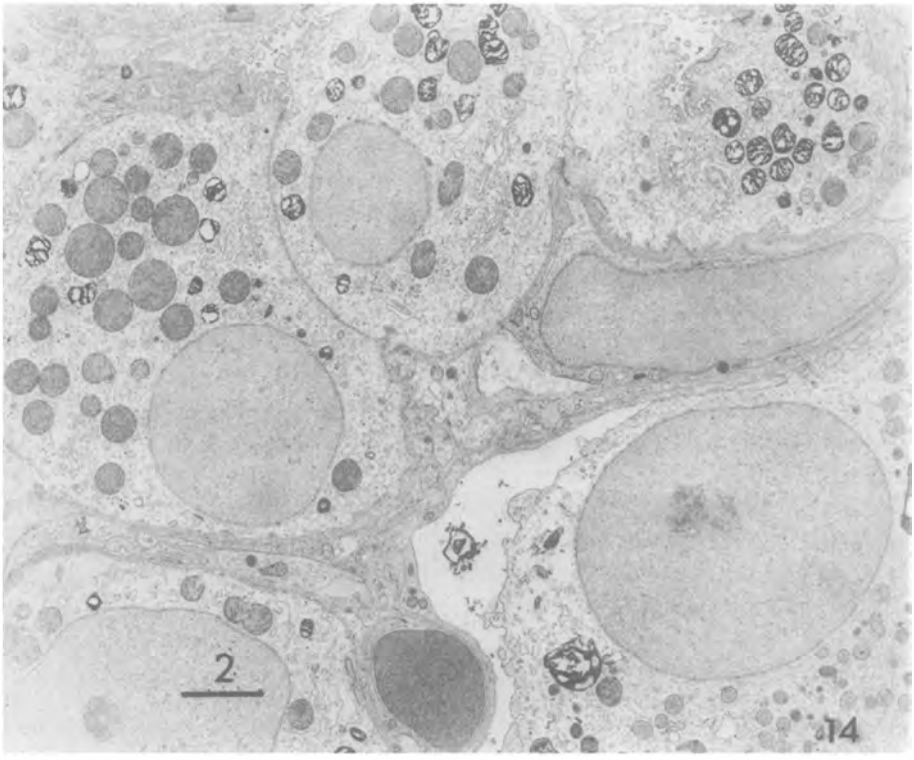


FIG. 13. A part of a macrophage. Two cholesterol crystalloids are shown. Group III; 2500 ppm 27,000.



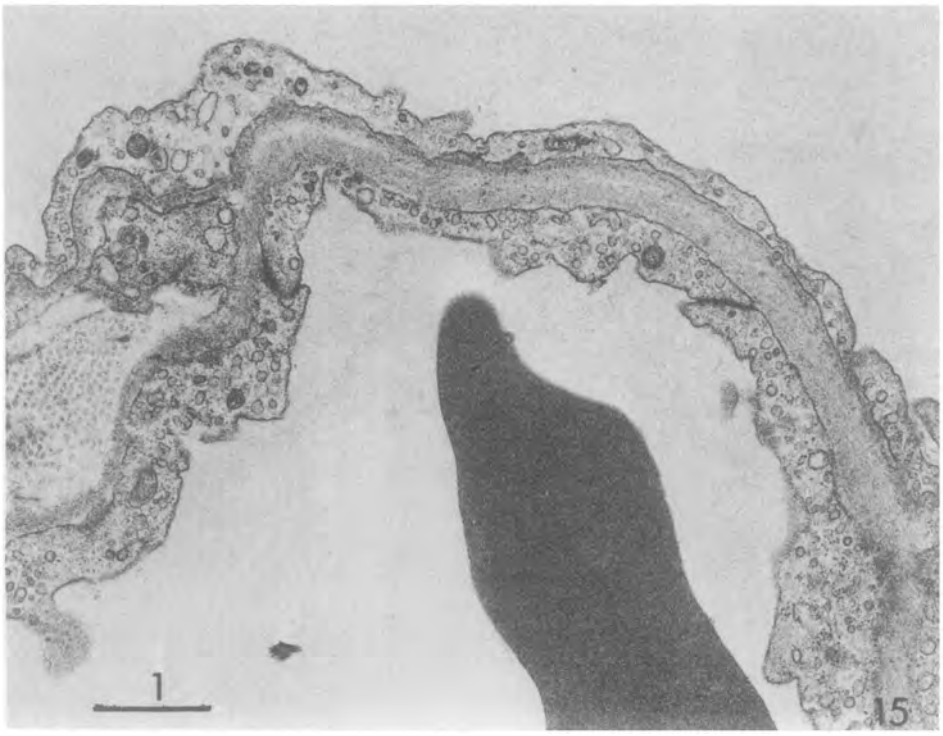


FIG. 15. Thickened basement membrane of an alveolar capillary. Group I: 6000 ppm: ×15,000.

