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Pathogenesis of pulmonary fibrosis induced by chrysotile asbestos

Longitudinal light and electron microscopic studies on the rat model

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Summary. A single instillation of 1 mg chrysotile B with a fiber length between 0.05 and 0.2 μm in 0.1 ml tricaprylin was made via a polyvinyl catheter into the lower lobe of the right lung of 120 six-week-old Wistar rats under anesthesia. The animals were killed at intervals between five minutes and two years. The lower lobes of the right lung were investigated by light and electron microscopy.

The process of pulmonary fibrosis induced by asbestos can be subdivided into four phases: these are the phase of phagocytosis (five to 15 min), the phase of granuloma formation (between one and two weeks), the phase of septal fibrosis (between two and six months) and finally the scar stage (after one year). After instillation of small asbestos fibers into the alveoli, a major proportion of these fibers is phagocytosed by alveolar macrophages after five minutes and leaves the lungs via the airways. A proportion of the fibers penetrates through the alveolar wall (mostly conveyed by type I pneumocytes) and reaches the interstitium of the lungs. There, the fibers are taken up by pulmonary tissue macrophages and giant cells. Within the phagolysosomes, the fibers are broken down into fragments less than 0.01 μm in length. Type II pneumocytes produce surfactant in excess. These cells become necrotic, tubular myelin and lamellar bodies pass into the alveoli and into the interstitium. Surfactant is phagocytosed by resident macrophages. These macrophages can break down. Besides asbestos and surfactant, mediators of fibrillogenesis are released. Macrophages following up from blood monocytes ingest surfactant and asbestos. This process is perpetuated up to complete scarring. After two years, small asbestos fibers less than 0.01 μm long are present in fibroblasts and pleural mesothelia.

Key words: Pulmonary fibrosis – Asbestosis – Chrysotile – Macrophages – Electron microscopy

Introduction

"Asbestosis" is a form of pneumoconiosis which has been a separate disease entity for more than 60 years. The first description of pulmonary fibrosis due to asbestos dust was published by Cooke (1924). He initially published a short note, and then in 1927 a detailed description of the disease course and the autopsy finding in a woman who died at the age of 33 years and who had worked for 13 years in an asbestos factory. He termed the lung changes "pulmonary asbestosis". Subsequent papers (Gloyne 1933; Egbert 1935; Heard and Williams 1961; Hourihane and McCaughey 1966) discussed whether the pathogenesis of pulmonary fibrosis is elicited by mechanical irritation by the asbestos needles or by chemical induction due to magnesium silicate. This question is still unanswered today. In 1960, Wagner et al. recognized the connection between inhalation of asbestos dust and development of mesotheliomas of the pleura and peritoneum.

Cooke (1924; 1927) had already seen the structures which are today termed "asbestos bodies" in his case of pulmonary asbestosis: he described them as "curious bodies". However, these bodies were mentioned for the first time by the German pathologist Felix Marchand, who reported "peculiar pigment crystals" in 1906 before the German Pathological Society. In a paper accompanying Cooke's publication (1927), McDonald described the "foreign bodies" very precisely. He mentioned the distinct yellowish-brown color, the lack of stainibility with anilin dyes and the positive prussian blue reaction of the surrounding "plasmodial masses" with potassium ferrocyanide. Stewart (1928) designated the bodies as "asbestosis bodies". Since the structures were also seen in asbestos workers without pulmonary fibrosis, he shortened the term to "asbestos bodies". Reviews on this topic are to be found at Davies (1964); Gaenssler and Addington (1969) and Becklake (1976).

Stewart (1930) performed the first very simple animal experiments for clarification of the pathogenesis of an asbestos fibrosis: he exposed guinea pigs to the dust from asbestos factories: meticulous experiments with different kinds of asbestos and different applications were first undertaken by Vorwald et al. (1951). The majority of the subsequent animal experiments simulate the conditions of human asbestos exposure. Rodents were exposed to inhalation of asbestos in dust chambers for different lengths of time (Davis 1963; Holt et al. 1964; 1966; Adee and Laidler 1973; Wagner et al. 1974; Tetley et al. 1976; Brody and Hill 1981; Brody et al. 1981; 1983; Barry et al. 1983, Ogisho et al. 1984). Begin et al. (1981; 1982) gave intratracheal injections of asbestos to sheep. Experiments with intrapleural and intraperitonal asbestos injections for induction of tumors as well as the problem of asbestos-induced carcinomas and mesotheliomas will not be dealt with here.

The pathogenesis of pulmonary fibrosis due to asbestos, the nature and origin of the cells involved and the fate of the asbestos fibers in the lungs can only be elucidated in long-term investigations in animal models. Despite the imitation of extrinsic conditions in humans, exposure of rats to asbestos in dust chambers has several disadvantages. The amount of asbestos inhaled

is difficult to control. Rats breathe through the nose, which is why a large part of the asbestos dust is already trapped in the upper airways. The mucociliary clearance in the trachea is very rapid: back-transport amounts to several millimeters per minute (Berke and Roslinski 1971). In inhalation, most asbestos fibers do not reach the pulmonary alveoli, but are trapped in the bifurcations fo the bronchioles (Brody et al. 1981). Even dusting of all parts of the lungs of the experimental animals leads to adverse effects on respiration and pulmonary circulation, so that most animals cannot be kept under observation for two years. It is sufficient to follow the development of pulmonary fibrosis induced by asbestos and the fate of the introduced asbestos fibers in *one* lobe of the lungs. For this reason, the method of a single intrabronchial instillation of asbestos in tricaprylin by means of a polyvinyl catheter into the right lower lobe of the lung of the Wistar rat with subsequent light and electron microscopic investigations at time intervals between five minutes and two years was chosen for these investigations.

Methods

Asbestos. Of the different kinds of asbestos, the main types amosite, anthophyllite, crysotile and crocidolite are processed industrially, chrysotile accounting for 90%. The experimental investigations were therefore only carried out with chrysotile. The serpentine asbestos chrysotile is a fibrous hydrated magnesium silicate ($3MgO.\ 2SiO_2.\ 2H_2O$) with 43.5% SiO_2, 43.5% MgO and 13% H_2O (Miller 1978). The fibers consist of tubular crystals with cylindrically curved, electron microscopically translucent layers and a fine central capillary (Holt et al. 1966; Langer et al. 1974). Whittaker (1956) distinguishes various crystallographic forms (clino-chrysotile, para-chrysotile, ortho-chrysotile). The fibers occurring in chrysotile have different lengths between 2 mm (Miller 1978) and 0.25 µm (Marconi et al. 1984). The diameter varies between 0.4 and 0.05 µm, the mean fiber length is between 0.08 and 1.6 µm, and light microscopic detection is limited at 0.4 µm. 70% of the chrysotile fibers in asbestos dust are shorter than 0.25 µm and can thus be rendered visible neither in the light microscope nor in the scanning electron microscope (Rood and Streeter 1984). Long fibers are already held back in the upper airways in inhalation, and do not play any role in the reaction of the alveolar wall. For the trials described here, ground Canadian chrysotile B with a fiber length of 0.05 – 0.2 µm (Fig. 1) in accordance with UICC standard reference samples of asbestos (Timbrell et al. 1968) was used.

