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THE LONG TERM FIBROGENIC EFFECTS OF CHRYSOTILE AND CROCIDOLITE ASBESTOS DUST INJECTED INTO THE PLEURAL CAVITY OF EXPERIMENTAL ANIMALS

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SUMMARY.—In a series of experiments on the effects of chrysotile and crocidolite asbestos dust injected into the pleural cavities of experimental animals, the following results were obtained. Both dusts produced large granulomas in all the species used, but the histological patterns of these lesions varied. In guinea-pigs both chrysotile and crocidolite produced granulomas that consisted mainly of giant cells, mixed with a few macrophages and fibroblasts, and each granuloma was surrounded by a distinct capsule of fibrous tissue. In mice and rats, however, relatively few giant cells were present in the asbestos granulomas, and the lesions consisted only of macrophages with some fibroblasts. In these 2 species the granulomas were not surrounded by a distinct capsule. In all cases the granulomas were eventually replaced by masses of fibrous tissue, but the time sequence of this change was variable. In guinea-pigs the granulomas remained cellular for 6–12 months, and after this period there was evidence of the invasion of the lesions by fresh macrophages which rephagocytosed dust left behind by the death of previous cells. In mice some areas of the granulomas remained cellular for 12–15 months, but others were completely fibrosed by 6–9 months. In rats fibrosis of the asbestos granulomas was very rapid, and most lesions consisted of old almost acellular collagen by 3–4 months. In all species a few animals showed massive pleural fibrosis outside the main granulomas. In some cases this appeared to be associated with an infection spreading from the lung tissue, but in others no such association could be traced.

ASBESTOSIS is a pathological fibrosis of lung tissue caused by the inhalation of asbestos dust. In the early stages a collagenous interstitial thickening of the alveolar septum is found, but later the alveolar structure over quite large areas is destroyed and replaced by masses of irregularly arranged fibrous tissue. Often the fibrosis involves the pleural surfaces which become greatly thickened and adherent to one another. In addition to its fibrogenic properties, asbestos is now believed to be associated with the formation of bronchial carcinomas (Doll, 1955), and mesotheliomas (Wagner, Sleggs and Marchand, 1960).

It had been previously shown (Davis, 1963*a*, *b*) that when asbestos dust came into contact with tissues, the initial cell reaction consisted entirely of macrophages. These were the only cells involved in the phagocytosis of the dust, and each cell could ingest many particles. When several macrophages came into close proximity in an area containing dust they combined to form giant cells, and later some of them at least appeared to become directly converted to fibroblasts.

In these experiments, however, the lung tissue had been dusted by inhalation, and little was known about the cell response to dust in the pleural cavity.

Smith, Miller, Elsasser and Hubert in 1964, however, injected asbestos samples directly into the pleural cavity of hamsters. The main purpose of these studies was the experimental production of mesotheliomas, but the early dust lesions were described as follows: " Extensive pleural and pericardial adhesions were found in hamsters treated with each of three varieties of asbestos tested. Asbestos fibres were numerous in these adhesions, and there were occasional asbestos bodies. There was marked pleural thickening and calcification. In specimens removed early in the experiment, adhesions consisted of granulomatous inflammation. Multinucleated giant cells, often filled with asbestos then became a prominent feature. In later specimens, cellular infiltrate was sparse and the adhesions consisted principally of fibrous tissue."

In 1967 Roe, Carter, Walters and Harington injected asbestos dust s.c. into mice, but found that the dust was often moved from this site and selectively deposited in the submesothelial tissues of the thorax and abdomen. In these sites the dust produced fibrous adhesions between organs. It was reported that " In some animals the adhesions were infiltrated with granulation tissue and chronic inflammatory cells, but the latter were never seen in large numbers." These animals were, however, examined 40 or more weeks after dust injection and so it was not certain whether the early submesothelial lesions consisted of chronic inflammatory cells or not.

In order to study the full range of histological changes produced by the presence of asbestos dust in the body cavities, and to check on species differences in these reactions, it was decided to undertake a new study involving the intrapleural injection of both chrysotile and crocidolite asbestos dust into guinea-pigs, rats and mice. The results from this study relating to the production of mesothelial tumours will be published separately. The histological pattern of the asbestos lesions themselves is described here.

MATERIALS AND METHODS

Chrysotile and crocidolite asbestos dust was injected into the lower right pleural cavity of guinea-pigs, rats and mice. The dose used in each case was 25 mg. for the guinea-pigs and rats, suspended in 1 ml. of distilled water, and 10 mg. for the mice, suspended in 0·5 ml. of distilled water. The dust was sterilized by quickly raising the temperature of the distilled water to 85° for about 1 min. and then quickly cooling to room temperature. This procedure proved satisfactory for sterilization and it was hoped that chemical changes due to heating the asbestos in water were kept to a minimum. The animals were examined at intervals from 7 days to 4 yr after dust injection. The oldest guinea-pigs survived 49 months from the start of the experiment, and the oldest mice for 25 months. The rats used in this study had unfortunately all died by 13 months after injection owing to an outbreak of pneumonia. Since, however, no changes had been observed in the rat lesions between 6–13 months it was considered that the results obtained gave a true picture of the pleural response to dust in this species and the results are included in this paper.

At autopsy granulomas or fibrous lesions were found in the pleural cavities of all animals injected. In each case the lungs were sectioned and examined to see if they had been penetrated by the injection needle, but this was found to have occurred in 2 cases only. Both these animals were guinea-pigs injected with chrysotile dust, and the histological findings from the animals were excluded from the present series. The pleural lesions found in the animals were each divided into two halves. One was fixed in formol saline for light microscope study, and the other was fixed in buffered osmium tetroxide for electron-microscope examination. The light microscope sections were stained as appeared appropriate with either,

haematoxylin and eosin, Perl's method for iron, van Gieson's method for collagen, Gordon Sweet's reticulin technique, or Hale's method for acid mucopolysaccharides. The osmium fixed material was embedded in Araldite and stained with lead citrate. It was unfortunately found, however, that while it was quite possible to cut thin sections of tissue containing chrysotile asbestos dust, the much tougher crocidolite produced such difficulties that no systematic electron-microscope study was possible. The observations on crocidolite dust were therefore based on light microscope examination alone.

The dust samples used in these studies may be categorized as follows. They were supplied by Dr. P. F. Holt of Reading University, and had been prepared for use in his inhalation chambers. The milling apparatus used was described by Holt and Young in 1960. The dust consisted almost entirely of fibrous asbestos material, which varied in size from large bundles 100 μ long and 10 μ in diameter down to single crystals less than 1 μ long. By far the majority of the dust, however, was in the form of crystal bundles less than 5 μ in length and between 0·1–1 μ in diameter.