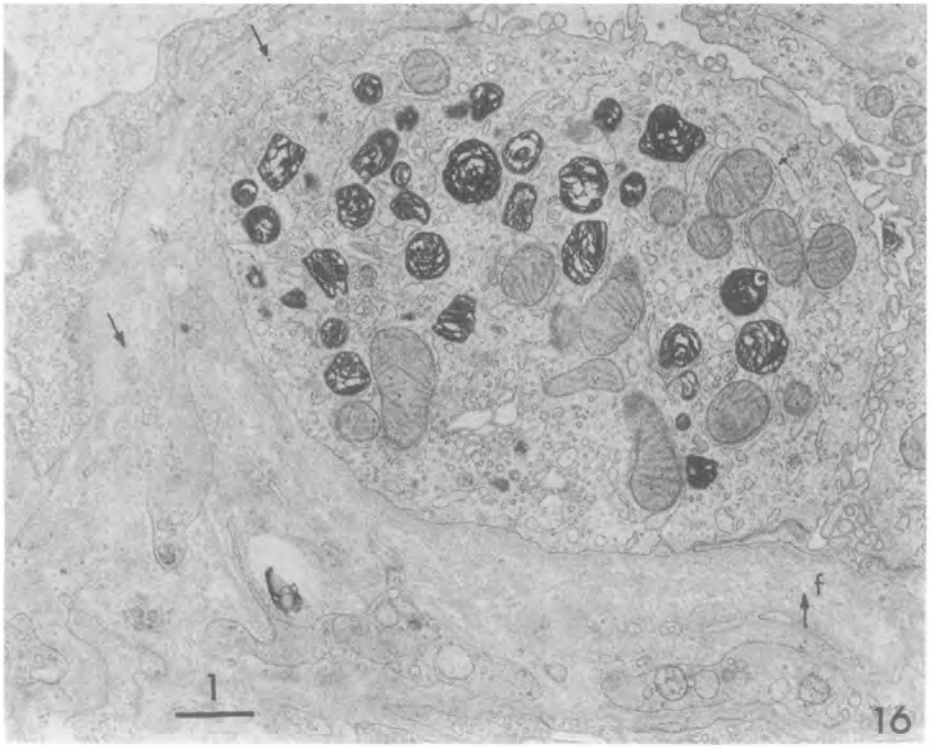


FIG. 16. Cell debris of degenerated alveolar septal cells (arrows) and fibrillar appearance of a basement membrane ("f" with an arrow) are illustrated. Group I: 6000 ppm: ×10,300.

