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A STUDY BY ELECTRON MICROSCOPY OF THE EFFECTS OF CHRYSOTILE AND MAN-MADE MINERAL FIBRES ON RAT LUNGS

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INTRODUCTION

In undertaking the experimental programme of the European Insulation Manufacturers Association contract with the IARC, Institute of Occupational Medicine and Medical Research Council Pneumoconiosis Unit, it was decided that further information could be obtained by transmission electron microscopy. This paper reports preliminary results on the effects of inhaled man-made mineral fibres on the ultrastructure of the rat lung. A comparison is drawn with the changes resulting from inhalation of chrysotile.

MATERIALS AND METHODS

Twelve adult SPF Fischer rats were used: two were exposed to an atmosphere containing 10 mg/m^3 of each dust for seven hrs a day for five days each week for 50 weeks and then had a four-month post-inhalation period; two further animals were maintained under similar but dust-free conditions. The dusts used were UICC chrysotile B, glass fibre (Johns Manville Code 100), rock wool, resin-coated glass wool and uncoated glass wool. The physical dimensions of these dusts are reported elsewhere in this volume (Wagner et al.[1]). The animals were sacrificed by an overdose of anaesthetic (ether). The right lung was removed, cut into 2-mm

[1] See p. 363

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s and fixed by immersion in 3% buffered glutaraldehyde; 2-mm
e cubes were processed conventionally for electron microscopy.

RESULTS

The lungs of all exposed animals were characterized to variable
es by focal fibrosis, type II alveolar-cell proliferation, locali-
egeneration of the lining of the alveolar air space, macrophages
ining fibres and hyperplasia of alveolar and bronchiolar cells.

The extent of the fibrosis varied considerably (Figs 1-4). In
ly affected regions there were increased amounts of collagen in
nteralveolar septae compared with the normal lung (Fig. 1). In
ely affected areas the prominent collagenous masses were often
ascular and extended into adjacent alveolar walls, where the
li were compressed and lined by a few aberrant type II cells (Fig.
Fibrosis was evident with all the dusts but was more marked with
otile, although all cases showed the above spectrum of changes.

The areas of fibrosis were associated with increased numbers of
II alveolar cells. Following inhalation of chrysotile, the type
ls were often abnormal, with grossly distended rough endoplasmic
ulum and a large pale nucleus (Fig. 4). Animals exposed to other
had increased numbers of relatively normal type II cells; however,
gions where intra-alveolar accumulation of dust-laden macrophages
ed, a covering of abnormal type II cells was present (Fig. 8).
lal type II cells occasionally replaced the squamous epithelial
of respiratory bronchioles in fibrotic regions.

In the fibrotic lesions the type I alveolar cells were often
erate, the cell cytoplasm was oedematous and the cell membrane
the alveolus was occasionally ruptured.

In all exposed animals the intra-alveolar air space contained
lations of cellular debris and numerous myelin figures (Figs
; these were more marked following exposure to glass fibre.

Macrophages containing dust particles occurred in the alveolar air
, in the interstitium and perivascularly (Figs 2 & 5-8). The
hages filling the alveolar air spaces were associated with little
collagen, and an occasional giant cell was seen. The intra-
lar fibres were either membrane-bound, in secondary lysosomes
e in the cytoplasm. Free fibres that were seen occasionally
interstitial matrix were commonly chrysotile (Figs 7 & 10).
ing exposure to glass fibre, some of the macrophages appeared to
ergoing a process of lysis.

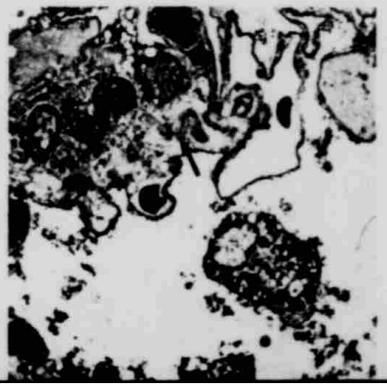
FIG. 1. ELECTRON MICROGRAPH SHOWING ADJACENT TYPE II CELLS OF RELATIVELY NORMAL APPEARANCE FOLLOWING INHALATION OF RESIN-COATED GLASS WOOL

The interalveolar septum contains slightly more collagen than normal (arrows) (× 3000).



FIG. 2. ELECTRON MICROGRAPH OF AN ALVEOLAR AIR SPACE CONTAINING MACROPHAGE AND CELLULAR DEBRIS

Perivascular thickening is evident (arrow) (glass fibre; ×1200).



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FIG. 3. ELECTRON MICROGRAPH OF A DEBRIS-CONTAINING ALVEOLAR AIR SPACE

The alveolar walls are considerably thickened (rock wool; × 3000).