Animal model. 120 Wistar rats of both sexes (six weeks old and bred in sibling pairs at the Borstel Research Institute) received a single instillation of 1 mg chrysotile B in 0.1 ml tricaprylin (Serva) via a sterile Portex polyvinyl catheter PP 60 (Portex Ltd., Hythe, Kent, GB) into the lower lobe of the right lung under Ketavet anesthesia (Parke-Davis). The glycerol tricaprylate (tricaprylin) has da depot function for chrysotile and is intended to prevent transport away of the asbestos fibers via the airways. Then rats of the same age received only tricaprylin administered in the same way as a control. To test the tissue reaction after the instillation of chrysotile, two animals each were killed and autopsied after 5, 10, 15, 30, 60, 120, 180 min and after 24 h. Afterwards, one animal was killed and autopsied per week between one week and 24 months. The control animals (only instillation of tricaprylin) were killed after 5, 30, 180 min, 24, 72, 120 h, 1, 4, 23 weeks and after one year. All animals were killed in ether.

Light and electron microscopic investigations. From each autopsied animal, lungs, heart, lymph nodes, spleen, liver and kidneys were embedded in Paraplast after conventional dehydration and examined by light microscopy (stains: haemalum-eosin, van Gieson, Turnbull blue reaction). For electron microscopic investigations (transmission electron microscopy), small tissue

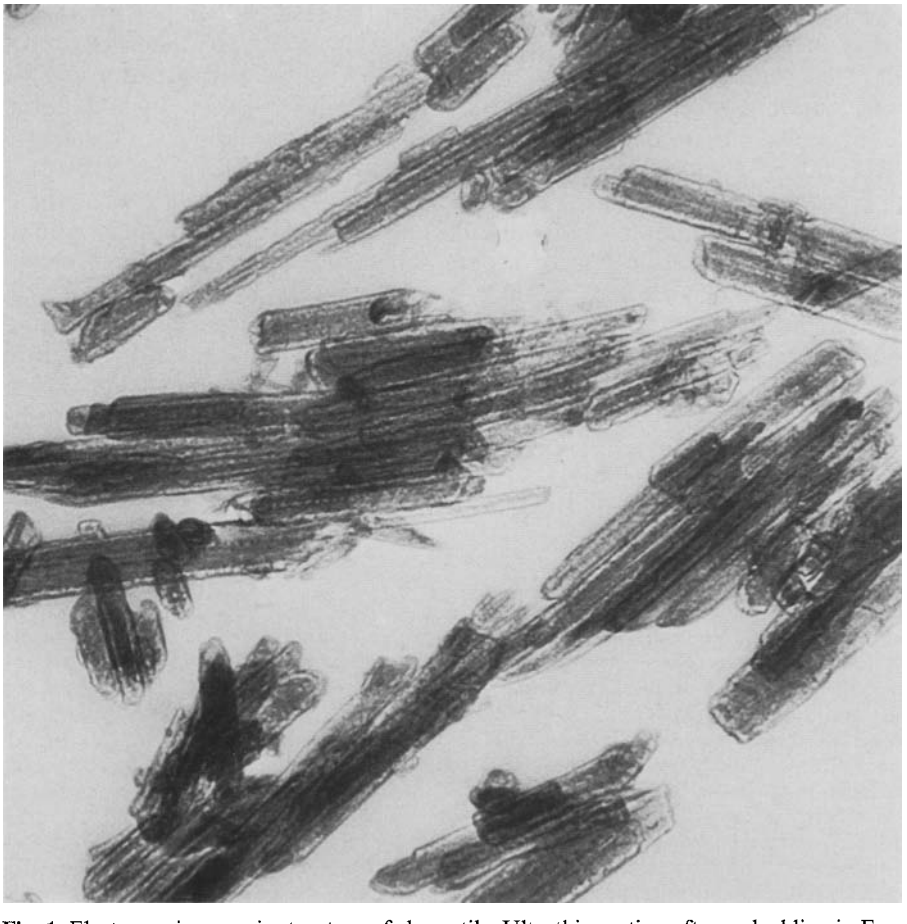


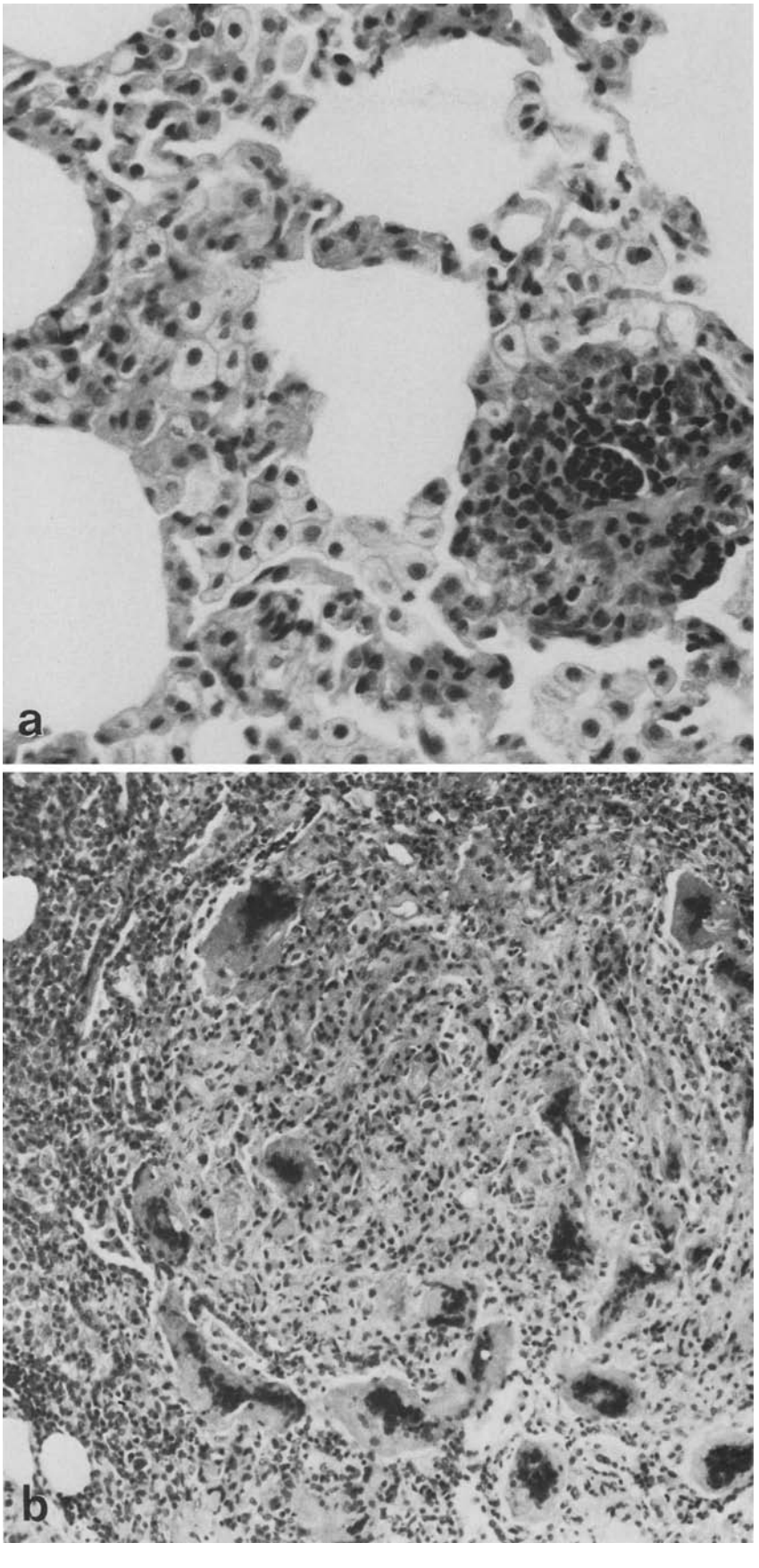
Fig. 1. Electron microscopic structure of chrysotile. Ultrathin section after embedding in Epon. No fixation, no contrasting. TEM, magnification × 70,000

