Br. J. exp. Path. (1972) **53**, 190.

THE FIBROGENIC EFFECTS OF MINERAL DUSTS INJECTED INTO THE PLEURAL CAVITY OF MICE

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Received for publication November 9, 1971

Summary.—A series of mineral dusts were injected into the pleural cavities of mice in order to test their relative fibrogenicity. It was found that long fibre dust specimens produced widespread cellular granulomata which formed firm adhesions between the lungs, diaphragm and chest wall. These granulomata were gradually replaced by fibrous tissue and in old animals the dust was often found embedded in masses of acellular collagen. When the same mineral samples were more finely ground and sieved so that no long fibres remained in the dust, the resulting granulomata were much smaller and did not form adhesions. The small granulomata did, however, fibrose eventually and the dust was embedded in small nodules of collagen. Non-fibrous mineral rocks when finely ground and sieved also produced small non-adherent granulomata. The actual size of these granulomata depended on the number of cells attracted to the dust and this did vary with the different samples tested. The final degree of fibrosis within the granulomata was very closely correlated with the initial cellularity of the lesions. Those that were very cellular produced considerable amounts of collagen while little was produced if the initial granulomata had contained few cells.

MANY mineral dusts are able to cause tissue damage and eventual fibrosis. Without experimental manipulation, however, only the lung is readily accessible to dusts and with few exceptions diseases produced in humans by dust inhalation are varieties of lung fibrosis and grouped under the general heading of pneumonoconioses. Silicosis was known in ancient Egypt and coal workers pneumoconiosis has been recognized for as long as coal has been mined. More recently, asbestos, graphite, haematite, and beryllium have been shown to cause lung fibrosis and it is likely that many other minerals are potentially dangerous although they are not subject to industrial use in a way that would cause dust build up in the lungs. Industrial usage can change, however, and it is therefore important to understand the mechanisms by which any mineral is able to damage tissues.

Most work on this subject has involved experiments with silica or asbestos, but even in these two cases it has been extremely difficult to discover the exact properties of the minerals that cause tissue damage and eventual fibrosis. In the case of silica there have been many theories on this subject. Initially it was suggested that the toxic action of silica dust was due to its rough abrasive surface, but this mechanical theory was rejected when it was found that many more abrasive dusts had little cytotoxic effect. Later it was suggested by King (1947) that silica dust dissolved slowly in tissue fluids to produce silicic acid, and that this was the real cytotoxic chemical. Curran and Rowsell (1958), however, showed that silica particles implanted into the peritoneum in diffusion chambers did not produce

any fibrogenic effect although silicic acid was liberated from the chambers. Vigliani and Pernis (1963) suggested that silica dust might react by way of an auto-immune reaction but this idea has been difficult to substantiate and at present it would appear that the most likely method is that proposed by Allison, Harington and Birbeck (1966). Allison suggested that the damaging effect of silica dust depended on its ability to cause the rupture of phagosome membranes. Silica, like all dust of suitable size, is rapidly taken up into the phagosomes of macrophages and the cell secretes lysosome enzymes into these vacuoles. This is the normal sequence of events after phagocytosis, and these enzymes normally remain in the phagosome or its residual body. If silica has been ingested, however, the dust causes the rupture of the phagosome membranes and the lysome enzymes are liberated into the cell cytoplasm causing cell death. These findings alone would not explain the production of fibrous tissue after macrophage death but Heppleston (1967) showed that macrophages killed by silica liberated a substance that stimulated the production of collagen by fibroblast cultures.