FIG. 4. ELECTRON MICROGRAPH OF A COMPRESSED ALVEOLUS LINED BY ABERRANT TYPE II CELLS CONTAINING VACUOLES (ARROWS)

Collagen is prominent (chrysotile; × 1500).

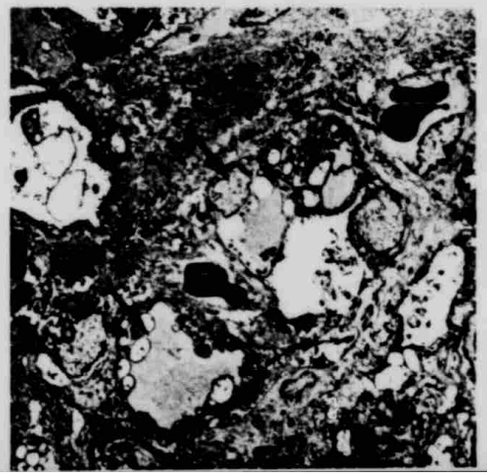
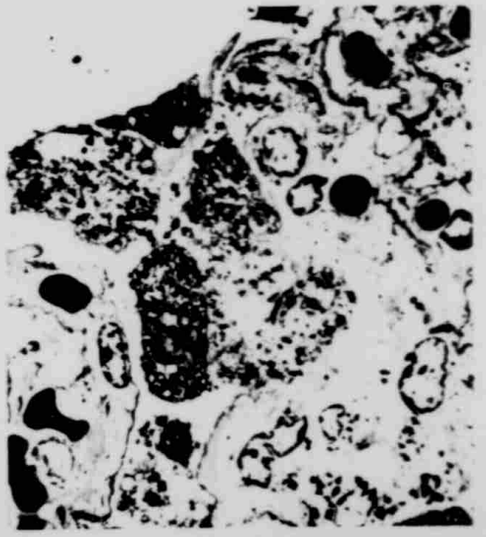


FIG. 5. ELECTRON MICROGRAPH OF A PERIVASCULAR MACROPHAGE CONTAINING RESIN-COATED GLASS-WOOL FIBRE (× 3000)



FIG. 6. ELECTRON MICROGRAPH OF DUST-LADEN MACROPHAGES FREE IN THE ALVEOLAR AIR SPACE (GLASS FIBRE; × 1500)



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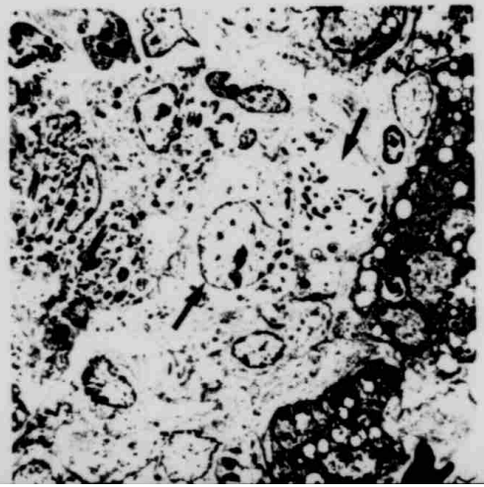
FIG. 7. ELECTRON MICROGRAPH OF CHRYSOTILE FIBRE FREE IN THE INTERCELLULAR MATRIX

Insert shows a cross-section of a ferruginous body (× 15,000).



FIG. 8. ELECTRON MICROGRAPH OF AN INTERALVEOLAR MASS OF MACROPHAGES CONTAINING GLASS FIBRES

The macrophages are surrounded by type II cells (arrows) (× 1500).



In five of the ten exposed animals there were discrete areas of hyperplastic cells. In both of the chrysotile-exposed animals, nodules of Kulschitzky-type cells occurred; these cells contained the characteristic neurosecretory granules (Fig. 9). In one animal, the cells were found lining an alveolus (Fig. 9); in the other, the cell mass was

FIG. 9. ELECTRON MICROGRAPH OF A MASS OF KULSCHITZKY-LIKE CELLS LINING THE ALVEOLAR AIRSPACE (× 3000)

Insert shows typical neurosecretory granules (× 40,000).



overlying type II cells lining a respiratory bronchiole (Fig. 10). In one animal exposed to resin-coated glass wool, a nodule of hyperplastic type II cells occurred (Fig. 11), the cells of which were characterized by a few small cytoplasmic lamellar bodies and a general similarity to the cells overlying the macrophage-filled alveolar air spaces. In the other animal exposed to resin-coated glass wool and in an animal exposed to glass fibre, there was hyperplasia of the ciliated cells lining the terminal bronchiole (Fig. 12) close to the junction with the respiratory bronchiole.