blocks from the lungs, the thoracic lymph nodes and the spleen were fixed in osmium tetroxide immediately after killing the animals, washed out, blockcontrasted with uranyl acetate, dehydrated and embedded in Epon. The ultrathin sections were postcontrasted with lead citrate and examined in the EM 300 (Philips) transmission electron microscope.

Results

Light microscopic investigations. Under the light microscope, the lungs of the animals killed 5, 10 and 15 min after the instillation of chrysotile show

Fig. 2a–d. Light microscopic details from the right lower lobe of the rat after instillation of chrysotile. **a** after five minutes. Phase of phagocytosis. Numerous alveolar macrophages in the alveoli, few lymphocytes. Haemalum-eosin, magnification × 480. **b** After two weeks. Granuloma formation with numerous giant cells of foreign-body type. Haemalum-eosin, magnification × 320. **c** After three months. Septal fibrosis interspersed by dilated bronchioli and alveoli. Haemalum-eosin, magnification × 120. **d** After two years. Scar stage, remnants of macrophages and pneumocytes. Haemalum-eosin, magnification × 480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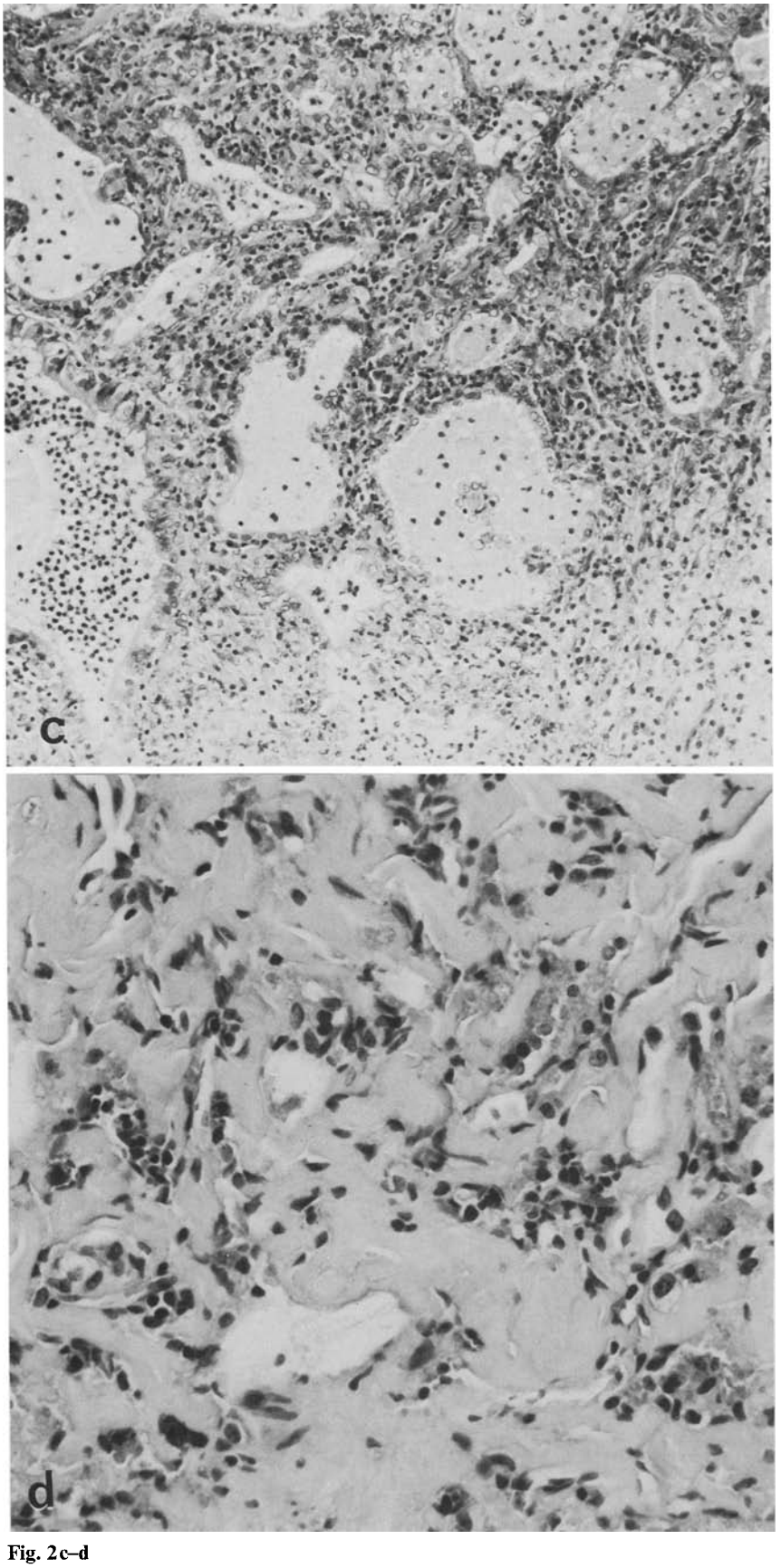