The method by which asbestos is able to damage tissues is less clearly understood, but the initial stages must be different from silica since in physiological conditions at least asbestos dust has little if any cytotoxic effect on macrophage cultures (Allison, 1970). Asbestos is, however, known to be very fibrogenic in the long term and a considerable number of studies have been published in which dust has been administered to animals both by inhalation and injection. These papers were reviewed by Vigliani in 1968, and the theories of asbestos induced fibrosis discussed. It is considered likely that there is a factor produced by dust carrying macrophages that stimulates collagen production by fibroblasts but as yet experimental evidence on this idea is lacking. All asbestos samples appear to be fibrogenic but the relative fibrogenicity of the different types is still uncertain. Both Wagner and Skidmore (1965) and Morris *et al.* (1966) observed that chrysotile was less fibrogenic than chrocidolite or amosite, but Holt, Mills and Young (1965) found no difference between the amount of fibrosis produced by chrysotile, crocidolite, amosite or anthophilite. It seems likely that fibre length is more important than difference in the asbestos types. This was first suggested by Gardner (1938) and the idea supported by King *et al.* (1946). King reported that long fibres produced interstitial and focal fibrosis with alveolar collapse, while short fibres produced only slight peribronchial fibrosis. Vorwald, Durkan and Pratt (1951) also suggested that only chrysotile fibres longer than 3 μm were fibrogenic and more recently Webster (1965) has supported the idea that long asbestos fibres are more important in fibrogenesis than short ones. The only report that might contradict this hypothesis is that by Holt *et al.* (1965). These workers reported considerable fibrosis in the lungs of guinea-pigs treated with very fine asbestos dust and suggested that long fibre dust did not result in more severe lesions.

In order to better understand the processes by which mineral dusts cause fibrosis it was decided to undertake a series of experiments in which asbestos could be compared with minerals associated with it in the mining areas and also with a number of man-made fibres that are being used industrially. In theory inhalation experiments would have produced the most meaningful results in relation to clinical medicine but facilities for the treatment of large numbers of animals by this method were not available at the time. Moreover no experimental inhalation studies had yet produced lung fibrosis as severe as human asbestosis, so there was no certainty that even inhalation experiments would give clinically meaningful

results. Since asbestos at least was known to produce pleural fibrosis and these lesions had already been studied in experimental animals (Davis, 1970), it was decided to use the technique of intrapleural injection for administering the dust and mice were chosen as experimental animals as large numbers of these could be housed quite easily.

MATERIALS AND METHODS

The minerals used in these experiments were as follows: those chosen because of their association with asbestos in rock formations were, brucite, chlorite, chromite, forsterite, magnetite, olivine, pyroxene, serpentine, and talc. Man-made fibres studied were glass fibre of varying thickness, silica fibres, and 3 man-made insulation fibres.

Chrysotile asbestos was used as a control specimen and, in order to examine the effects of variations of this basic mineral, samples of synthetic chrysotile were included in the series and also a sample of chrysotile that had been fragmented by ultrasonic vibration until it consisted solely of single crystals 1 μm or less in length.

Most of the dust samples were produced by dry grinding in a " Grindex " mechanical mortar and pestle followed by sieving the dust through a 250 mesh copper sieve. All the minerals tested were treated in this way except for the synthetic chrysotile which was already very small and the chrysotile fragmented by ultrasonic vibrations. The dust samples produced by these methods were quite satisfactory but it was found that fibrous minerals were being so finely ground that only fibres of very short length remained.

Alternative grinding methods were tried for these samples, and it was found possible to produce suitable dust by grinding bulk samples saturated with distilled water in a small hand mortar. These samples consisted almost entirely of long fibres often several hundred μm in length and very few small particles were present at all. Because of this it was possible in several cases to compare samples of the same mineral that consisted of either long or short fibres. Brucite although a fibrous mineral could not be effectively ground in a hand mortar and so a sample of this, grindex ground, was sieved through a 50 mesh sieve rather than a 250 mesh one. This did not of course produce a pure sample but many long fibres were found to be present and this allowed some comparisons with a 250 mesh sample that contained no long fibres at all.

For all mineral samples the dosage was 10 mg suspended in 0·5 ml of distilled water. This was injected into the upper right pleural cavity of Balb/C mice and batches of 25 mice were used for each sample. Animals were killed at intervals of from 2 weeks to 18 months after injection and the lesions removed for histological study after fixation in formol saline. (Sections were stained with either H. and E. or Van Gieson's method for collagen.) In most cases the density of tough mineral particles in the tissue precluded electron-microscope examination, but this was possible with the chrysotile samples and tissue from these animals was fixed in buffered osmium tetroxide before embedding in " Araldite ".