RESULTS

After intrapleural injection of chrysotile asbestos dust the main reaction in guinea-pigs, rats and mice was the formation of large granulomas. These granulomas were well formed by the end of 7 days and did not appear to increase in size after 14 days. In most cases they were firmly attached to either the lung surface, the diaphragm or the chest wall. In guinea-pigs the granulomas consisted mainly of giant cells, but among these a few fibroblasts were always present, and some single macrophages (Fig. 1). Plasma cells, lymphocytes and neutrophil polymorphonuclear leucocytes were also present, but only in small numbers. In the rat and mouse granulomas, however, few giant cells were formed, and these were always smaller than those from guinea-pigs. The lesions in these animals were made up largely of mononuclear cells closely packed together (Fig. 3, 4). Some of these cells were spindle shaped, but most had an irregular outline and looked like macrophages. A few plasma cells, lymphocytes and neutrophils were always present in both species, and in addition rat lesions usually contained a number of mast cells.

A reticulin network was formed in the granulomas of all 3 species during the first 7 days after dust injection, but this was noticeably more dense in the rat lesions than in those from guinea-pigs and mice. In the earliest granulomas no collagen was present, but a faint network of van Gieson positive material had been formed in all species by about 14 days. In guinea-pigs the structure of the granulomas did not change very much between 14 days and 6–8 months after injection, although there was a gradual increase in collagen during this period. Between 6–8 months after injection, however, the giant cells gradually died off and were replaced by collagen. Although this fibrous tissue eventually became almost acellular, there appeared to be a period after giant cell death when some single macrophages remained among the fibroblasts. The process of giant cell death was rather haphazard and scattered clumps of giant cells were found in some lesions as much as 2 yr after dust injection. In most animals, however, these granulomas were replaced by fibrous tissue within 18 months. The mouse chrysotile lesions also remained almost unchanged in cell content for about 6 months after their formation, although during this period there was a gradual increase in collagen. After this period there was a loss of cells from some areas accompanied by an accelerated production of collagen. In the mouse granulomas, however, some parts always remained cellular even in the oldest animals, and these areas contained relatively little collagen. The rat lesions were replaced by fibrous tissue much quicker than those of guinea-pigs and mice, and by 2 months after dust injection

some areas had begun to lose their cells. By 3–4 months most rat lesions had been replaced by almost acellular collagen.

In the guinea-pig granulomas, large numbers of Perl positive asbestos bodies were formed in the giant cells between 14 days and 6 weeks after dust injection, but after this time their numbers increased slowly, if at all. When giant cell death occurred in the ageing granulomas, the asbestos bodies, and any uncoated dust particles from the cells, were left behind lying free among the collagen fibrils. In the mouse granulomas a few bodies were found between 3–8 weeks after dust injection, but in the rat granulomas no asbestos bodies at all were found during these studies. The process of asbestos body formation within the guinea-pig and mouse granulomas is described separately (Davis, 1970*b*).

The main guinea-pig granulomas fibrosed slowly over a period of 18 months, but outside these granulomas a variable amount of collagenous fibrous tissue was present within a few days of injection. This tissue was first seen as an accumulation of young spindle-shaped fibroblasts at about 7 days. By 14 days the fibrous tissue had begun to " age ", the cells were thinner and more elongated, and a considerable amount of collagen was present between the cells. The amount of fibrous tissue present outside the main granulomas varied from animal to animal. A distinct capsule was always present around each granuloma and large areas of the visceral pleura were also frequently fibrosed and thickened (Fig. 2). In many cases enough fibrous tissue was formed to produce widespread adhesions between the various lung lobes, the diaphragm and the chest wall. During these experiments a total of 6 animals was found in which extremely extensive fibrosis was present in the pleural cavity. In these animals the chest contents at death were in the form of a solid mass of fibrous tissue in which were embedded the heart and lungs. In 3 of these animals, death occurred only 14 days after injection, and appeared to be due to intrapleural haemorrhage. In the others which died at 5, 9 and 11 months respectively, there was evidence of lung infection that had spread to the pleura.

In mouse granulomas a little rapidly maturing fibrous tissue was sometimes present near to the main lesions, but a distinct capsule was seen much more rarely than in guinea-pigs. However, the granulomas themselves often formed firm adhesions between the various lung lobes, the diaphragm and the chest wall. On some occasions areas of the visceral pleura, well away from the granulomas, became thickened with collagenous fibrous tissue within 2–4 weeks of dust injection. In a few cases there was very rapid fibrosis throughout the pleural cavity between 9–12 months after injection, and the chest contents were surrounded and compressed by large amounts of fibrous tissue. In some of these cases there was evidence that a lung infection had spread to the pleural cavity, but in other cases this did not appear to have occurred. Rat granulomas in common with those in mouse were seldom surrounded by a distinct capsule of fibrous tissue, although the granulomas themselves often formed strong adhesions between the chest contents. Some areas of the pleural surface did, however, become thickened by the accumulation of fibroblasts during the first 2–4 weeks after dust injection.

From about 18 months after injection the old fibrosed granuloma sites in guinea-pigs frequently became calcified (Davis 1970*a*). The resulting areas of calcification appeared very similar to the calcified pleural plaques frequently found in cases of human asbestosis. No signs of calcification were seen in either mouse or rat lesions during these experiments.

The initial response to crocidolite asbestos dust injected into the pleural cavity of experimental animals was similar to that found with chrysotile dust. Large granulomas were formed within 7–10 days of dust injection. In contrast to chrysotile dust, however, which was invisible to the light microscope when in tissue, much of the crocidolite was easily visible, both as long fibres and as smaller granular aggregates within the cells (Fig. 5). In guinea-pigs, the early granulomas were made up mainly of distinct giant cells, with some single macrophages and a few fibroblasts scattered between them. Some of the long crocidolite fibres within giant cells became coated with Perl positive material to form asbestos bodies, but this happened much less frequently than with chrysotile dust. In rats and mice the initial response to crocidolite was similar to that found with chrysotile dust in the same species. The granulomas contained very few giant cells, and the main cell response consisted of macrophages and fibroblasts. Very few asbestos bodies were found in the granulomas of rats and mice injected with crocidolite.