Fig. 2c–d

large numbers of alveolar macrophages in the alveoli (Fig. 2a). In some cases, these cells adhere to the inside of the septa, and in other cases they completely fill the alveoli. Moreover, small accumulations of lymphocytes occur within the septa, and complete granulomas cannot yet be detected. In the lungs of animals killed after one and two weeks, circumscribed granulomas of mononuclear macrophages, multinuclear giant cells of foreign-body type and lymphocytes (Fig. 2b) are found in the interalveolar septa. In these granulomas, formation of collagenous fibers is already shown. The nodules are frequently situated in the vicinity of small bronchi, but not in the bronchial walls. After two months, these granulomas have disappeared. The lung tissue now shows the picture of chronic interstitial inflammation with fibrosis (Fig. 2c). The tissue is interspersed with connective tissue strands of different widths. Between these, there are dilated respiratory bronchioli and parvocystic alveoli. The interstitium is infiltrated by mononuclear macrophages, lymphocytes and plasmacytes. The dilated alveoli which are left behind contain alveolar macrophages, lymphocytes and desquamated pneumocytes. After one year, the right lower lobe of the lung shows the picture of complete scarred fibrosis (Fig. 2d). Between broad bundles of collagenous connective tissue fibers, there are residues of respiratory epithelium, some mononuclear macrophages and a few lymphocytes. Alveoli and bronchi are no longer to be discerned. At no time in the investigations can stuctures be detected in the pulmonary tissue which might be designated as "asbestos bodies". The lungs of the control animals in which only tricaprylin was instilled show a moderate increase of intra-alveolar macrophages in the first hours under the light microscope. Giant cells are lacking. After 24 h, the lungs are once more unremarkable. No alterations are found in the intrathoracic lymph nodes and in the large parenchymal organs.

Electron microscopic investigations. The electron microscopic investigation carried out chronologically in parallel reveals the location of the chrysotile in the individual phases of the pulmonary alterations. In the first three hours after instillation of chrysotile, the alveoli contain needlelike asbestos fibers situated singly or in bundles. Due to the respiratory excursion of the alveolus, these fibers penetrate the alveolar wall at circumsribed sites which are evidently fortuitous; type I pneumocytes are injured in this perforation (Fig. 3). In the damaged alveolar epithelium, the fibers are not ingested into phagovacuoles or phagosomes, but they are situated free in the cytoplasm. The smooth endoplasmic reticulum in the immediate vicinity is enlarged. Moreover, a rough endoplasmic reticulum with ribosomes is formed in the vicinity of the fibers. Lysosomes are absent (Fig.3). Occasionally, fibers which have penetrated into the cell nuclei are also seen in the alveolar epithelia. Intra-alveolar asbestos fibers are already phagocytosed by alveolar macrophages in the first 30 min. The fibers ingested by phagocytosis into these cells are very much smaller than the intra-epithelial structures. In the alveolar macrophages, the fibers are first of all situated freely in the cytoplasm (Fig. 4a), and then they are engulfed by phagovacuoles

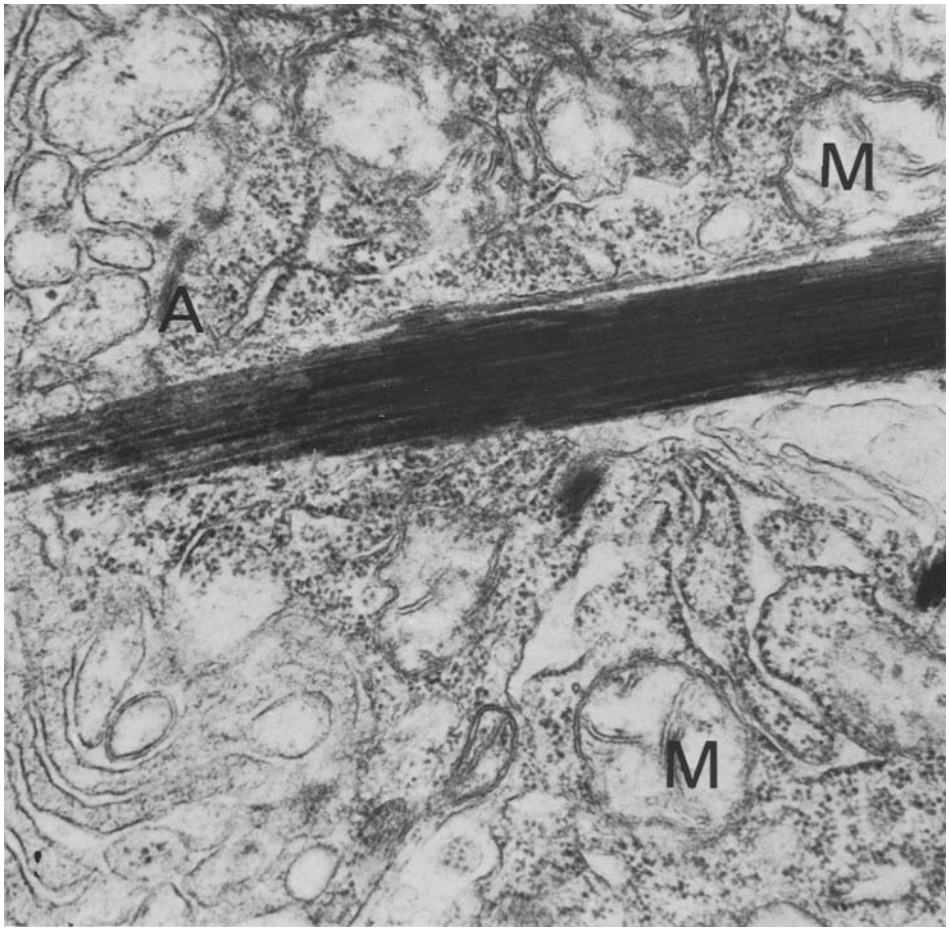
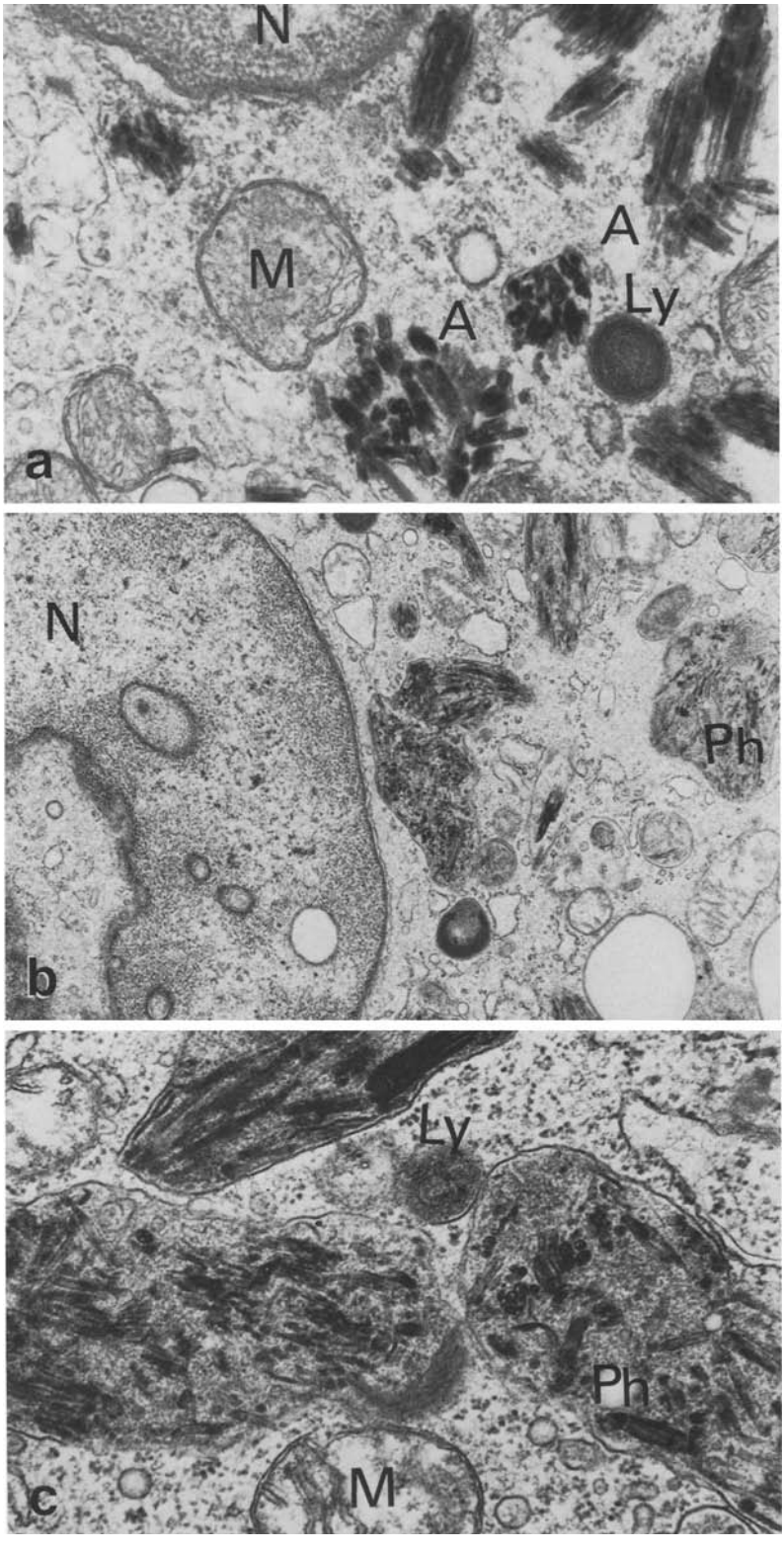