OBSERVATIONS A

Details of Dust Samples Used in These Experiments

(1) *Brucite* $Mg(OH)_2$

The sample used was fibrous brucite and consisted of approximately 95 per cent $Mg(OH)_2$ with 5 per cent of various impurities. After grinding and sieving through a 250 mesh sieve the brucite sample consisted for the most part of irregularly shaped pieces that ranged from 0·2 μm to 2 μm in diameter. No large diameter fibres were present, but there were some short single crystal fibres with diameters of about 0·5 μm and lengths up to 2 μm. A sample sieved through a 50 mesh sieve still contained many small irregular particles, but in addition there were large numbers of long crystalline fibres from 0·5 μm to 3 μm in diameter and up to 50 μm in length.

(2) *Chlorite* $(Mg, Al, Fe)_{12}$ $(Si, Al)_8O_{20}(OH)_{16}$

The chlorites are a group of minerals with a layered structure which in many respects resemble the micas. After grinding the dust sample consisted mostly of irregularly shaped particles 1–3 μm in diameter, although some very small pieces no more than 0·1 μm in diameter were present.

(3) *Chromite* $FeO\ (Cr\ Al)_2O_3$

Chromite is a member of the spinel group (like magnetite) and is an accessory mineral of both igneous and metamorphic rocks. After grinding bulk samples of this material the dust consisted of irregularly shaped particles from 0·1 μm to 5 μm in diameter. The average particle diameter was about 1 μm. Fig. 1 is an electron-micrograph of this sample.

(4) *Forsterite* $3Mg_2SiO_4$

The sample used was produced by the thermal decomposition of chrysotile and therefore contained about 12 per cent of silica in addition to forsterite. After grinding the dust consisted mainly of short cylindrical particles 1–2 μm in diameter and up to 10 μm long. Electron-microscope examination showed that the basic chrysotile crystals had become distorted and partially fused with one another and that grinding had broken these multicrystal bundles into short lengths. A few single crystals or small groups of crystals were always present, but far fewer than normally found in unheated chrysotile samples.

(5) *Magnetite* Fe_3O_4

Magnetite is one of the ubiquitous and abundant oxide minerals in igneous and metamorphic rocks and is the principal magnetic ore.

After grinding, the magnetic dust used in these experiments consisted of irregularly shaped particles which ranged from 0·05 μm to 5 μm in diameter. The average particle diameter was about 2 μm. Fig. 2 is an electron-micrograph of this sample.

(6) *Olivine* $2(Mg\ Fe)\ O.SiO_2$

Olivine is an orthosilicate and commonly occurs in basic igneous rocks. The finely ground samples used in this study consisted of irregularly shaped particles which ranged from 0·1 μm in diameter to about 3 μm in diameter. The majority of the particles appeared to be approximately 0·5 μm in diameter. Fig. 3 is an electron-micrograph of this dust sample.

(7) *Pyroxene*

Pyroxenes are single chain structure silicates and this particular sample was based on Mg(Ca)Fe silicate. The dust sample used consisted of irregularly shaped particles from 0·1 μm to 3 μm in diameter. Many particles were elongated and had jagged pointed ends, but they appeared to be fragments of large crystalline masses rather than broken pieces of fibre. Figure 4 is an electron-micrograph of this dust.

(8) *Serpentine*

The sample used in this study was serpentized dolomite and consisted of 60 per cent antigorite (serpentine, $Mg3Si_2O_5(OH)_4$) plus 4 per cent calcite, $CaCO_3$. After sieving the dust consisted of very small irregularly shaped particles from 0·05 μm to 1 μm in diameter. Fig. 5 is an electron-micrograph of this type of dust.

(9) *Talc*

Talc is a sheet like silicate of approximate composition $Mg6(Si_8O_{20})(OH)_4$. The dust sample used in these experiments consisted mostly of irregularly shaped plates 1–10 μm in length. Mixed with the talc plates were small quantities of asbestos fibres from 0·05 μm to 0·5 μm in diameter and up to 2 μm in length. An electron-micrograph of this sample is shown in Fig. 6.