Within the crocidolite granulomas in guinea-pigs, a reticulin network was laid down among the giant cells during the first 7 days after dust injection. By 14 days some collagen was present and this gradually increased with time. Apart from this increase in collagen content, the granulomas underwent little change for the first 6 months. Between 6–8 months after injection, however, the giant cells in some areas began to die off and were replaced by collagen. When this happened the dust was left behind among the collagen fibrils. In contrast to the chrysotile granulomas, however, it was rare for the whole lesion to become acellular and replaced by fibrous tissue. Large cellular areas containing giant cells were found in some animals 3 yr after dust injection. In some of these areas a collagen network was present between the giant cells, but in others the giant cells were separated from each other by clear spaces that contained neither collagen nor reticulin. It was noticeable in these areas that the giant cells were both smaller and rounder than those seen in the early lesions (Fig. 6). In mice a similar ageing process was seen with the crocidolite granulomas. A reticulin network was present within 7 days and some collagen by 14 days after injection. Between 14 days and 3 months, the collagen in the granulomas increased, but during this period there was little loss of cells from the lesions. From 3 months onwards, however, there was a gradual loss of cells from some areas accompanied by accelerated collagen production. By 6 months after injection some areas consisted mainly of collagen—and the dust that had originally been present in the cells was now found free, embedded in the collagen mass. In other areas of the same granulomas, however, it was common to find that the cells remained alive for up to 18 months after injection. In these areas the amounts of collagen present were never very great. In rats, the crocidolite granulomas matured much more rapidly than crocidolite lesions in guinea-pigs and mice. The initial reticulin network was very dense, and some of it had been converted to collagen by 14 days. From then onwards there was a gradual loss of cells, accompanied by increased collagen production, until by 4–5 months after injection most of the lesions had been converted to fibrous tissue.

The guinea-pig crocidolite granulomas were usually surrounded by a distinct capsule of young fibrous tissue after a few days. At the same time large areas of the visceral pleura became thickened by the accumulation of young fibroblasts. Both the granuloma capsule and the areas of pleural fibrosis matured much more

quickly than the granulomas themselves, and usually consisted of acellular collagen by 3 months after injection. The amount of fibrous tissue in these areas was usually enough to form distinct adhesions between the granulomas and the other chest contents. In rats and mice the young crocidolite granulomas were seldom surrounded by a distinct capsule of fibrous tissue, but the granulomas themselves formed strong adhesions between the various lung lobes, the diaphragm and the chest wall. These adhesions became firmer and more marked as the amount of

EXPLANATION OF PLATES

FIG. 1.—An area from a guinea-pig pleural granuloma produced in response to chrysotile asbestos dust injected 2 weeks previously. The granuloma consists mainly of large multinucleate giant cells, but it is surrounded by a capsule of fibrous tissue (F). ×225.

FIG. 2.—An area of fibrous tissue causing pleural thickening in a guinea-pig 2 weeks after injection with chrysotile asbestos dust. The original line of the pleura is indicated by the subpleural layer of smooth muscle (M). ×225.

FIG. 3.—Part of a granuloma produced on the visceral pleura of a mouse by the injection of chrysotile asbestos dust 2 weeks previously. No obvious giant cells are visible in this specimen which consists of irregularly shaped mononuclear cells. Lung tissue is labelled (L). ×225.

FIG. 4.—A section of a granuloma formed on the diaphragm of a rat 2 weeks after injection with chrysotile asbestos dust. This granuloma does not appear to contain giant cells and consists mainly of irregularly shaped mononuclear cells. ×225.

FIG. 5.—An area from a granuloma in a guinea-pig pleural cavity 4 weeks after injection with crocidolite asbestos dust. Many giant cells are present and these are packed with crocidolite fibres which are easily visible in the light microscope. ×225.

FIG. 6.—An area of granulation tissue from a guinea-pig pleural cavity 15 months after injection with crocidolite dust. Most of the dust containing cells have several nuclei and are therefore giant cells, but they are much smaller than those seen in the early crocidolite lesions. ×225.

FIG. 7.—An electron micrograph of part of a guinea-pig pleural granuloma produced by the injection of chrysotile dust 14 days previously. Many of the cells are already multinucleate, but lines of interdigitated phagocytic process indicate that giant cell fusion is still going on. These areas of partial fusion are indicated by arrows. ×2400.

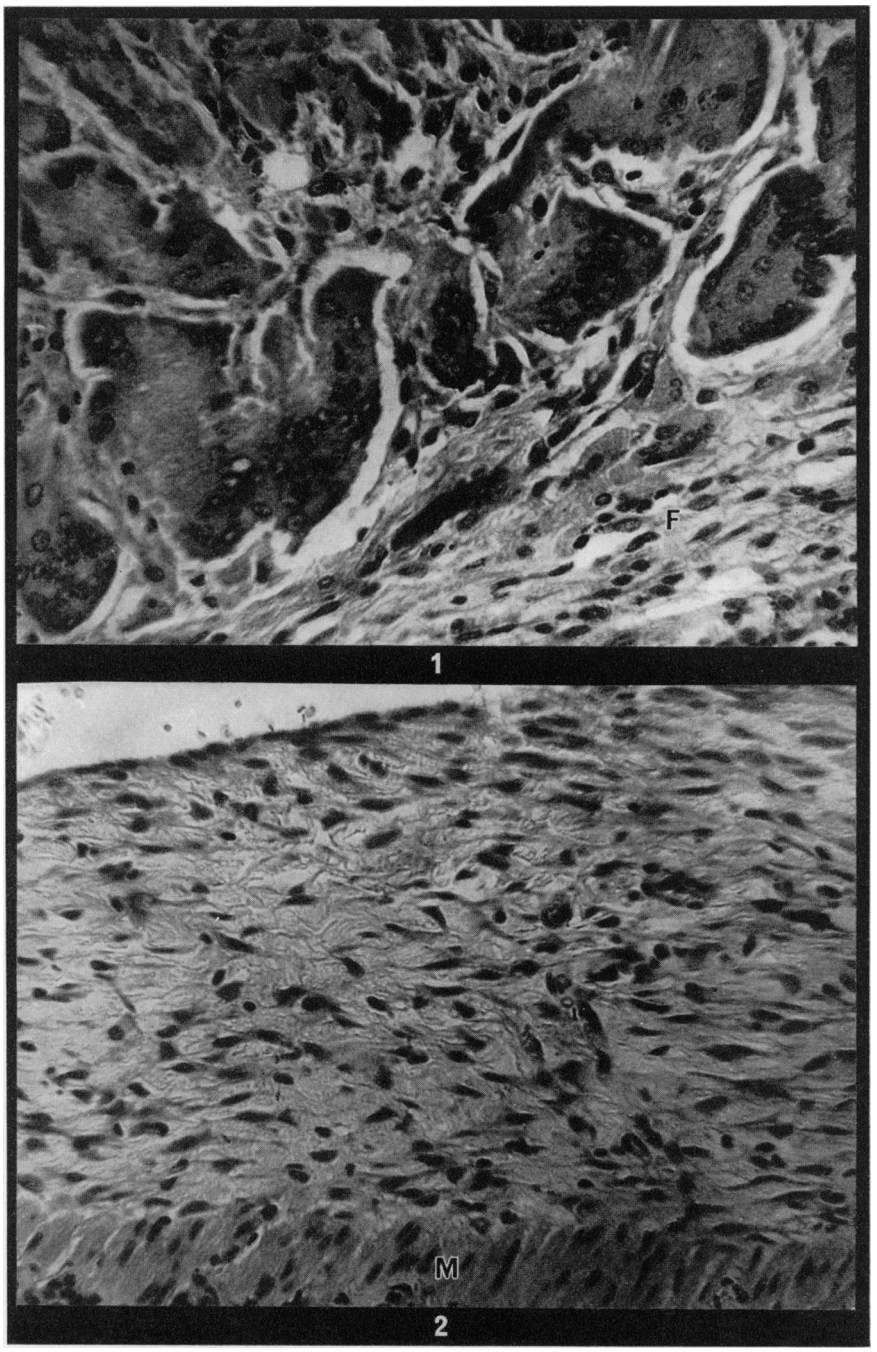
FIG. 8.—Rephagocytosis of chrysotile asbestos crystals in a guinea-pig granuloma 15 months after dust injection. In this region most of the giant cells had died and the dust was left behind in masses of collagen. It can be seen that in order to reach the dust (A), the macrophage phagocytic process (B) has had to find a way through densely packed collagen fibrils. In doing so it has produced a clear space (C) where the collagen has either been pushed aside or removed. ×38,000.