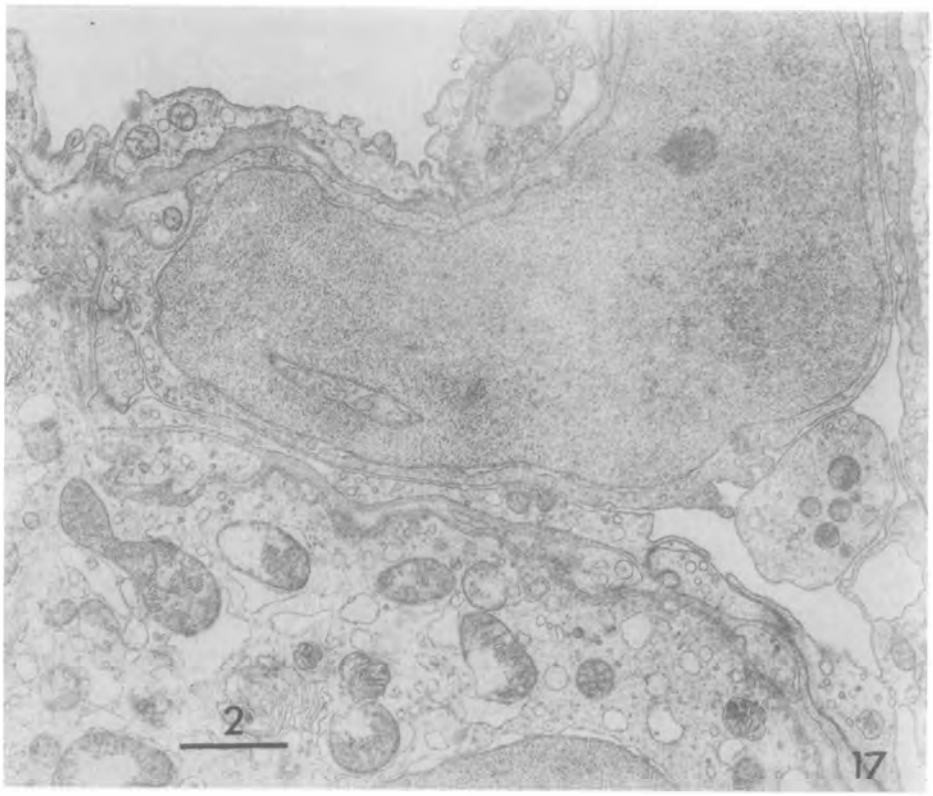


FIG. 17. A large nucleus in the alveolar capillary endothelium. Group II; 2500 ppm; ×7900.

and 6 months), obviously showed bronchiolo-alveolar changes. These alterations were recognized in almost all of the treated animals regardless of difference in doses (2500 and 6000 ppm), duration of exposure (5 and 6 months), and recovery time (2, 6, and 37 days).

The types of pulmonary cells which were involved in the structural alterations varied. It was interesting that the ultrastructural changes seen in Clara cells and to some extent in the ciliated cells were similar to those of hepatic cells of animals exposed to vinyl chloride; cellular hypertrophy and proliferation of the endoplasmic reticulum were common responses, seen in both the bronchiolar epithelium and the hepatic cells. The endoplasmic reticulum of the hepatic cell has been suggested as the site where vinyl chloride is metabolized, to be transformed into a chemically reactive metabolite which is the ultimate carcinogen (1, 2, 4, 5, 21, 22). Although it has not been shown that lung has the capacity to metabolize the chemical to produce the carcinogen; if it were so, the bronchiolar cells, particularly Clara cells may provide this mechanism: the cells are normally equipped with well-developed smooth-surfaced endoplasmic reticulums and the organellae have shown a proliferative response after mice are exposed to vinyl chloride. Recently, Kaufman *et al.* (11) have reported that Clara cells of mice developed into neoplasms which could occur in a malignant form, after transplacental exposure to

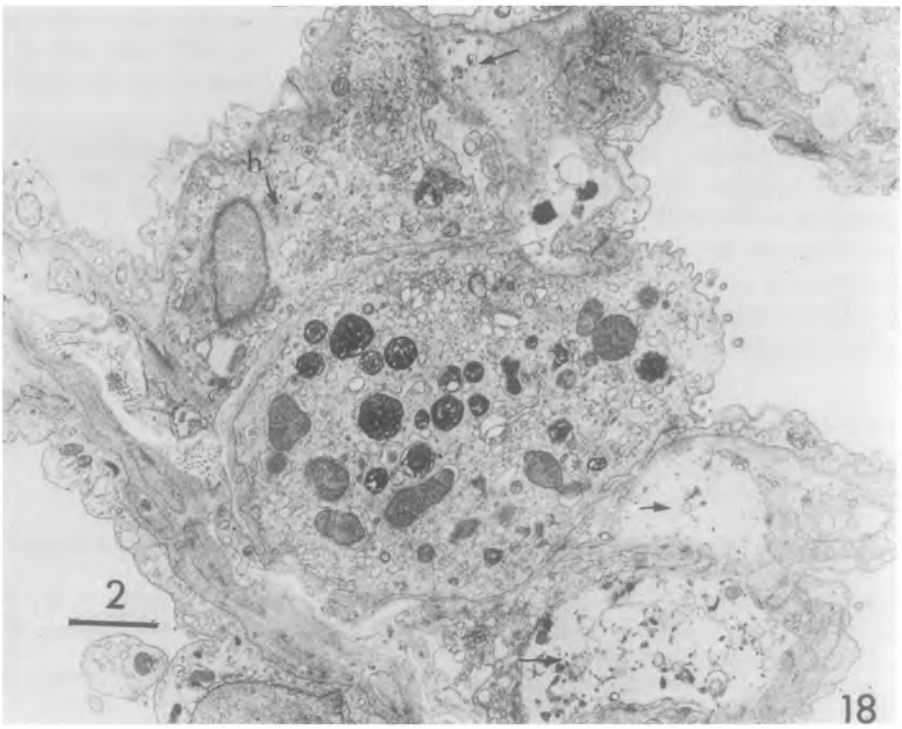


FIG. 18. Showing degenerative alterations of the alveolar septal cells (arrows) and a hypertrophic septal cell ("h" with an arrow). Group I: 6000 ppm; ×6300.

ethynitrosurea. However, such a Clara cell tumor was not produced in our material.