(10) *Glass fibre*

The glass samples used were of boron silicate glass, and were initially received as a felted mass. One sample had an average fibre diameter of 0·05 to 0·1 μm and the average diameter of the other was from 2·5–4 μm. The overall size range was, however, quite large. The thin fibre sample contained many fibres as much as 1 μm in diameter, and also some up to 100 μm long. Figure 7 is an electron-micrograph of a sample of the fine fibre glass prepared in this way. When the same glass samples were mechanically ground and sieved through a 250 mesh sieve it was found that the size range of the fibres had been considerably changed. All the fibres had been broken into quite short lengths and few were found more than 10 μm long. The thinnest fibres had, however, been more severely affected than the thick ones and many of the very fine fibres had been ground to a non-fibrous powder. The result of this was that the machine ground samples consisted of short lengths of fibre with an average diameter greater than that of the original samples. This can be seen by comparing Figs. 7 and 8.

(11) *Silica fibre*

This fibrous material consisted of 98 per cent silicon dioxide and the average fibre diameter was about 1 μm. A considerable number of fibres were, however, as small as 0·1 μm in diameter while some were as thick as 3 μm. When this material was gently ground in a hand mortar the dust samples consisted almost entirely of quite long fibres from 10–100 μm in length (Fig. 9). When, however, the dust was mechanically ground and sieved through a 250 mesh sieve, the larger fibres were broken up into short lengths of from 1–5 μm while the finest fibres were almost completely destroyed. These were often ground to a fine non-fibrous powder (Fig. 10).

(12) *Man-made insulation fibres*

Three samples were used in this study. The first was a pure alumino-silicate and had an average fibre diameter of about 4 μm. However, a few fibres were as fine as 0·75 μm and some were as large as 15 μm in diameter. The second sample was a type of calcium silicate with an average fibre diameter of 10 μm. A few fibres were, however, as fine as 1·5 μm in diameter while some were as much as

10 μm. The third sample was a calcium alumino-silicate with an average fibre diameter of 4 μm. The finest fibres were, however, only 0·5 μm in diameter while the thickest were as much as 10 μm. These fibrous minerals behaved in exactly the same way as glass fibre when subjected to different methods of grinding. Gentle grinding in a hand mortar produced samples consisting almost entirely of long fibres, some up to 200 μm in length, while mechanical grinding and sieving produced samples containing large number of short fibres mixed with irregularly shaped non-fibrous particles. As with glass fibre the smallest fibres had disintegrated the most.

(13) *Normal chrysotile*

This dust sample was the same one used in the series of experiments described by Davis (1970). It was prepared by Dr P. F. Holt of Reading University (Holt and Young, 1960). The dust consisted almost entirely of fibrous chrysotile which varied in size from large bundles of crystals 200 μm long and 10 μm in diameter down to single crystals less than 1 μm long and 25–30 μm in diameter. By far the majority of the dust, however, was in the form of crystal bundles less than 5 μm in length and between 0·1 and 1 μm in diameter.

(14) *Short fibre chrysotile*

This sample of chrysotile had been subjected to ultrasonic vibration over long periods while it was suspended in water. In the final sample the fibre bundles had completely broken down to single crystals and these crystals had been broken until few were more than 1 μm long. From Fig. 11 and 20, however, it will be seen that these short crystals had a strong tendency to aggregate into large irregular masses.

(15) *Synthetic chrysotile*

This sample consisted of single short chrysotile crystals mixed with some amorphous material. There was, however, a considerable range of crystal diameter. The largest crystals appeared tubular and were often only 40–50 μm in diameter, while the smallest ones were " solid " needles only 15–20 μm in diameter. When suspended in water the particles tended to aggregate into large masses and these masses were apparently retained in the tissues. A separated sample of synthetic chrysotile crystals is shown in Fig. 12.

OBSERVATIONS (B)

All dust samples used in these experiments produced granulomata in the pleural cavities of the mice. The number of granulomata produced in any animal varied considerably and depended on the degree of dust dispersion after the initial injection. With any one injection material, however, the amount of granulation tissue produced in response to 10 mg of dust was very similar in all animals of the group. In all cases the cellular response to the dust consisted initially of macrophages but later a few giant cells were often found together with a variable number of fibroblasts. There was, however, great variation in the magnitude of the cell response, and while some granulomata were very small and encapsulated others were large and formed firm adhesions between the various organs in the chest cavity.

