Virchows Arch (1995) 427:77–83

ORIGINAL ARTICLE

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Ultrastructural changes in the lung in Niemann-Pick type C mouse

Received: 22 November 1994 / Accepted: 20 May 1995

Abstract The biochemical and morphological aspects of BALB/c mice with many features of the Niemann-Pick disease type C in man (NP-C mouse) have been studied extensively. However, the pulmonary pathology has not been studied extensively and we describe here some unique ultrastructural features of the lung in the NP-C mouse. Ultrastructurally, macrophages in younger mice contained osmiophilic dense granules and annulolamellar structures, but larger multilamellar concentric structures increased in the macrophages of older mice. In contrast, endothelial cells and type I pneumocytes showed membrane-bound bodies with dense granules and vesicular or vesiculogranular structures as well as amorphous materials. Type II pneumocytes were unremarkable throughout. Our study suggests that endothelial cells and type I pneumocytes are the major site of metabolic derangement resulting in pronounced morphological changes with granular and round membranous structures in the lungs of NP-C mouse. Alveolar macrophages with multilamellar concentric structures may be a result of disturbed disposal of surfactant material from type II pneumocytes rather than that from storage material of type I pneumocyte.

Key words Niemann-Pick disease · Mouse · Lung · Electron microscope · Ultrastructure

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Introduction

Niemann-Pick disease is a clinically and biochemically heterogeneous group of autosomal recessive lysosomal sphingomyelin-cholesterol storage disease [14]. Niemann-Pick disease, type A and type B are due to primary sphingomyelinase deficiency. Niemann-Pick disease, type C (NP-C), however, does not represent a primary sphingomyelinase deficiency, but reflects an, as yet, unidentified mutation that blocks lysosomal cholesterol transport. In 1980, Pentchev et al. [12] discovered a strain of BALB/c mice with many features of NP-C in humans. The basic metabolic defect of these human and murine disorders lies in an impairment of intracellular transport of low-density lipoproteins (LDL)-derived cholesterol, resulting in a massive accumulation of unesterified cholesterol within the lysosome [13, 17]. Pathologically, the presence of foamy cells is a relatively constant feature of Niemann-Pick disease and may be found in a variety of organs such as reticuloendothelial and nervous system as is the case in NP-C [12, 18]. Although electron-microscopic studies have been reported, reports of changes in the respiratory system are very limited. A report by Skikne et al. [16] was the only detailed study which depicted ultrastructural changes of the lung from a young patient with putative Niemann-Pick disease of unspecified type. Shio et al. [15] very briefly described electron microscopic features of alveolar macrophages in NP-C mice. Using NP-C mouse models, Higashi et al. described topographical and chronological aspects of morphological changes in neuropathological lesions [7] as well as degenerative changes of the cerebellum [8]. Here we report ultrastructural features of the lung in the same mouse model.

Materials and methods

Mutant BALB/c mice, which are known as a murine model of NP-C disease, have been maintained by interbreeding of heterozygotes in the animal factility of the Developmental and Metabolic Neu-

rology Branch, National Institute of Neurological Disorders and Stroke, National Institute of Health, United States Public Health Service.

In total 7 each (2 each at 20 and 40 days of age, and 1 each at 50, 60, and 80 days of age) of homozygous (NP-C) and litter-mate control mice were used for this study. They were sacrificed by the perfusion of a fixative made of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer through the left ventricle under deep ether anaesthesia. The chest was opened to remove the lungs in toto. Generally, lungs were so small that the sampling sites could not be specified. Representative tissue blocks of about 1 mm^3 were fixed in the same fixative for 1 h and post-fixed in Dalton's fixative for 2 h, dehydrated with graded ethanol and em-

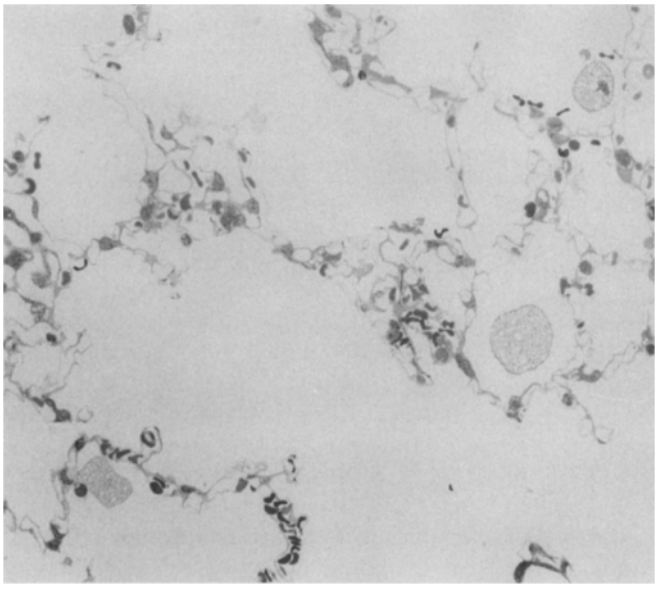

Fig. 1 Lung at day 20. A few scattered macrophages with vacuolated cytoplasm are seen. Toluidine blue, ×200