The fact (32) that vinyl chloride has a tendency to be soluble in lipid substances suggests that lipid-rich pulmonary cells such as type II cells (rich in osmiophilic lamellar bodies) and alveolar septal cells (containing lipid granules) may bind to vinyl chloride. If this assumption is correct, at least some of ultrastructural alterations seen in those cells might resulted from this mechanism. Both type II cells and Clara cells have been known as surfactant factor-producing cells (23). Hyperproduction of the surfactant factor was suggested, since hyperplasia of those cells was commonly seen in our material. Osmiophilic lamellar bodies and cholesterol crystalloids, which were seen in alveolar macrophages, may represent lipid substances bound to vinyl chloride. They may be removed from lung as part of the clearance mechanism for vinyl chloride. It is well accepted that mesenchymal elements such as bone, connective tissue, and blood vessels are involved in responses to vinyl chloride in various organs (8, 20, 22, 28). Above all, malignant transformation of the blood capillary endothelium, induction of hemangioendothelioma in liver, and the subcutaneous connective tissue, lung, and adipose tissue have been well documented (10, 15, 16, 22).

It is reasonable to assume that the alveolar capillary endothelium and the septal cell suffered toxic effects by vinyl chloride, since these cells are part of the mesenchymal elements in lung, sites of uptake and excretion of the chemical and its metabolites (30, 31).

A dose–response relationship has been suggested in the production of alveologenic tumors by vinyl chloride. The same relationship has been seen in occurrence of alveolitis (mouse) and bronchopneumonia (rats) with the chemical (19). A delayed appearance of these inflammatory changes after periods of recovery, following exposure to the chemical, has been postulated. A threshold dose for the induction of the nonneoplastic pulmonary lesions which are reported here has not been established.

ACKNOWLEDGMENTS

The author would like to express his appreciation to Dr. I. J. Selikoff for his review and suggestions on this manuscript. The author also thanks Mr. C. Din and Mr. R. Ashley for their excellent technical assistance.

REFERENCES

1. Antweiler, H. (1976). Studies on the metabolism of vinyl chloride. *Environ. Health Perspect.* **17**, 217–219.
2. Bartsch, H., Malaveille, C., Barbin, A., Bresil, H., Tomatis, L., and Montesano, R. (1976). Mutagenicity and metabolism of vinyl chloride and related compounds. *Environ. Health Perspect.* **17**, 193–198.
3. Block, J. B. (1974). Angiosarcoma of the liver following vinyl chloride exposure. *J. Amer. Med. Ass.* **229**, 53–54.
4. Bolt, H. M., Kappus, H., Buchter, A., and Bolt, W. (1976). Disposition of 1,2-C-vinyl chloride in the rat. *Arch. Toxicol.* **35**, 153–188.
5. Bolt, H. M., Laib, R. J., Kappus, H., and Buchter, A. (1977). Pharmacokinetics of vinyl chloride in the rat. *Toxicology* **7**, 179–188.
6. Buffer, A., Wood, S., Eifler, C., Suarez, L., and Kilian, D. J. (1979). Mortality experience of workers in a vinyl chloride monomer production plant. *J. Occup. Med.* **21**, 195–202.
7. Creech, J. L., Jr., and Johnson, M. N. (1974). Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J. Occup. Med.* **16**, 150–151.
8. Cook, W. A., Giever, P. M., Dinman, B. D., and Magnuson, H. J. (1971). Occupational acro-osteolysis II: An industrial hygiene study. *Arch. Environ. Health* **22**, 74–82.
9. Heston, W. E. (1949). Induction of pulmonary tumors in strain A mice with methylbis (B-chloroethyl) amine hydrochloride. *J. Nat. Cancer Inst.* **10**, 125–130.
10. Holmberg, B., Tronevi, T., and Winell, M. (1976). The pathology of vinyl chloride exposed mice. *Acta Vet. Scand.* **17**, 328–342.
11. Kauffman, S. L., Alexander, L., and Sass, L. (1979). Histologic and ultrastructural features of the Clara cell adenoma of the mouse lung. *Lab. Invest.* **40**, 708–716.
12. Keplinger, M. L., Goode, J. W., Gordon, D. E., and Calandra, J. C. (1975). Interim results of exposure of rats, hamsters and mice to vinyl chloride. *Ann. N.Y. Acad. Sci.* **246**, 219–220.
13. Lee, C. C., Bhandari, J. C., Hause, W. B., Woods, J. B., and Dixon, R. L. (1976). Inhalation toxicity of vinyl chloride (VC) or vinylidine chloride (VDC) in rats and mice. *Pharmacologist* **18**, 245.
14. Lilis, R., Anderson, H., Nicholson, W. J., Daum, S., Fischbein, A. S., and Selikoff, I. J. (1975). Prevalence of disease among vinyl chloride and polyvinyl chloride workers. *Ann. N.Y. Acad. Sci.* **246**, 22–44.
15. Maltoni, C., and Lefemine, G. (1975). Carcinogenicity bioassays of vinyl chloride: Current results. *Ann. N.Y. Acad. Sci.* **246**, 195–218.
16. Maltoni, C. and Lefemine, G. (1974). Carcinogenicity bioassays of vinyl chloride. I. Research plan and early results. *Environ. Res.* **7**, 387–405.
17. McNamara, B. P., Personal communication.