FIG. 9.—A group of macrophages from a mouse pleural granuloma 6 weeks after the injection of chrysotile asbestos dust. Each macrophage has produced many phagocytic processes but these have folded back on to their parent cells without interdigitating with the processes from neighbouring cells. The area inset at high magnification shows that the processes refuse readily with their own cell surface, but there is no sign of fusion with the macrophages on either side. The lines of the surface membrane of each macrophage are arrowed. ×22,000. Inset ×44,000.

FIG. 10.—Giant cell formation in a mouse pleural granuloma 8 weeks after dust injection. Some complete fusion of macrophages has occurred and there are no signs of the original cell boundaries between the 4 nuclei labelled N. Some macrophages are, however, still only partially fused to the main cell body, and their lines of fusion are indicated by large areas of vesiculation (V). ×10,000.

FIG. 11.—A macrophage from a mouse pleural granuloma 12 months after chrysotile dust injection. Most of the original cells in this area have died by this time, but this macrophage is still actively phagocytic as indicated by the many elongated processes on the cell surface. At point A some of these processes, having folded back on to the cell surface, have partially refused with the main plasma membrane. This cell is probably rephagocytosing dust left behind by the original cells. At point B 2 asbestos fibres can be seen lying free among the collagen fibres. ×28,000.

FIG. 12.—An area from a rat pleural granuloma 2 weeks after the injection of chrysotile dust. The macrophages have many phagocytic processes on their surface membranes, but already there is a dense reticulin network in the granuloma and the cells are separated by bundles of reticulin fibres (A). ×11,000.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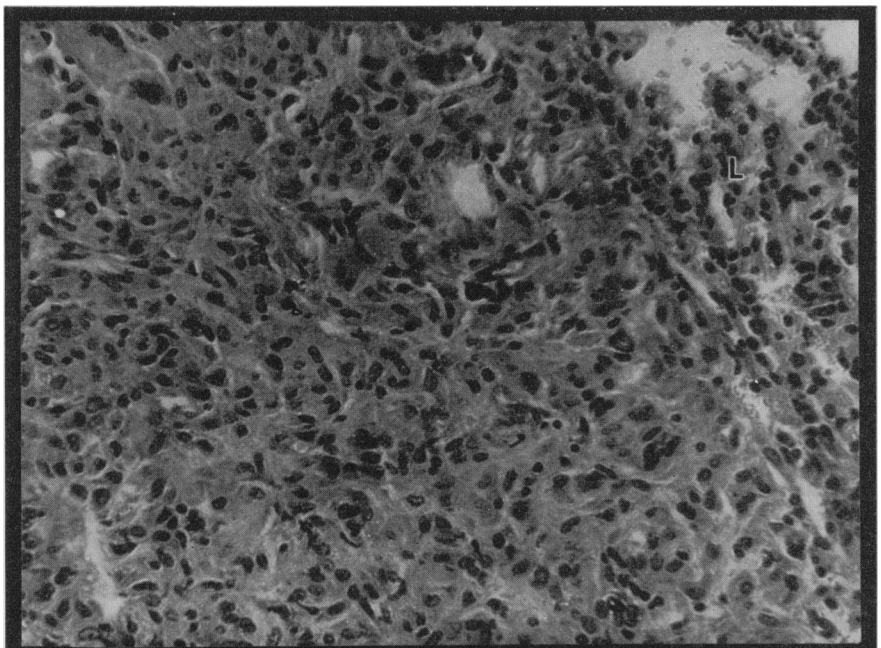

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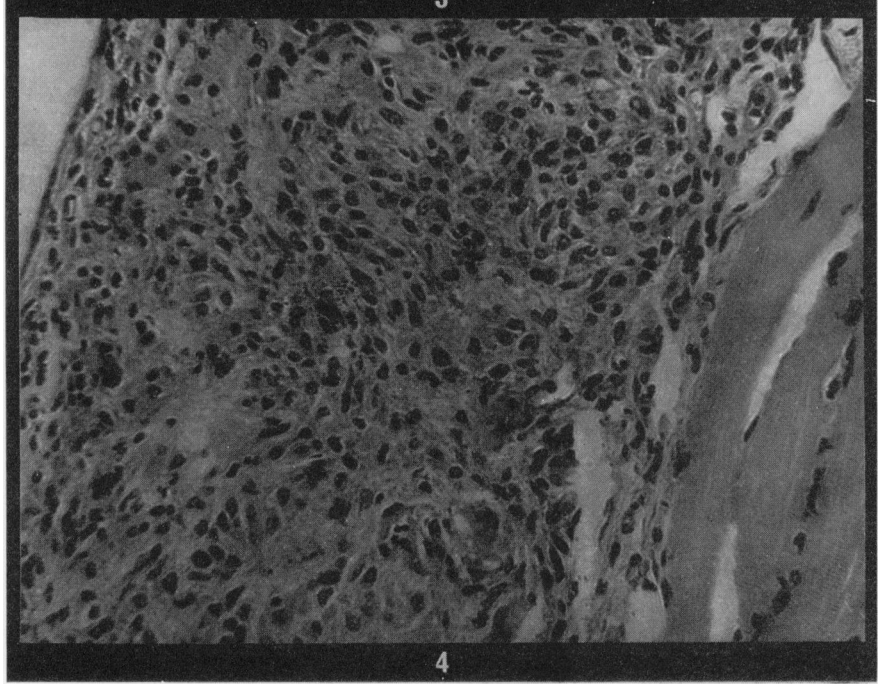