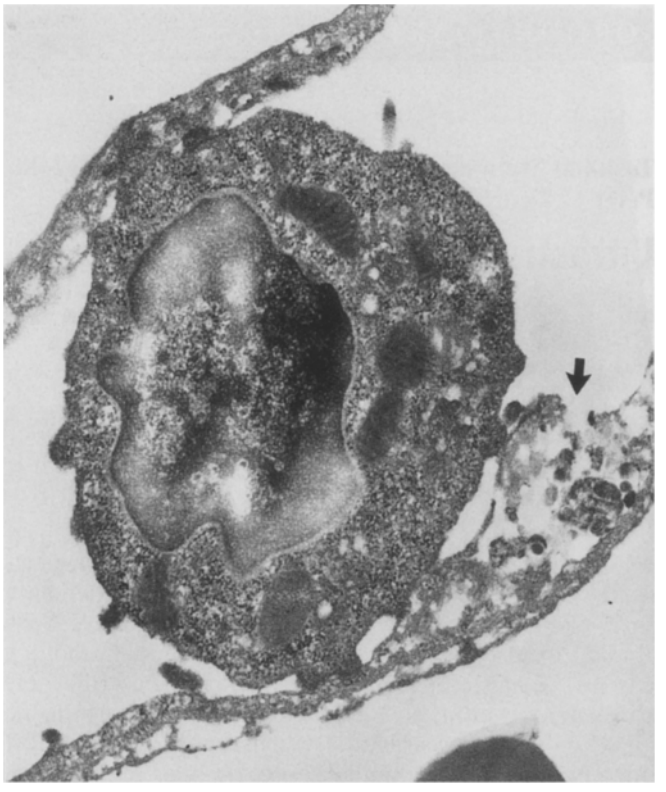

Fig. 3 Affected mouse at day 20. Note a mononuclear cell in alveolar capillary. An *arrow* indicates the thickened portion of the endothelial cell which contains few granular materials. The mononuclear cell seems to attach to endothelial cells with short and blunt cytoplasmic processes. ×22,000