Fig. 3. Electron microscopic details from the lower lobe of the right lung ten minutes after instillation of chrysotile. Bundled asbestos fibers which have penetrated into a type I pneumocyte (*A*). *M*, mitochondria. Magnification × 35,000

with a monocontoured membrane (Fig. 4b). Lysosomes are closely apposed to the phagosomes, and a fusion to phagolysosomes (type II lysosomes) appears possible (Fig. 4c). In the phagosomes, the fibers are enveloped in a fine dense granular substance, and larger fibers appear to break down into small fragments (Fig. 4c).

After penetrating the alveolar wall, the fibers pass into the tissue of the lung skeleton. In the phase of granuloma formation (two weeks after

Fig. 4a–c. Electron microscopic details from alveolar macrophages one hour after instillation of chrysotile. **a** Free asbestos particles (*A*) in the cytoplasm, mostly smaller than mitochondria (*M*). *Ly*, lysosome. *N*, nucleus. Magnification × 18,000. **b** Asbestos particles in phagosomes (*Ph*). *N*, nucleus. Magnification × 11,000. **c** Besides phagosomes (*Ph*) with small fragments of asbestos fibers, a lysosome (*Ly*). *M*, mitochondrion. Magnification × 35,000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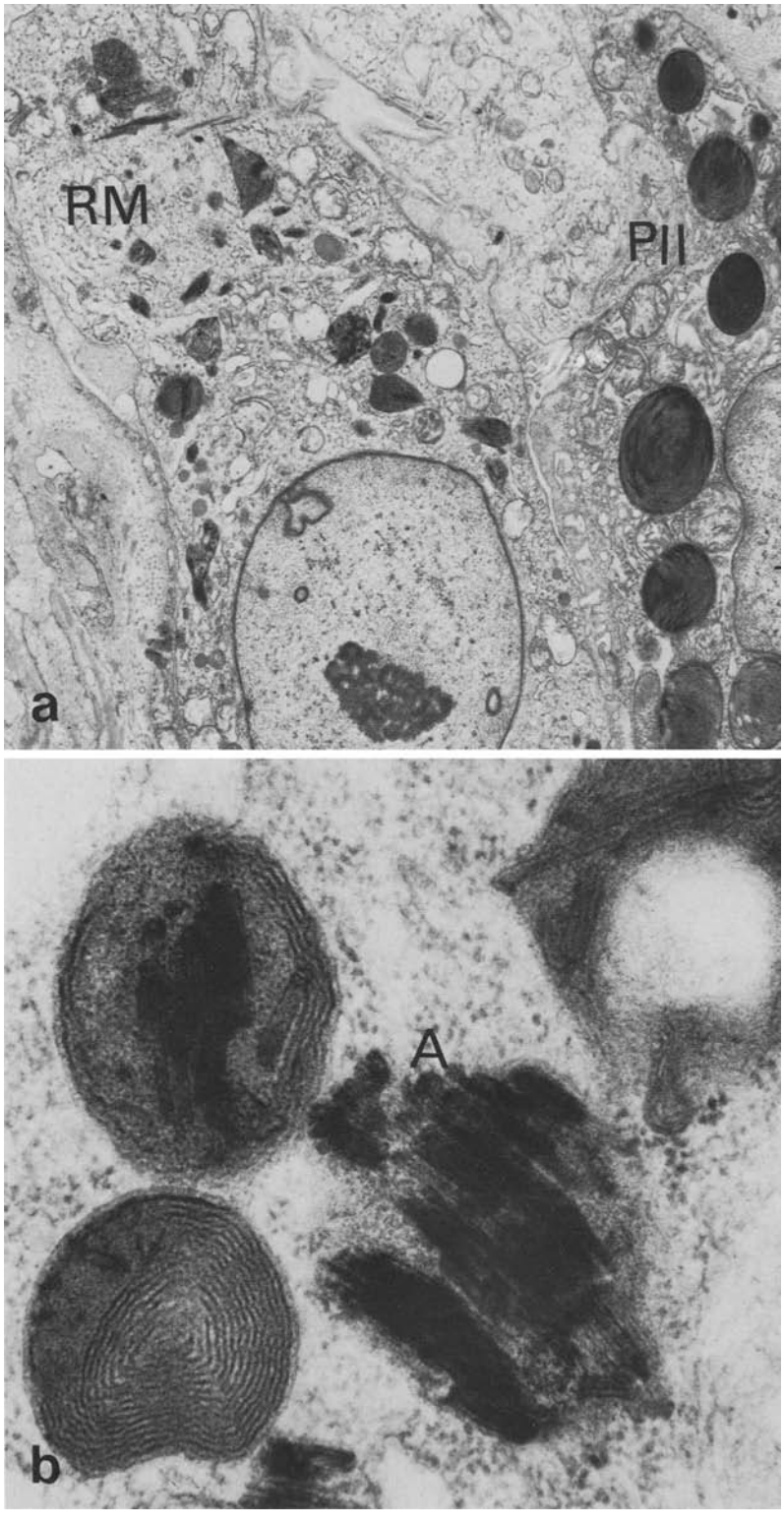
RM
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instillation) only fiber fragments up to 0.2 μm in length are seen. These structures are ingested by giant cells and by mononuclear macrophages (pulmonary tissue macrophages). The fibers are initially also freely suspended in the cytoplasm in these cells, or in the lumina of the enlarged endoplasmic reticulum. Moreover, in the resident macrophages another process of cellular struggle with the foreign substances is manifested. Around small groups of fiber fragments, membranes in a parallel annular arrangement develop with a dense finegranular interstitial substance (Fig. 5b). Between these concentrically layered membranes, chrysotile is evidently largely dissolved, and lamellar residual bodies are left behind.