Of all the samples tested, chromite and magnetite stimulated the poorest cellular response. These were the two heaviest minerals used and since the dose was calculated by weight small granulomata were expected. With these two specimens, however, macrophage accumulation was extremely small. The dust particles became aggregated into small compact masses, and only a few cells penetrated the closely packed dust (Fig. 13). As shown in Fig. 1 and 2, these dust samples consisted mainly of irregularly shaped small particles, although some larger fragments were always present. The larger dust particles were of course

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EXPLANATION OF PLATES

FIGS. 1 to 12.—Electron-microscope photographs of the dust samples used in this study. Samples 1 to 6, 8 and 10 were prepared by mechanical grinding followed by sieving through a 250 mesh copper sieve. Samples 7 and 9 were prepared by gentle grinding in a hand mortar, and sample 11 was produced by prolonged ultrasonic vibration while the mineral was suspended in water. The minerals concerned are Fig. 1 chromite × 10,000, Fig. 2 magnetite × 10,000, Fig. 3 olivine × 10,000, Fig. 4 pyroxene × 10,000, Fig. 5 serpentine × 10,000, Fig. 6 Talc × 10,000, Fig. 7 long glass fibre × 10,000, Fig. 8 short glass fibre × 10,000, Fig. 9 long silica fibre × 10,000, Fig. 10 short silica fibre × 10,000, Fig. 11 short fibre chrysotile × 25,000, and Fig. 12 synthetic chrysotile × 45,000.

FIG. 13.—A small granuloma produced in a mouse pleural cavity in response to chromite dust injected 6 weeks previously. The lesion consists mainly of large opaque aggregations of dust particles, and the cell response to the dust is minimal. The granuloma is suspended in the pleural cavity by a very thin band of connective tissue (A). × 450.

FIG. 14.—Part of a granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of some machine ground glass fibre (average fibre diameter 0·05 μm). The dust particles are closely packed together, but some macrophages have been able to penetrate the dust mass and these have phagocytosed many of the the smaller fragments. The surface of the lesion is smooth and is composed of a very thin layer of fibrous tissue. × 450.

FIG. 15.—Part of a granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of some talc dust. The cellular response to this dust is very great and the smaller dust particles are invisible among the large numbers of macrophages. A few large elongated plates of talc are however, visible and are marked T. This granuloma is firmly attached to the lung surface and lung tissue is labelled L. × 450.

FIG. 16.—Part of the granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of serpentine dust. Serpentine granulomata are very cellular, but the dust particles are mostly 1 μm or less in diameter so that they are invisible in the light microscope. × 450.

FIG. 17.—Part of a granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of pyroxene dust. There has been a considerable macrophage response to this dust, and although most of the dust particles are invisible in the light microscope some large dust aggregations are visible and are marked P. The surface of this granuloma is smooth and consists of a very thin layer of fibrous connective tissue. × 450.