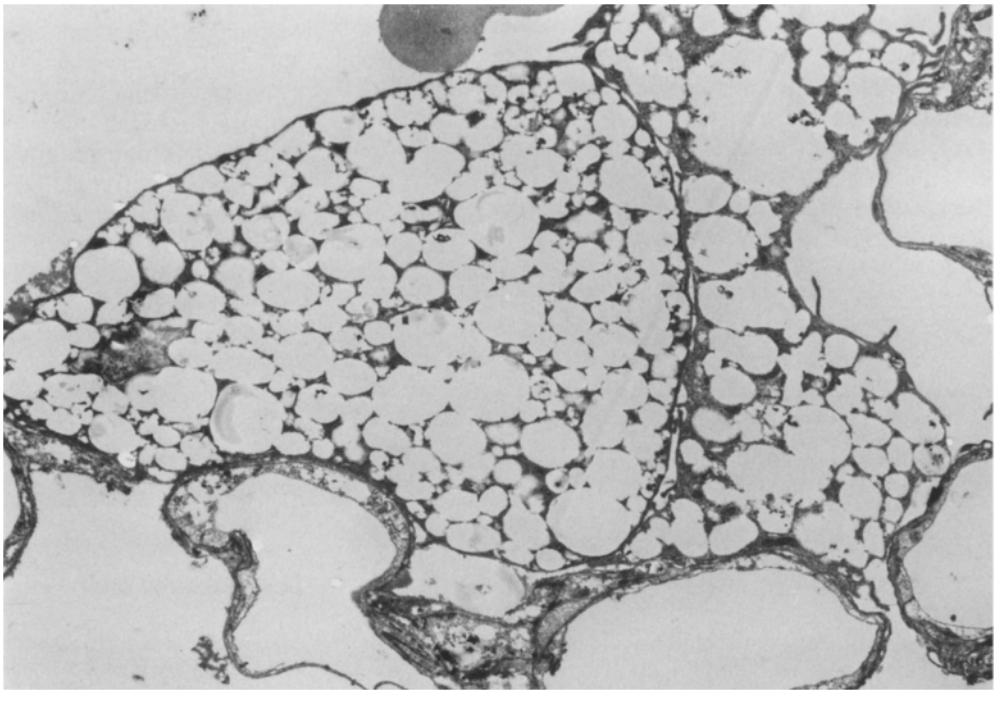

Fig. 2 Foamy macrophages in the alveolar space from affected mouse at day 20. Vacuoles are mostly empty, but peripheral semilunar areas of low electron density and a few dot-like bodies are occasionally seen. Note that no membranous and/or lamellar structures are present. ×6,000

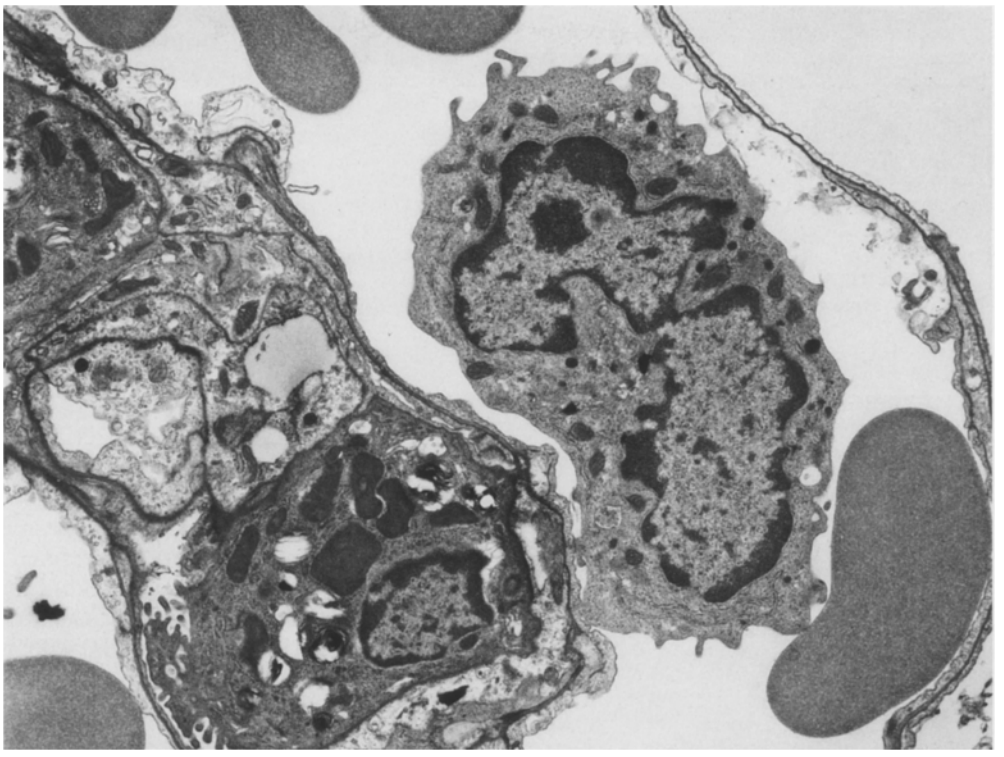

Fig. 4 Affected mouse at day 20. Endothelial cells in alveolar capillary have disintegrated in places, and a polymorphonuclear leukocyte abuts on the disrupted plasma membrane. The type II pneumocyte is unremarkable. ×8,700

bedded in EM bed 812 (Electron Microscopy Science, Fort Washington, Pa., USA) as Higashi et al. [8] have described separately. Sections 1 μm thick were stained with toluidine blue. Ultrathin sections were double-stained with lead citrate and uranyl acetate and examined in a Hitachi 500 electron microscope.

Results

The lungs of affected mice at day 20 showed alveolar architecture to be well-preserved with the occasional presence of a few foamy cells in the alveolar spaces (Fig. 1), while foamy cells were never seen in any control mice. Electron-microscopically, these foamy cells were macrophages with multivacuolated cytoplasm (Fig. 2). The vacuoles were mostly empty and not membrane-bound. In areas, however, some empty vacuoles showed vague peripheral thin semilunar foci of homogeneous low electron density, possibly representing neutral fat. One or two dot-like electron-dense microstructures were occasionally seen within the vacuoles. No membranous or lamellar structures were noted in these cells. On occasion, endothelial cells were focally thickened with vacuoles containing a few granular and round membranous structures (Fig. 3). There were focal areas where endothelial cells had disintegrated (Fig. 4). In those areas, mononuclear cells and polymorphonuclear leukocytes appeared adherent to the plasma membrane of endothelial cells with short processes. Type I pneumocytes were unremarkable. The cytoplasm of type II pneumocytes were filled with lamellar and concentrically lamellar inclusion bodies, but there were no vesiculogranular structures seen in endothelial cells.