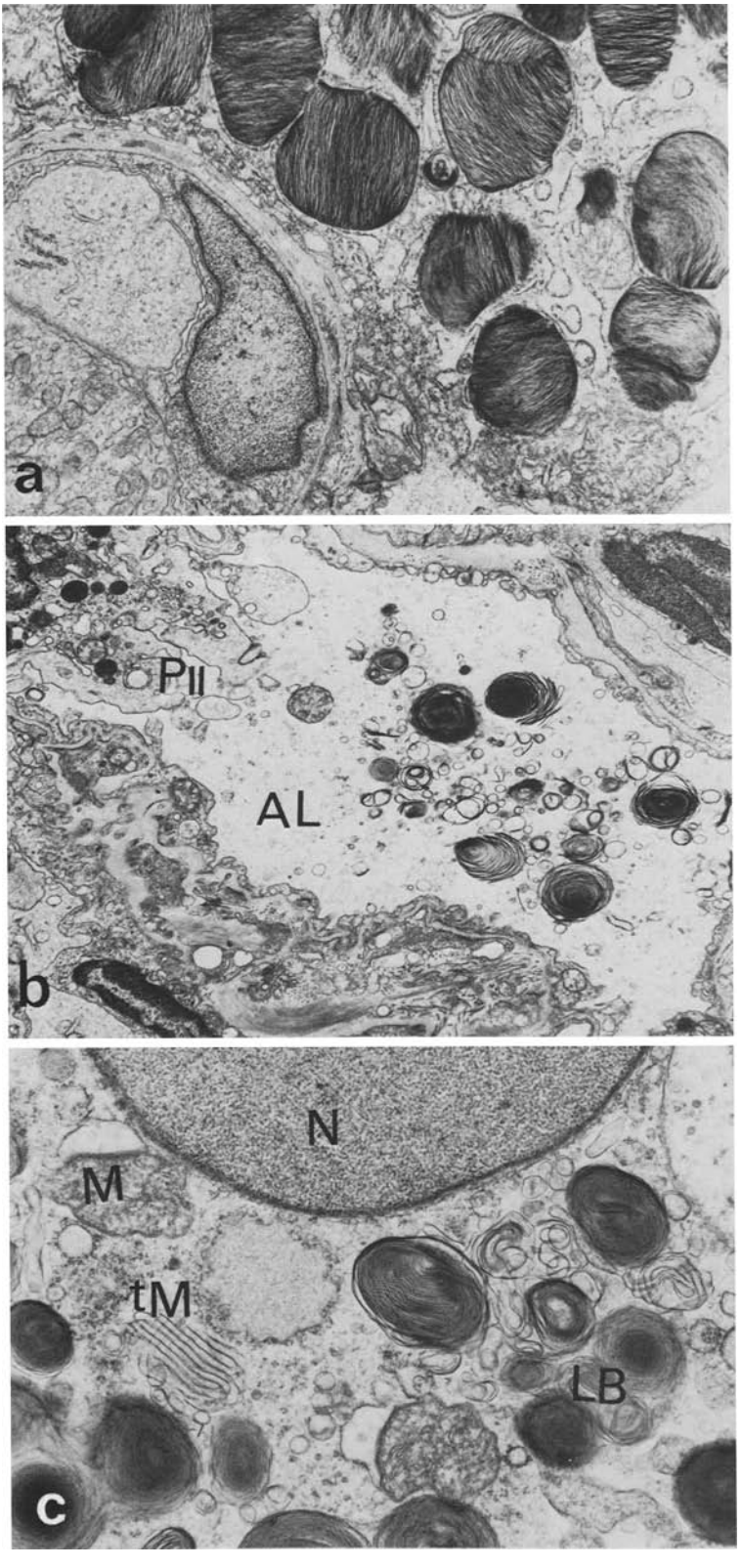
In the phase of fibrosing (two month after the instillation), many resident macrophages with fragments of asbestos fibers in phagosomes and phagolysosomes are situated within the widened septa (Fig. 5a). The remaining type II pneumocytes are severely swollen, and in the cytoplasm there are numerous very large lamellar bodies with surfactant (Fig. 6a). The overloaded epithelial cells become necrotic, lamellar bodies and tubular myelin pass into the lumina of the alveoli (Fig. 6b). This material is in turn phagocytosed by alveolar macrophages, but not degraded. The cytoplasm of the macrophages contains free tubular myelin and free lamellar bodies. Lysosomes do not come into contact with these structures, and phagosomes and phagolysosomes are absent (Fig. 6c). Many of the alveolar macrophages break down, so that lamellar bodies and tubular myelin once more pass into the alveolar lumina. The process perpetuates itself. The tissue still contains fragments of asbestos fibers in the final cicatriceal stage (between one and two years after the instillation). These are located in some cases in the cytoplasm of macrophages engulfed in the scar tissue, and in some cases even free in the tissue. These may derive from necrotically destroyed macrophages. Two years after the instillation, small asbestos fibers are seen in the cytoplasm of fibroblasts and within the mesothelial cells of the visceral pleura (Fig. 7a, b).