18. Miller, A., Teirstein, A. S., Chuang, M., Selikoff, I. J. and Warshaw, R. (1975). Changes in pulmonary function in workers exposed to vinyl chloride and polyvinyl chloride. *Ann. N.Y. Acad. Sci.* **246**, 42–52.
19. Nicholson, W. J., Hammond, E. C., Seidman, H. and Selikoff, I. J. (1975). Mortality experience of a cohort of vinyl chloride-polyvinyl chloride workers. *Ann. N.Y. Acad. Sci.* **246**, 225–230.
20. Popper, H. and Thomas, L. B. (1975). Alterations on liver and spleen among workers exposed to vinyl chloride. *Ann. N.Y. Acad. Sci.* **246**, 172–194.
21. Remmer, H. (1978). Metabolism of carcinogens: Its significance for the initiation of liver tumors. *In* "Primary Liver Tumors" (H. Remmer, Ed.), MTP Press Limited Falcon House, Lancaster, England.
22. Schaffner, F. (1978). Effect of long term vinyl chloride exposure in mouse liver structure. *In* "Primary Liver Tumors" (H. Remmer, Ed.), pp. 189–199. MTP Press Limited Falcon House, Lancaster, England.
23. Smith, P., Heath, D., and Moosavi, H. (1974). The Clara cell. *Thorax* **29**, 147–163.
24. Stewart, H. L., Dunn, T. B., and Snell, K. C. (1970). Pathology of tumors and non-neoplastic proliferative lesions of the lungs of mice. *In* Morphology of Experimental Respiratory Carcinogenesis (P. N. Nottesheim, M. G., Hanna, and J. W. Deutherage, Eds.), Atomic Energy Commission Symposium Series, No. 21, pp. 161–168.
25. Suzuki, Y. (1978). Pulmonary tumors induced in mice by vinyl chloride monomer. *Environ. Res.* **16**, 285–301.
26. Tabershaw, L. R., and Gaffey, W. R. (1974). Mortality study of workers in the manufacture of vinyl chloride and its polymer. *J. Occup. Med.* **16**, 509–518.
27. Thomas, L. B., Popper, H. Berk, P. D., Selikoff, I. J., and Falk, H. (1975). Vinyl chloride-induced liver disease. *N. Engl J. Med.* **292**, 17–22.
28. Veltman, G., Lange, C. E., Jühe, S., Stein, G., and Bachner, U. (1975). Clinical manifestation and course of vinyl chloride disease. Toxicity of vinyl chloride-polyvinyl chloride. *Ann. N.Y. Acad. Sci.* **246**, 6–21.
29. Watanabe, P. G., McGowan, G. R., Madrid, E. O., and Gehring, P. J. (1976). Fate of [^{14}c] vinyl chloride following inhalation exposure in rats. *Toxicol. Appl. Pharmacol.* **37**, 49–59.
30. Watanabe, P. G., and Gehring, P. J. (1976). Dose-dependent fate of vinyl chloride and its possible relation to oncogenicity in rats. *Environ. Health Perspect.* 17, 145–152.
31. Waxweiler, R. J., Stringer, W., Jones, J., Wagoner, J. K., Falk, H., and Carter, C. (1976). Neoplastic risk among workers exposed to vinyl chloride. *Ann. N.Y. Acad. Sci.* **271**, 39–48.
32. Wolff, M. S. (1976) Evidence for existence in human tissues of monomers for plastics and rubber manufacture. *Environ. Health Perspect.* **17**, 183–187.