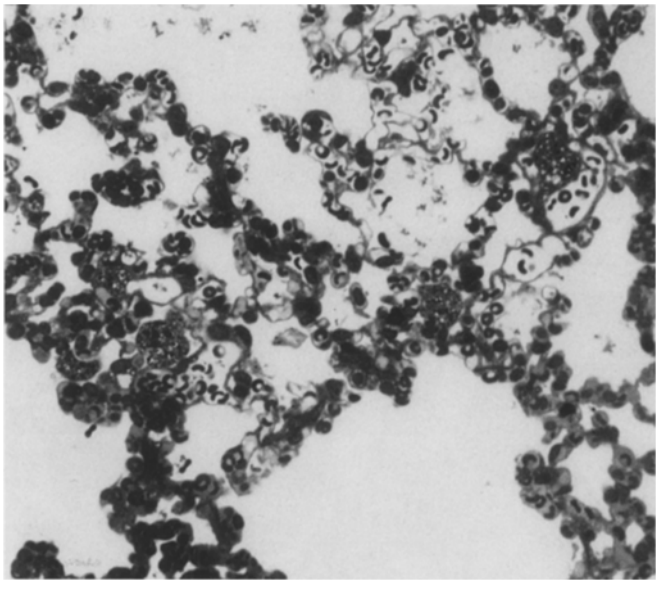

Fig. 5 Lung at day 50. Macrophages contain granular material in their cytoplasm. Toluidine blue, ×200

Successively from day 40 through day 80, macrophages in alveolar spaces increased in number, and alveoli there tended to be collapsed (Fig. 5). The cytoplasm of these macrophages no longer contained empty vacuoles. Instead, they appeared granular under light microscopy. At the electron microscopic level, osmiophilic dense granules, small concentrically lamellar and annulolamellar structures were seen in these cells of young