In all cases of development of a pulmonary fibrosis, the asbestos fibers are found to be uncoated. Coated fibers in the sense of "asbestos bodies" cannot be demonstrated in the rat. In the thoracic lymph nodes and in the spleens of the experimental animals, asbestos fibers cannot be detected by electron microscopy.

Discussion

The staggered investigations over a period of two years after a single instillation of 1 mg chrysotile into the lower lobes of the right lungs of the rat provide information on the following questions: Which cells are involved

Fig. 5a, b. Electron microscopic details from the interstitium of the lungs two months after instillation of chrysotile. **a** Resident macrophage (*RM*) with phagosomes and phagolysosomes with fragments of asbestos fibers. Besides this, type II pneumocyte (*P II*) with abundant lamellar bodies. Magnification × 7,100. **b** Detail from a resident macrophage. Formation of residual bodies around fragments of asbestos (*A*). Magnification × 53,000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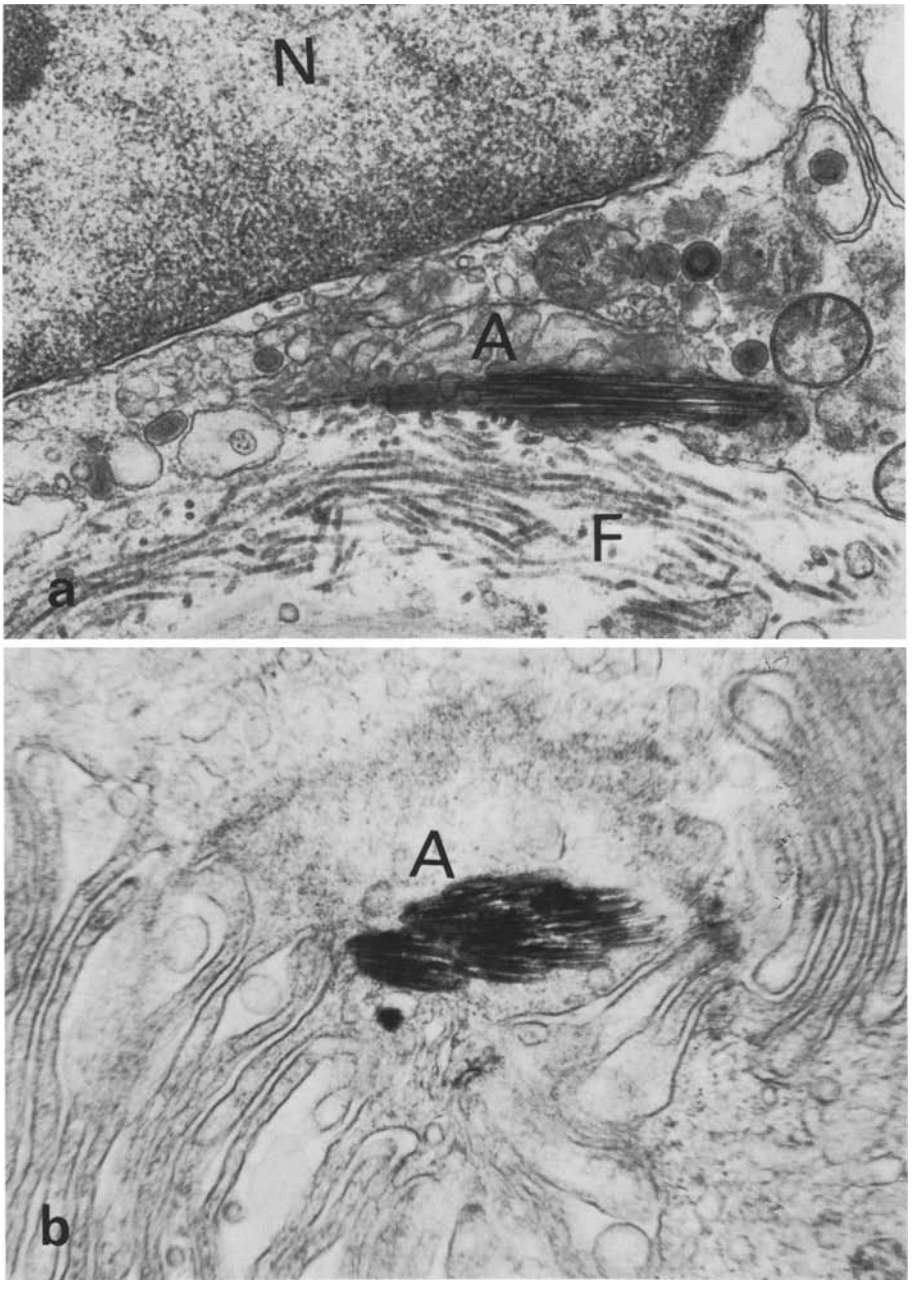

Fig. 7a, b. Electron microscopic detail from the right lower lobe two years after instillation of chrysotile. **a** Asbestos fibers (*A*) in a fibroblast. *F*, fibrils, *N*, nucleus. Magnification × 14,000. **b** Fragments of asbestos fibers (*A*) in a mesothelial cell of the pleura. Magnification × 14,000

Fig. 6a–c. Electron microscopic details from the right lower lobe four months after instillation of chrysotile. **a** Section of a type II pneumocyte with large lamellar bodies. Magnification × 11,000. **b** Alveolar wall with degrading type II pneumocyte (*P II*). In the alveolus, there are lamellar bodies and tubular myelin. Magnification × 7,100. **c.** Detail from a macrophage after phagocytosis of tubular myelin (*tM*) and lamellar bodies (*LB*). *N*, nucleus, *M*, mitochondrion

in the formal genesis of pulmonary fibrosis induced by asbestos? Does the genesis of the fibrosis depend on the size of the asbestos fibers? Where do the introduced asbestos fibers remain during the two-year observation period?

In the pathogenesis of asbestos fibrosis, four phases can be distinguished. These are firstly the phase of phagocytosis by alveolar macrophages (onset five to 15 min), secondly the phase of granuloma formation (between one and two weeks), thirdly the phase of septal fibrosis (between two and six months) and fourthly the scar stage (after one year).