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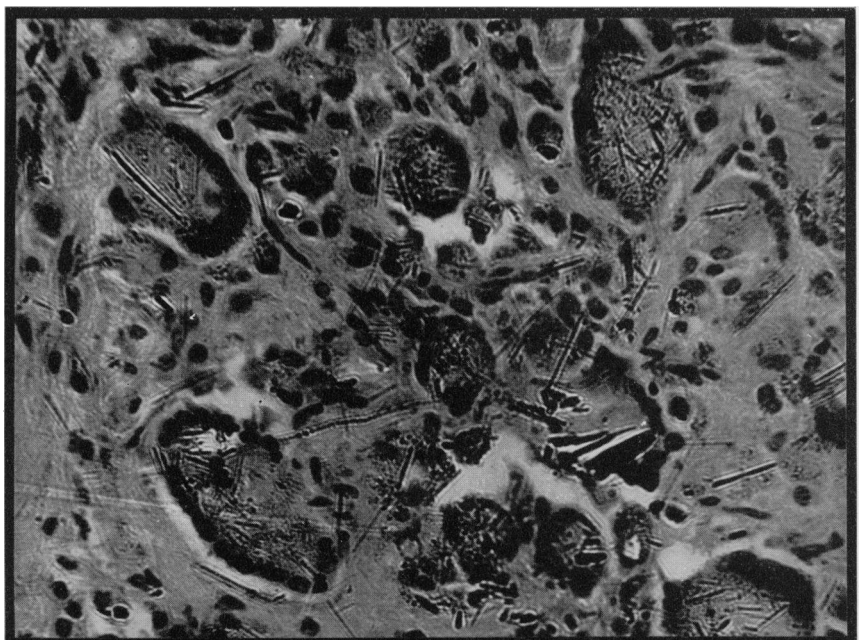

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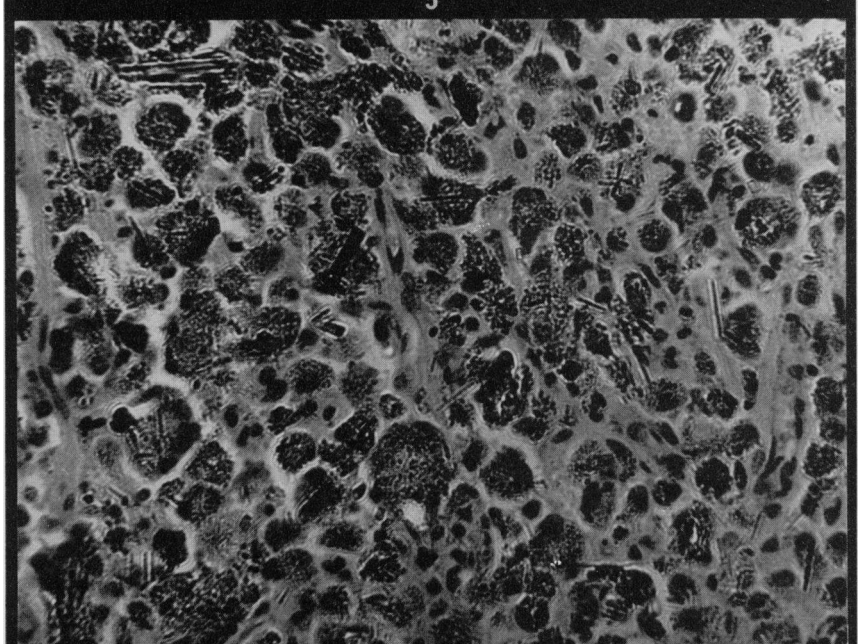

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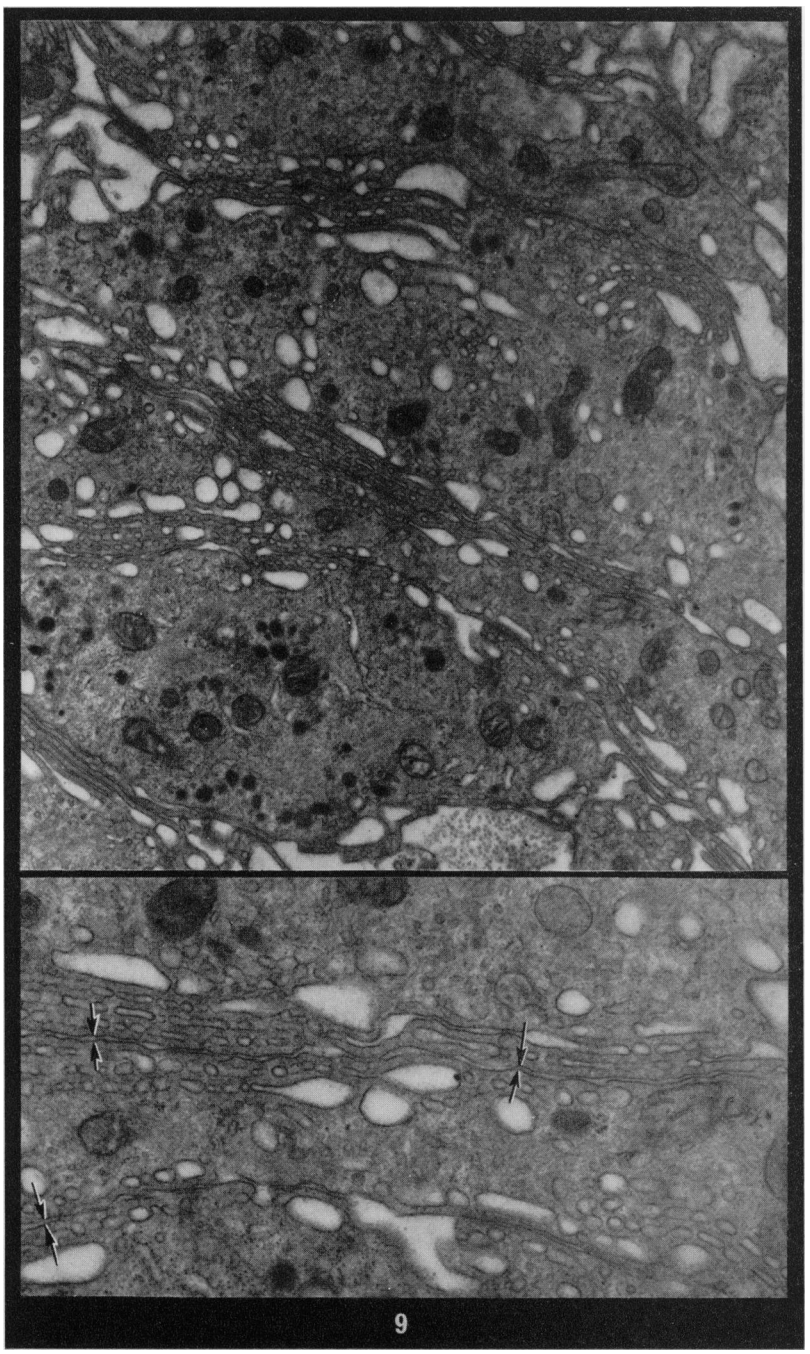