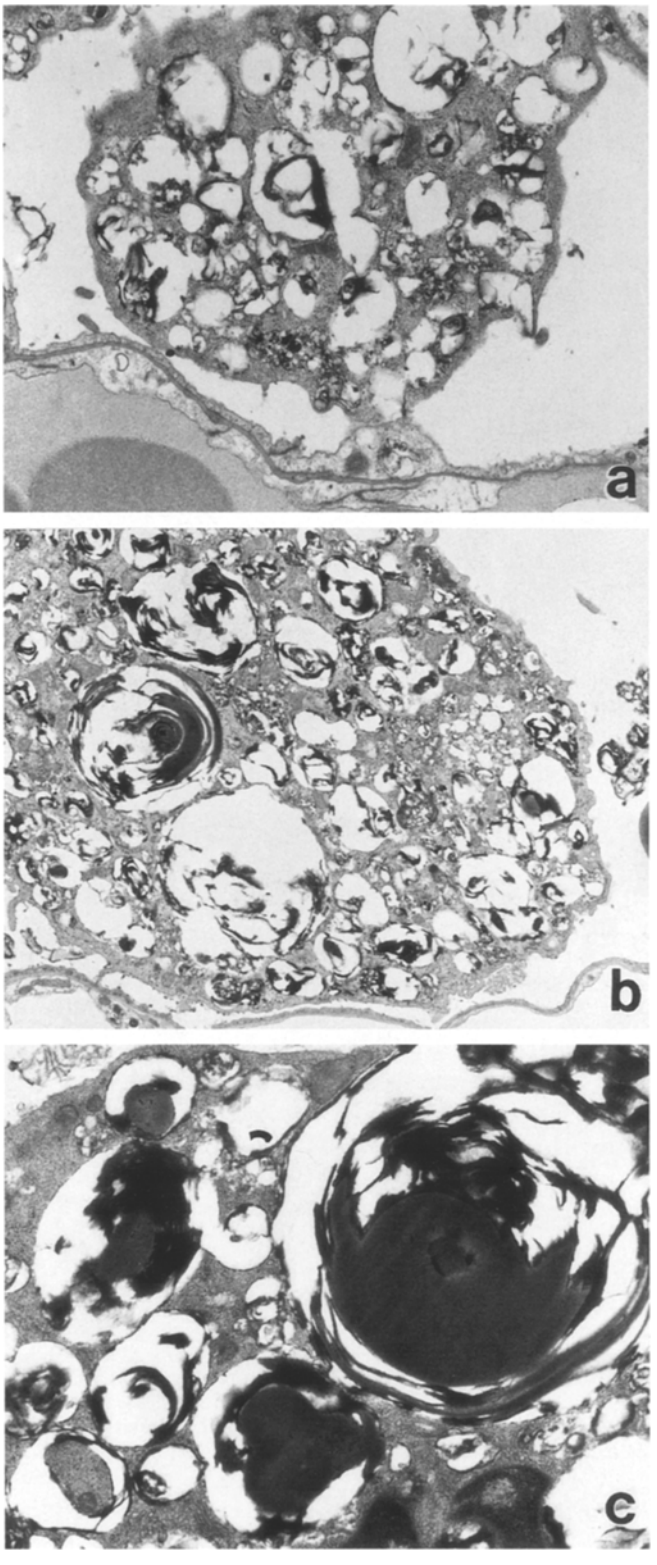

Fig. 6 Alveolar macrophages at day 50 (**a**) and day 60 (**b**). Osmiophilic dense granules, as well as concentrically lamellar structures are prominent in the affected mouse at day 50, whereas large multilamellar concentric structures predominate at day 60. Osmiophilic multilamellar structures (**c**) are reminiscent of lamellar bodies in type II pneumocytes. **a** ×5,000; **b** ×5,700; **c** ×16,000

mice (Fig. 6a). In older mice larger multilamellar concentric structures were conspicuous in the cytoplasm of macrophages (Fig. 6b, c). Some inclusions were made up of aggregates of multiple, small, round electron-dense structures. In older mice, dense granules, round bodies and annulolamellar structures were scanty within the macrophages. Tubular myelin-like figures were never seen in these cells. In the alveolar spaces, fragments of lamellar structures similar to those in the type II pneumocytes and macrophages were scattered with numerous tubular myelins. Endothelial cells often showed membrane-bound structures which were filled with either dense granules, membranous round to ovoid structures of variable size (vesicular and vesiculogranular structures) or amorphous materials (Fig. 7a). With time, these substructures became prominent and increased in number and amount (Fig. 7b). Sometimes, limiting membranes of these vacuoles appeared to be connected with plasma membranes of the luminal side. The content of these vacuoles were seen within the vascular spaces on occasion as if they had been extruded into the vascular lumen. Mononuclear cells with prominent lysosomal granules were tightly adherent to such endothelial cells in places. With time, mononuclear and polymorphonuclear leukocytes within blood vessels became swollen with multivesicular and granular bodies, and filled the vascular space (Fig. 8a, b).

Type I pneumocytes also began to have similar vesiculogranular structures but not as many as were seen in the endothelial cells. Type II cells, in contrast, did not show any of these structures. Lamellar inclusion bodies were consistently unremarkable.

Ciliated epithelia in the bronchioles also showed small, multilamellar inclusions and multivesiculogranular bodies within their cytoplasm (Fig. 9). They were increased in number and amount with time.

Discussion

The ultrastructural features of Niemann-Pick diseases have been described in man and in animal models. In general, homogeneous lucent or low density lysosomes and membranous deposits were associated with sphingomyelinase deficiency, while vesicular or annulolamellar deposits were seen with NP-C disease [3]. These studies were mostly carried out using tissues from the reticuloendothelial system [2], nervous system [11, 19] and liver [1, 4]. Pulmonary pathology, has not usually been studied, in particular by electron microscopy.

In our study, there were two salient features in the lung tissue with NP-C; namely multivesicular and vesiculogranular structures in endothelial cells and a few type I pneumocytes and multilamellar and annulolamellar structures in the alveolar macrophages. The former structures in the endothelium were membrane-bound and on occasion contained dense-core granules which abutted on the smooth endoplasmic reticulum and pinocytotic vesicles without any continuity. The limiting membrane