Within the first 30 min after chrysotile instillation, there is an accumulation of macrophages in the alveoli. These derive from the monocytes of the blood (Van Furth 1980), and their accelerated passage into the pulmonary alveoli proceeds in parallel with an increased formation of monocytes in the bone marrow (Adamson and Bowden 1980). Fibers of a length between 0.2 μm are at first freely suspended in the cytoplasm in the macrophages or in phagovacuoles which may fuse with lysosomes to form phagolysosomes (type II lysosomes). Within such organelles, the fibers are broken down into smaller fragments with a length of less than 0.01 μm. The comparable results in the literature are not uniform. Davis (1967), observed fibers in the alveolar macrophages within 30 min after asbestos injection in organ cultures of lungs isolated from guinea pigs. He interprets free fibers in the cytoplasm as resulting from a rupture from the phagosomes. Ogisho et al. (1984) saw only crocidolite in the alveolar macrophages but chrysotile in the vessel walls in rats after intermittent inhalation of crocidolite and chrysotile. According to Hilscher (1972), large asbestos fibers over 1.7 μm long cannot be phagocytosed and transported away and are therefore thought to produce fibrosis mechanically. The intra-alveolarly situated macrophages containing chrysotile frequently have no contact with the alveolar wall, and they are without significance for the genesis of pulmonary fibrosis. A proportion of these cells leaves the lungs via the airways, and other cells break down within the alveoli, chrysotile being released again.

In consequence of the respiratory excursions of the lungs, chrysotile fibers pass from the alveolar lumina into the interstitium. This is the prerequisite for the development of fibrosis. The epithelial cells of the inner alveolar wall are mechanically penetrated by the asbestos fibers. The cells themselves are not capable of phagocytosis. The asbestos fibers which have penetrated are not taken up into phagovacuoles or phagosomes, and lysosomes do not occur. Barry et al. (1983) observed asbestos fibers both in macrophages and in the alveolar epithelium in rats one day after inhalation of chrysotil. Occasional fibers are alleged to be visible in the type I pneumocytes as early as one hour after inhalation (Brody et al. 1981). In the pneumocytes, the asbestos fibers are not membrane bound, but they are transported through the epithelial cells into the interstitium by microfilaments containing actin (Brody et al. 1983). Davis et al. (1974) was able to demonstrate penetration electron microscopically neither through, nor between the epithelial cells of the rat intestine after feeding asbestos in butter. The process of penetration evidently depends on the thickness of the epithelial cells. For

this reason, penetration of the flat type I pneumocytes is more frequent than penetration of the more voluminous type II pneumocytes.

In the interstitium, the asbestos fibers are phagocytosed by the pulmonary tissue macrophages. Fiber fragments with a length of less than 0.01 µm are engulfed in phagosomes and broken down at least in part. The fibers become smaller and more transparent. Such a fiber breakdown depends on fusion with lysosomes and an activation of lysosomal enzymes. Davis et al. (1974) observed a rise in the lysosomal enzymes N-acetyl-β-D-glucosaminidase, β-glucuronidase and β-galactosidase in the culture medium in cell cultures of peritoneal macrophages of the mouse after exposure to chrysotile. Tetley et al. (1976) were able to measure an increase of cathepsin D, cathepsin B 1, lipase, acid phosphatase, ribonuclease and β-glucuronidase in the supernatant after lavage of asbestos-ventilated rat lungs. The release of hydrolases from macrophages in vitro is also possible with chrysotile, from which the magnesium was dissolved out with hydrochloric acid (Morgan et al. 1977). According to the investigation results presented here, the chrysotile fibers are broken down into smaller fragments with fiber lengths under 0.01 µm, but not completely degraded within two years. There are divergent answers in the literature to the question as to whether inhaled asbestos fibers might be broken down in the lungs in the course of time. Ashcroft and Hepplestone (1973) were unable to detect any breakdown of coated and uncoated fibers in the course of years. When asbestos fibers remain in the tissue for a long time, there may be a loss of MgO in the fibers (Langer et al. 1970; Pooley 1972b; Höhr and Friedrichs 1982; Jaurand et al. 1984). The loss of magnesium is probably the reason why the fiber fragments become more transparent electron microscopically in the resident macrophages.

The pulmonary tissue macrophages have a stimulatory effect on the development of pulmonary fibrosis. Macrophages release mediators which stimulate the fibroblasts to fibrillogenesis. The mediators include MSFA = "macrophage dependent fibroblast-stimulating activity" (Leibovich and Ross 1976) and MGF = "macrophage growth factor" (Naum et al. 1979). The process is not completed with phagocytosis of the instilled chrysotile. For two reasons, the process of fibrosis is perpetuated up to complete scarring. On one hand, macrophages can decompose again after phagocytosis of asbestos, and chrysotile is released together with the mediators of fibrillogenesis, and monocytes may pass into the lung tissue once more as macrophages from the bloodstream and the process is repeated. On the other, the increasing rigidity of the alveolar wall leads to a reactive proliferation of type II pneumocytes and an overshoot production of surfactant (Fasske and Morgenroth 1983). By the breakdown of type II pneumocytes, large amounts of surfactant pass into the lung tissue both as tubular myelin and as lamellar bodies. Tetley et al. (1976) were able to measure a rise of surfactant in the lavage fluid 15 to 20 weeks after asbestos exposure. Barry et al. (1983) observed an increase and swelling of the type II pneumocytes in rat lungs after inhalation of chrysotile. There are physiological interactions between the type II pneumocytes and the macrophages. An

overproduction of surfactant is compensated by phagocytosis (Zeligs et al. 1977; Nichols 1980; Fasske and Morgenroth 1983). The macrophages do not degrade surfactant, and they leave the lungs via the airways under physiological conditions. Within the fibrous tissue, the macrophages break down, surfactant is once more released into the tissue, monocytes leave the bloodstream, and the process begins anew. In this macrophage breakdown caused by surfactant phagocytosis, the mediators of fibrillogenesis are also released. Asbestos bodies are not involved in the development of pulmonary fibrosis. Vorwald et al. (1951) had already pointed out on the basis of comparative light microscopic investigations that numerous asbestos bodies are only to be found in human asbestosis, very few in rats, guinea pigs, cats and rabbits, and none in dogs. Moreover, asbestos bodies are only formed with a fiber length of more than 10 μm (Pooley 1972). The fibers are then enveloped in a protein coat containing iron: the iron components derive from the erythrocytes. Chrysotile not only has a cytotoxic effect, it also has a haemolytic action (Robock and Klosterkötter 1970). Light microscopic detection of asbestos bodies indicates only the uptake of asbestos into the lungs. The amount and the distribution of the asbestos fibers can only be demonstrated electron microscopically.

The results of the investigations allow the questions on which the study was based to be answered as follows. The development of fibrosis after uptake of asbestos into the lungs results from an interaction between macrophages and fibroblasts. The macrophages do not only phagocytose asbestos fibers, but also the surfactant which is formed in excess. With the macrophage breakdown, mediators of fibrillogenesis and phagocytosable material are released. Monocytes stream in from the circulation, and the process is perpetuated over months. This process is not triggered mechanically and therefore has nothing to do with the fiber size. The larger fibers which have penetrated through the alveolar wall are broken down in the macrophages into small fragments of less than 0.01 μm. Such fiber fragments can still be found in fibroblasts and in pleural mesothelia after two years.

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