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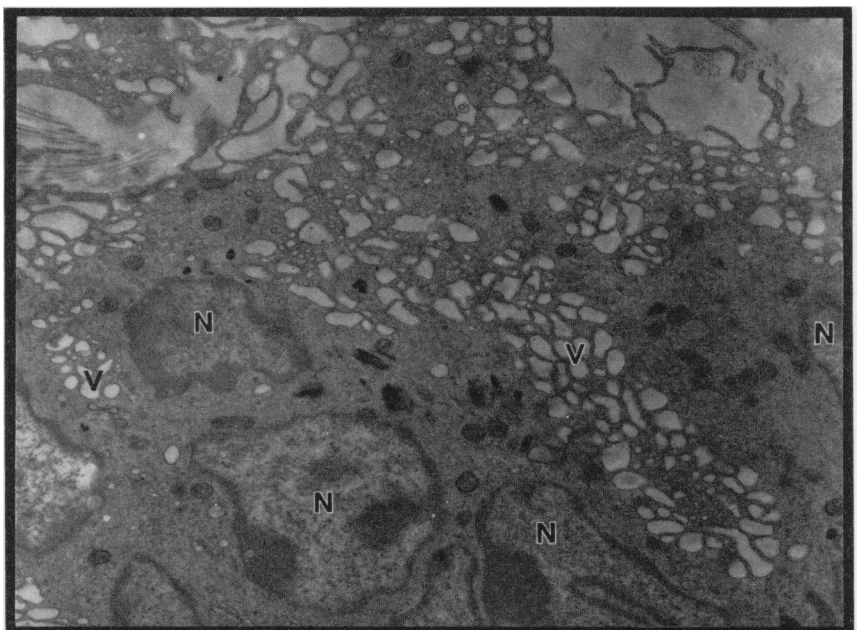

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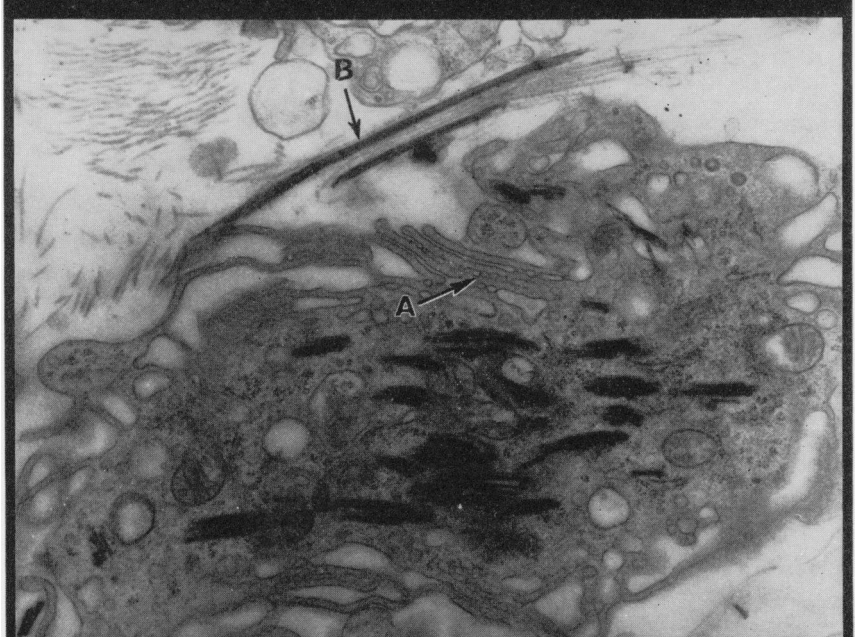

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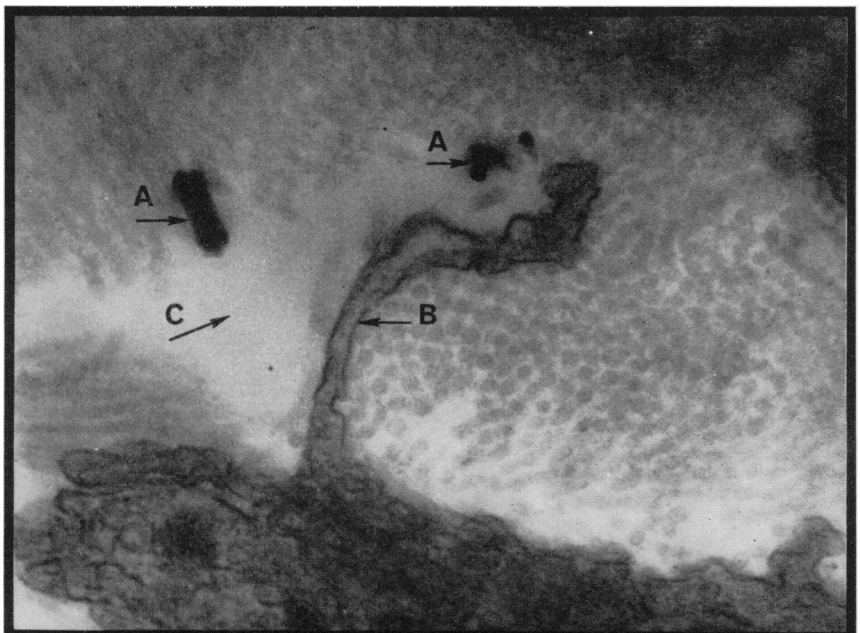