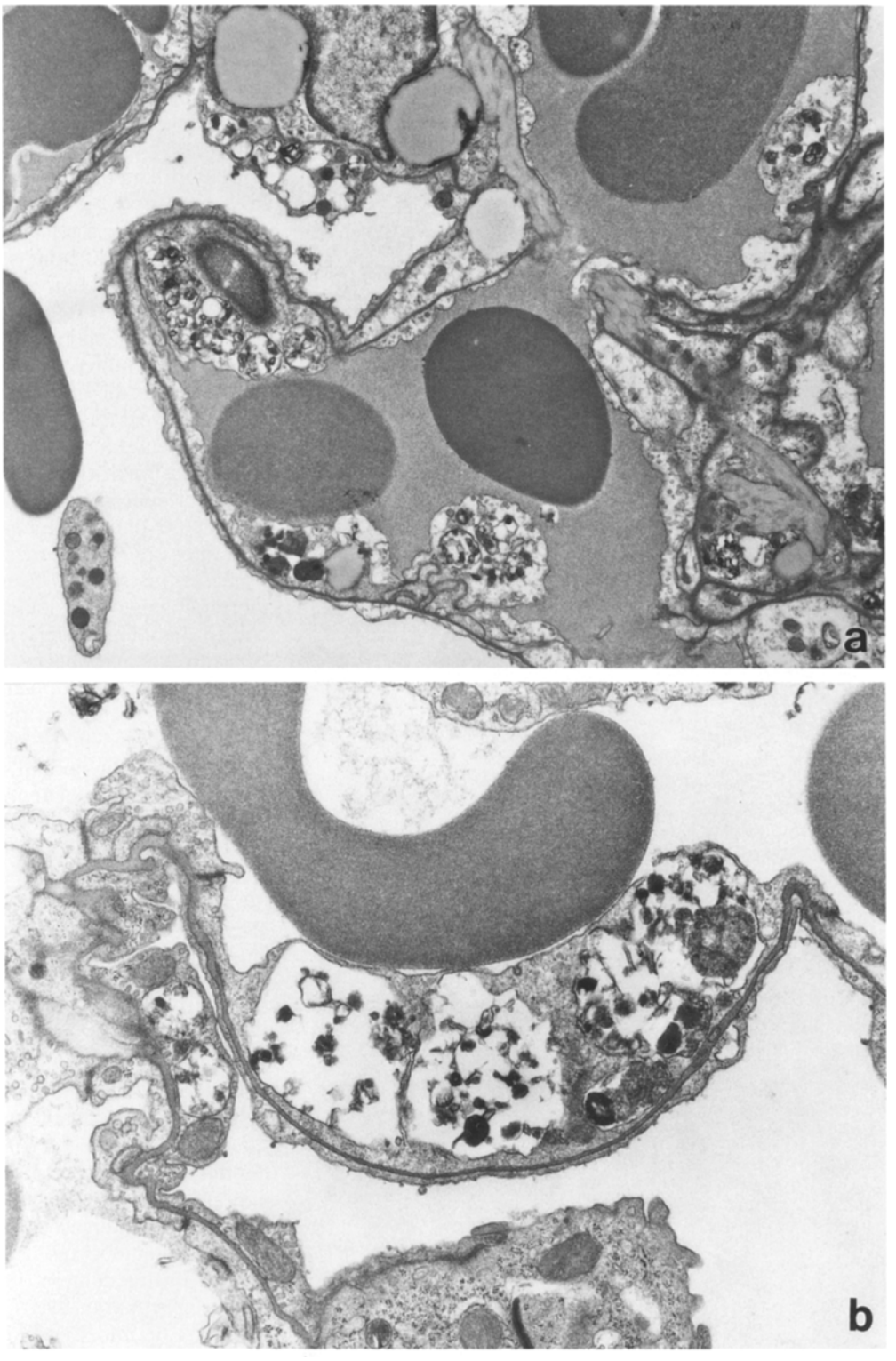

Fig. 7 Endothelial cells in alveolar capillaries. Membrane-bound multivesicular bodies are prominent at day 40 (**a**) and they become larger by a day 50 (**b**) with dense granules, membranous structures and amorphous materials. Sometimes they appear open to the lumen. **a** ×8,000; **b** ×17,000

of these structures were sometimes contiguous to the luminal plasma membrane. These findings suggest that they were produced within endothelial cells, because they were seen in mice as young as 20 days when no other cells, including mononuclear cells in the blood vessels, exhibited similar changes. It should be remembered that endothelium possesses no phagocytic activity [5, 6] and these cells may well be the major site of metabolic derangement in the lung of NP-C. After day 60, numerous mononuclear and polymorphonuclear leukocytes with multivesicular and granular inclusions were detected within alveolar capillaries. They never contained mul-