The overall impression gained from this study is that chrysotile was the most reactive material; similar reactions occurred with all of the man-made mineral fibres, except that the noncoated glass wool was more reactive than the coated variety.

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FIG. 10. ELECTRON MICROGRAPH OF A MASS OF KULSCHITZKY-LIKE CELLS OVERLYING TYPE II CELLS LINING A RESPIRATORY BRONCHIOLE, WITH A THICKENED WALL CONTAINING FREE FIBRES

rysotile; × 3000)

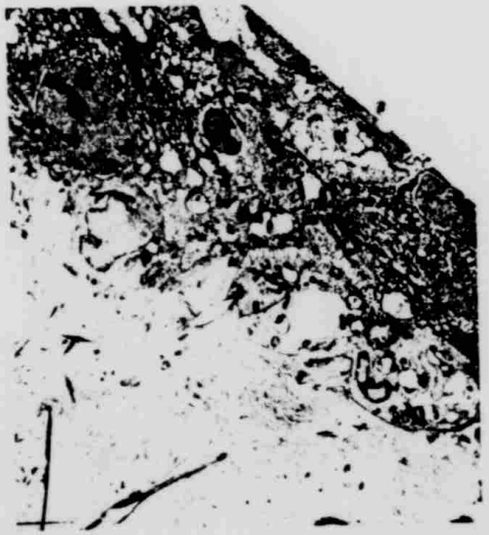


FIG. 11. ELECTRON MICROGRAPH OF A MASS OF HYPERPLASTIC TYPE II CELLS

(Resin-coated glass wool; × 2600)

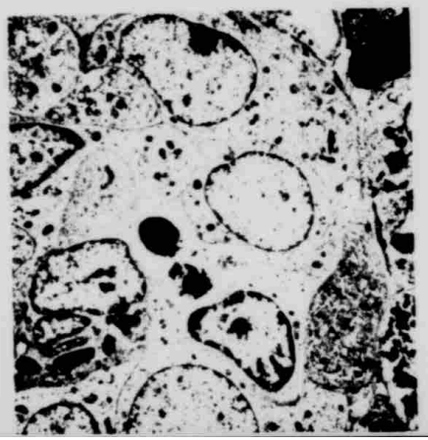


FIG. 12. ELECTRON MICROGRAPH OF A MASS OF HYPERPLASTIC CILIATED EPITHELIUM (ARROW)

(Glass fibre; × 1500)



DISCUSSION

These preliminary results show that some man-made mineral fibres can produce focal fibrosis, an accumulation of cellular debris and lipid material and hyperplasia of both alveolar cells and cells lining the terminal airways.

The degree of fibrosis seen with the man-made mineral fibres was less marked than that with chrysotile. The presence of fibrosis in the former cases is in contrast to the findings of Lee et al. (1979), who found no fibrosis following inhalation of glass fibre, although they described an accumulation of cellular material similar to that seen in this study. Schepers & Delahaut (1955) also found no fibrosis following inhalation of glass wool. However, in the study of Lee et al. (1979), few of the respirable fibres were longer than 3 µm; and fibres of less than 5 µm are usually devoid of fibrogenic activity and carcinogenicity (Maroudas et al., 1973). The respirable material in this study contained significant numbers of fibres 5 µm and greater in length (Wagner et al.[1]). There were considerably more fibres in the

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irable fraction of chrysotile than in that of the man-made mineral
es; this may, in part, account for the pattern of fibrosis seen in
study.

The presence of hyperplastic cells, both in the alveolus and ter-
l airways, suggests that both man-made and natural fibres may pro-
cellular irritation that results in a hyperplastic reaction in a
ion outlined by Spencer & Raeburn (1956).

SUMMARY

The effects of inhalation of rock wool, coated and uncoated glass
, glass fibre and UICC chrysotile B on the ultrastructure of the rat
have been investigated. All fibres produced focal fibrosis, type
ell proliferation, an accumulation of cellular debris and lipid
rial and hyperplasia of both alveolar cells and cells lining the
inal airways. The fibrosis was more marked following inhalation of
sotile than after exposure to man-made mineral fibre.

RESUME

Les auteurs ont étudié les effets de l'inhalation de laine minérale,
aine de verre, enrobée ou non, de fibre de verre et de chrysotile B
'UICC sur l'ultrastructure du poumon de rat. Toutes les fibres ont
oqué une fibrose focale, une prolifération cellulaire de type II,
accumulation de débris cellulaires et de matière lipidique et une
rplasie des cellules alvéolaires et des cellules revêtant les voies
iratoires terminales. La fibrose était plus marquée après l'inha-
on de chrysotile qu'après l'exposition à une fibre minérale arti-
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