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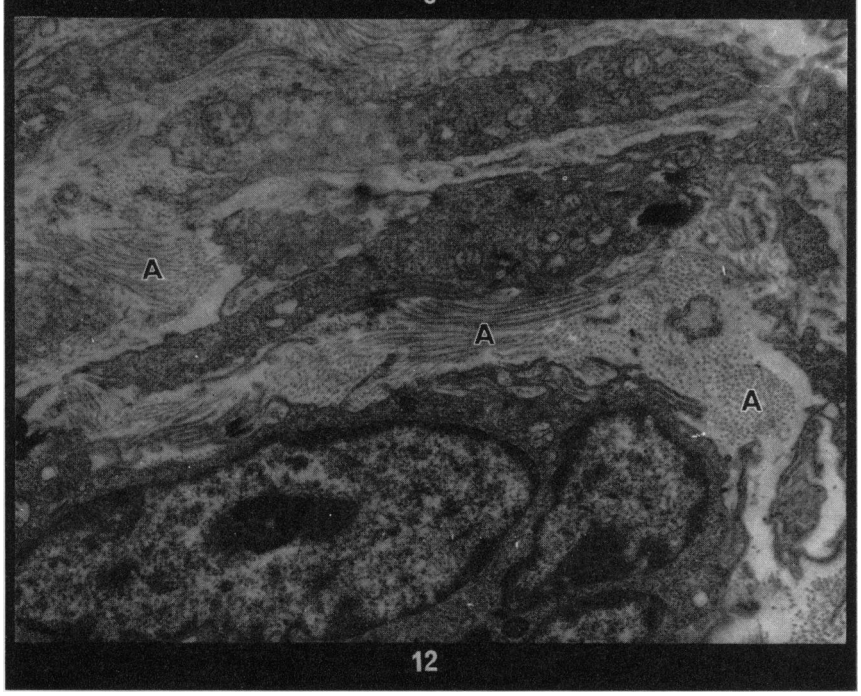

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collagen in the granulomas increased. Some areas of the pleural surface did, however, become thickened with fibrous tissue in both species during the first 14 days of the experiment. This tissue matured rapidly and by 3–6 weeks it had been converted to old almost acellular collagen. Small areas of calcification were found in the granulomas of only 2 guinea-pigs injected with crocidolite dust at 28 and 36 months after injection respectively. In rats and mice no signs of calcification were seen in the crocidolite granulomas during this study.

Electron-microscope observations

Electron-microscope studies showed that the uptake of dust in the guinea-pig pleural cavity is similar to that found in organ cultures of guinea-pig lung (Davis, 1967). The early cell accumulations consisted of macrophages, which engulfed small dust particles by encircling them with elongated phagocytic processes. These processes then folded back on to the cell surface to form a phagocytic vacuole or phagosome which contained the dust. During the first 7–10 days after injection most macrophages contained considerable numbers of phagosomes, but by 14 days most of these had contracted and the dust particles were surrounded by a close fitting membrane. Particles of asbestos dust, more than 3–5 μ in length could not be taken up by means of the elongated phagocytic processes. These were most frequently walled up within a forming giant cell as macrophages fused around them, but sometimes it appeared that a single macrophage could engulf a comparatively large dust particle by encircling it in amoeboid fashion. When this occurred the fibre was enclosed in a cytoplasmic vacuole similar to a phagosome. Giant cell formation occurred by the interdigitation of the phagocytic processes of adjoining macrophages (Fig. 7). After interdigitation these processes at first appeared to contract and pull the cells closely together. Later, however, the processes broke down into a series of cytoplasmic vesicles and true multinucleate giant cells formed. These giant cells, however, continued to produce large numbers of phagocytic processes on their free surfaces and no doubt continued to take up any small particles with which they made contact. Both giant cells and macrophages were often found containing hundreds of small dust particles. The formation of asbestos bodies from long dust fibres in guinea-pig granulomas is described separately (Davis, 1970*b*).

In the early granulomas it would appear that all the dust particles injected were taken up by cells as no free dust was found in these lesions. From about 6 months onwards, however, as the amount of collagen in the granulomas increased, it became quite common to find dust lying free among the collagen fibrils. This coincided with the gradual loss of giant cells from some areas of the granulomas. Where this occurred, however, it was usual to find a number of free macrophages that had invaded the collagen and were engaged in the rephagocytosis of the dust. In order to do this some macrophages became very elongated and some were found that measured 150 μ in length. The phagocytic processes of these cells appeared to have the ability to either push aside collagen fibrils in order to reach dust particles or to dissolve these by enzyme action. This is illustrated in Fig. 8. It can be seen that the dust particle was completely surrounded by collagen fibrils, but that the phagocytic process has made a space for itself to reach the dust. This process of rephagocytosis was noticeable in most granulomas to a variable extent from 6–18 months after dust injection. By 18 months, however, most of the granulomas had been completely replaced by old fibrous tissue, and

asbestos bodies and uncoated dust particles are left lying free among the collagen fibres.

Even in the earliest granulomas a few fibroblasts were present among the giant cells, and it was probably these which produced the reticulin network that was present 7 days after dust injection. From this time on, some fibroblasts were always found in the guinea-pig granulomas until these lesions had become completely converted to old fibrous tissue. Most of these cells contained a few particles of asbestos dust, so they must have been formed from phagocytic precursors that may have been macrophages. Since fibroblasts usually " age " and die during collagen production it seems likely that there was a gradual and continuous influx of new cells into the granulomas to correspond with the gradual increase of collagen content with time. It may be that these cells originally entered as macrophages involved in the rephagocytosis of dust.

Electron-microscope studies of mouse intrapleural granulomas showed that the first accumulation of cells consisted of macrophages very similar in structure to those found in the guinea-pig lesions. These cells took up small particles of dust by means of elongated phagocytic processes, and the processes of adjoining macrophages intertwined freely. In contrast to the guinea-pig lesions, however, the complete fusion of these macrophages to form giant cells occurred much less frequently. Some small multinucleate giant cells were always found from about 7 days after injection but more commonly areas of closely opposed macrophages were found whose phagocytic processes had interdigitated, but which showed no signs of fusion. In a few cases groups of macrophages were found whose processes had folded back on to the cell without interdigitating with those of neighbouring cells, even though the cells were in actual contact (Fig. 9). Even where complete fusion did take place, it was common in mice to find areas of giant cells in which the individual nuclei were separated from each other by patches of vesiculation which resulted from the fusion of phagocytic processes (Fig. 10). Similar regions are only occasionally found in guinea-pigs, probably because the process of fusion occurs much more quickly. In the mouse granulomas large dust fibres were usually found between the partially fused macrophages or within the few fully formed giant cells. As with guinea-pigs a few fibroblasts were always present from the initial granuloma formation, until the areas were completely replaced by old collagen. These cells usually contained small amounts of asbestos dust. Mouse granulomas changed little in appearance during the first 6 months, but after this there was a gradual loss of cells in some areas accompanied by an increase in collagen. When this occurred the asbestos dust particles were left behind among the collagen fibrils. Even in those areas that remained cellular, deposits of asbestos dust were often found amongst the collagen, indicating that the death of some macrophages had occurred. As in guinea-pigs this dust was rapidly rephagocytosed by other macrophages which may have newly infiltrated the granuloma (Fig. 11).