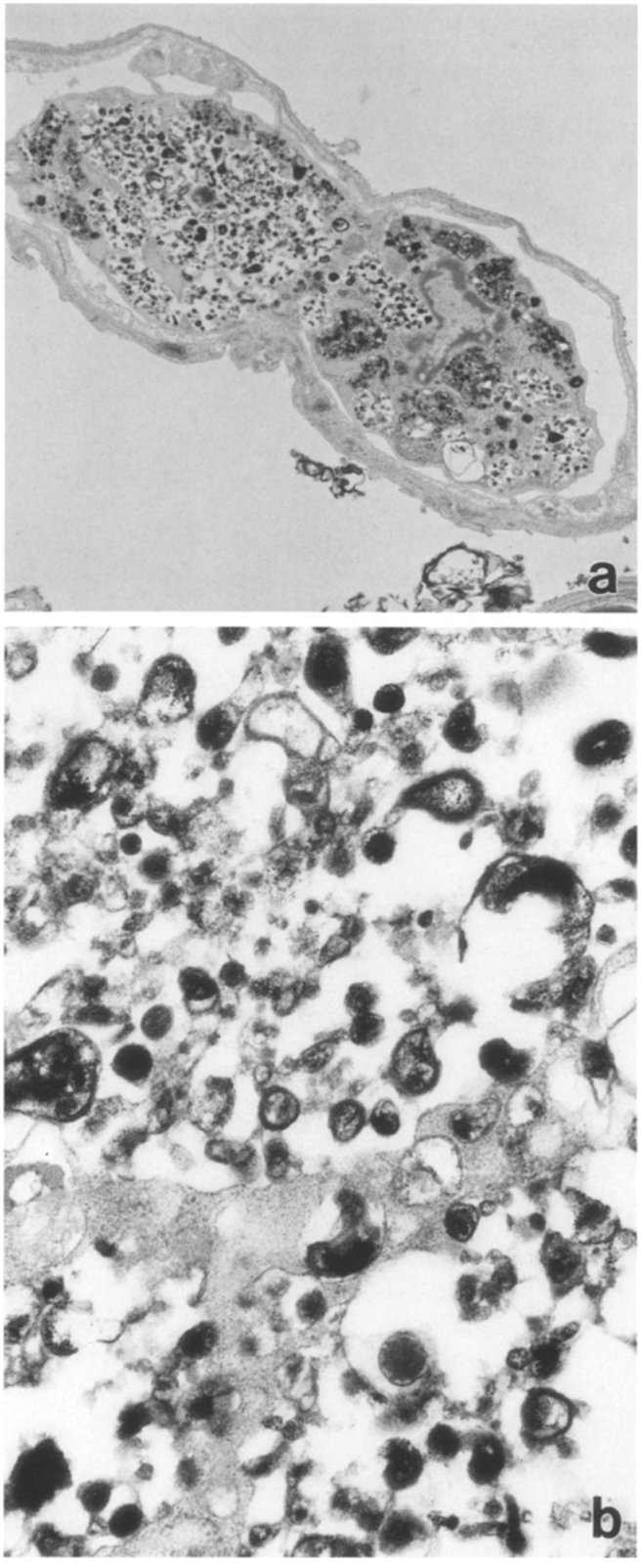

Fig. 8 After day 60, polymorphonuclear leukocytes are swollen with multivesicular and granular bodies (**a**). They often occlude the vascular lumen and possess short processes which attach to the endothelium. Multilamellar vesicles and granules reside within membrane-bound vacuoles (**b**). **a** ×6,700; **b** ×37,000

tilamellar structures. In younger animal, these cells were adherent to the endothelium by cytoplasmic projections and thus may be scavenger cells that engulf and remove these inclusions from the endothelium.