Rat pleural granulomas were similar to those produced in mice in that few giant cells were produced in response to asbestos dust, and those that were formed were much smaller than those from guinea-pig granulomas. Electron-microscope examination, however, showed that the reason for the lack of giant cells may be different in rats and mice. In the rat granulomas, examined between 7–10 days after dust injection, those giant cells that were found appeared completely fused and must have formed very quickly. The remaining macrophages had all produced

large numbers of phagocytic processes, and some of these were interdigitated with neighbouring cells. Most of them, however, were separated from each other by reticulin fibres as early as 7 days after injection and the deposition of reticulin at this period was much greater than in guinea-pigs and mice (Fig. 12). It seems likely that the dense reticulin network is at least partly responsible for the inability of the macrophages to fuse. In the rat granulomas, small dust particles of below 10 μ in length were rapidly taken up by macrophages and the few giant cells present and by 14 days after dust injection all dust in this size range was intracellular. In rats, however, it was found that many of the longer fibres were not taken up by cells at all, and were left embedded among the reticulin fibres. This was probably due to the inability of the macrophages to fuse around these fibres. From their earliest formation, rat asbestos granulomas contained a larger proportion of fibroblasts than was usual in guinea-pigs or mice, and most of these cells contained small amounts of asbestos dust. In addition to this, rat granulomas aged more rapidly and by 3 months after injection many of the macrophages and giant cells had disappeared and the granulomas consisted mainly of fibroblasts. By 6 months after injection most rat granulomas had been completely converted to old fibrous tissue. When this occurred, the asbestos dust was left lying free among the collagen fibrils, but in contrast to guinea-pigs and mice, there was no attempt at rephagocytosis by new populations of macrophages.

DISCUSSION

The examination of pleural granulomas produced in experimental animals by the injection of asbestos dust has shown that qualitatively at least the tissue response to asbestos in the pleural cavity is similar to that found in lung tissue. The illustrations published by Vorwald, Durkan and Pratt in 1951 and by Holt, Mills and Young in 1964 and 1965 are all similar to areas of the pleural granulomas seen in the same species in the present study. Electron-microscope examination of the pleural granulomas also shows that exactly the same cell types are involved in the response to the dust in this site, as were reported in asbestos lung lesions (Davis, 1963, 1967). Not only are the cell types similar but the individual macrophages, giant cells and fibroblasts from pleural lesions appear structurally identical to similar cells found in the lungs of the same species. Their behaviour is also identical in the 2 sites. The dust is phagocytosed by macrophages which may fuse to form multinucleate giant cells. Later variable amounts of fibrous tissue are formed and some of the fibroblasts involved always contain small particles of asbestos dust.

It is noticeable, however, that while those experimental asbestos lesions, so far produced in lung tissues, have all been relatively small and involved only a tiny fraction of the lung volume, those in the pleural cavity can be very large indeed. There are two possible reasons for this quantitative difference in response, both of which may be important. Firstly, the lung has clearance mechanisms which are extremely efficient at dust removal. It is believed that as much as 98 per cent of inhaled dust is quickly removed from the lungs. It seems likely, therefore, that relatively little dust is ever retained by the lung tissue long enough to cause granuloma formation. In the pleural cavity, however, although there may be some dust removal, especially to the lymph glands, the majority of the dust remains *in situ*, producing large lesions. The other possibility is that the lung

reacts differently to the pleural cavity in response to mineral dusts. Embryologically the lung is endodermal, while the body cavities develop from the mesoderm. Moreover, the lung is known to be very resistant to certain types of tissue reaction, especially the maturation of reticulin fibrosis to mature collagen. Comparisons based on embryological criteria are, however, confused by the fact that all the cells involved in an inflammatory response in the pleural cavity must come from outside, presumably *via* the blood stream. Pinkett, Cowdrey and Rowell in 1966 were also able to show that in the long term at least, the population of lung macrophages is partly maintained by transport from the haematopoietic system. It may be that the environment of the lung alveoli, which are in contact with the outside world, is more important in modifying cell response to dusts than any differences in the actual cell types involved.

Regardless of the difference in reaction to asbestos dust that may be seen in the lungs and the pleural cavity, the ability to examine large pleural granulomas of known age has made it possible to demonstrate marked differences between animal species in their response to asbestos dust. Thus guinea-pigs react to asbestos dust by the production of granulomas made up largely of giant cells. Around these lesions, however, there is usually a distinct capsule of fibrous tissue. Most of the dust is enclosed in the giant cell areas, but a small amount is always present in the fibrous capsule. Whereas this fibrous tissue capsule rapidly matures with the production of large amounts of collagen, the giant cell areas fibrose very slowly, and are seldom completely converted to fibrous tissue before 12–18 months after dust injection. The guinea-pig, therefore, appears to produce a dual response to asbestos dust. In mice, the distinction between granuloma and capsule is much less distinct, and the granuloma macrophages fuse less readily to produce giant cells. In rats none of the granulomas seen was encapsulated, and giant cell production was comparatively rare. This ability to produce giant cells readily in asbestos lesions does appear to closely parallel the ability of a species to produce asbestos bodies. This phenomenon is discussed in greater detail in a companion paper (Davis, 1970*b*), but it may be briefly stated that asbestos body production seems to start between the partially fused macrophages of a young giant cell. In guinea-pigs, where giant cell formation in asbestos lesions is a frequent occurrence, large numbers of asbestos bodies are produced. In mice, where giant cell production is far less frequent, few asbestos bodies are found, and in rats who also have difficulty in producing giant cells, asbestos bodies are very rare indeed and few were seen during the present study. The ability of a species to produce giant cells appears to vary somewhat according to the stimulus. Thus rats have difficulty in producing giant cells in both asbestos and tuberculous lesions (Francis, 1958), but Adams in 1966 found that giant cells formed freely in the lungs of rats injected with pine pollen.

Asbestos dust dissolves very slowly in tissues if at all, and in humans much dust may be found in the lung tissues many years after the last exposure. The present series of experiments and others have shown that asbestos is not rapidly toxic to cells, and some macrophages can be found containing over 100 small particles. Macrophages do eventually die in the asbestos lesions, however, whether from the toxic effects of the dust or other causes, and it was interesting to find that the liberated dust is still capable of exciting further cellular reaction. How many times any dust particle is rephagocytosed during the 12–18 months before the guinea-pig and mouse lesions fibrose completely is impossible to

discover, but if Heppleston's (1967) concept of macrophage death stimulating fibrosis holds good for asbestos lesions, then this rephagocytosis may be very important in deciding the amount of collagen production that eventually takes place. The dust need not itself be actually toxic to the macrophage, but if its presence can attract macrophages from outside an asbestos lesion, and these macrophages remain in the granuloma until they die, then a small amount of dust might stimulate a large amount of fibrosis over a long period. Since very old asbestos lesions eventually become almost acellular, however, it must be assumed that eventually either the dust loses its chemotactic stimulus or it becomes so deeply embedded in collagen that this stimulus is no longer able to attract macrophages from outside.

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