At day 20, macrophages in the alveoli were loaded with empty vacuoles, but by day 40, they became filled with multilamellar inclusions. Only a few vesiculogranular structures were present but there were no substructures suggesting a transition from vesiculogranular to lamellar. Within alveolar spaces, numerous multilamellar structures and tubular myelins were scattered separately or intermingled. However, the vesiculogranular structures as seen in endothelial cells and type I pneumocytes were seldom noticed in the alveolar spaces. Unfortunately, we have not been able to conduct a histochemical study to reveal the nature of these contents present at different stages. Morphologically, however, empty vacuoles with occasional low electron density at day 20 probably represent neutral fat and multilamellar structures of day 50 and 60 phospholipids. It seems unlikely that these structures are related to each other and have a direct relevance to this storage disease process. With the different morphologies of the inclusions at later stages, it is difficult to deny completely that the multilamellar materials of alveolar macrophages originated from lamellar bodies secreted by type II pneumocytes and phagocytized by macrophages, and that they had no relation with vesiculogranular bodies of endothelial cells and/or type I pneumocytes. In normal conditions, lamellar bodies of the surfactant secreted from type II pneumocytes may give rise to multilamellar bodies, tubular myelins and then invisible free molecules within the alveolar lining layer [10]. Finally, they constitute a surface molecular film to act as a surfactant, reducing surface activity. There may be several routes by which surfactant molecules are cleared from or catabolized within the lungs [9]. Some may be eliminated by mucociliary escalator system of bronchiolar and bronchotracheal trees, but some disposed by alveolar macrophages or transported across alveolar epithelia to the lymphatics, and some others recycled in the type II pneumocytes. In Niemann-Pick disease, surfactant materials may be disposed in the same way, but alveolar macrophages seem to play an important role in such a disposal function. This hypothesis may be supported by the fact that lamellar structures in macrophages increased with time whereas the amount in type II pneumocytes was constant during the entire course. In this context, then, our findings tempt us into further speculation that surfactant phospholipid metabolism may be impaired in these macrophages. Obviously, further extensive biochemical studies are needed in this respect.

Our findings are in contrast to those reported by Elleder et al. [4] in the liver of NP-C patients. In their study, vesicular or vesiculogranular structures with dense cores, which are somewhat similar to those seen in our mice, were prominent in Kupffer cells, a type of macrophages, and membranolucent or annulolamellar structures were numerous in hepatocytes. However, the pure

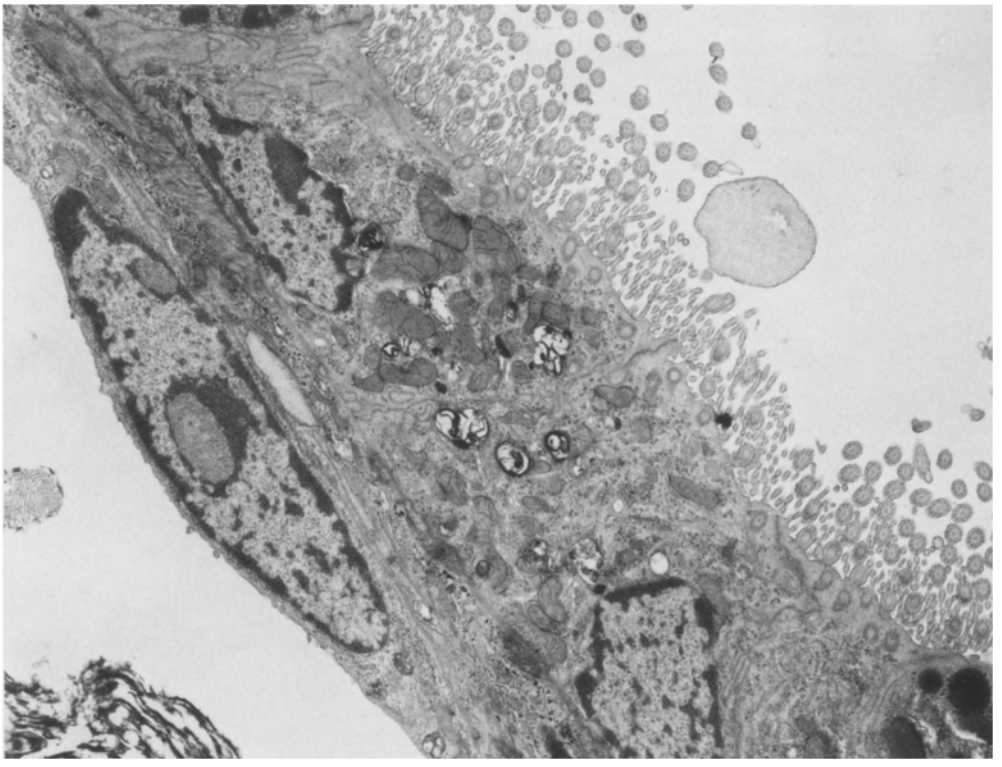

Fig. 9 Ciliated epithelia of the bronchioles exhibit the presence of small multilamellar inclusions and multivesiculo-granular bodies. ×8,600

lamellar inclusions found in our macrophages were not seen either in their macrophages or hepatocytes. This could be explained by either tissue difference or species difference, or may represent the heterogeneity in NP-C disease.

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