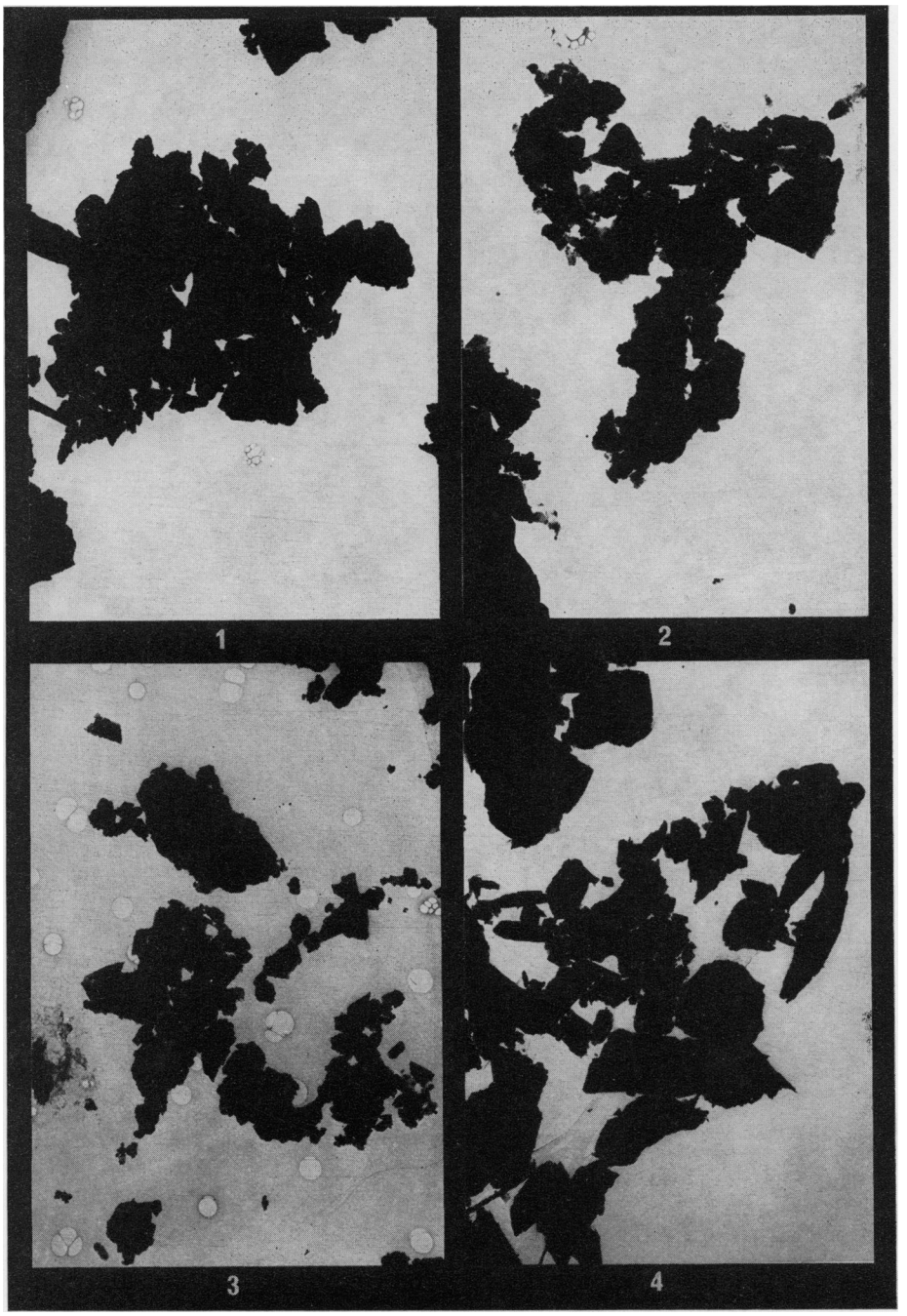
FIG. 18.—A low magnification photograph showing a large area of granulation tissue produced in the pleural cavity of a mouse 2 weeks after the injection of a sample of long glass fibres. (Average fibre diameter 0·05 μm.) This granuloma has formed a firm adhesion between the diaphragm D and the lung lobe L. × 150.

FIG. 19.—A high magnification photograph from the same granuloma as that shown in Fig. 18. Long glass fibres (G) are visible among the macrophages that make up the lesion, but they are quite widely separated and not compacted into solid groups. × 450.

FIG. 20.—An electron-microscope photograph from a granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of chrysotile dust that had been broken by ultrasonic vibration to an average crystal length of 1 μm. Some small clumps of chrysotile crystals have been phagocytosed by macrophages (C), but a large mass of crystals (M) has remained extracellular. × 40,000.

FIG. 21.—Part of a granuloma produced in the pleural cavity of a mouse 2 weeks after the injection of a sample of synthetic chrysotile. The lesion contains large eosinophilic masses (M) which are made up of closely packed crystals of synthetic chrysotile, and among these masses there has been moderate infiltration by macrophages and fibroblasts. × 450.

FIG. 22.—An electron-microscope photograph of a granuloma produced in response to synthetic chrysotile. Some small aggregates of crystals have been phagocytosed by macrophages (C), but two large dust masses (M) remain extracellular. They are surrounded by cells but in each case there is continuity between the dust and the the main tissue spaces. × 12,000.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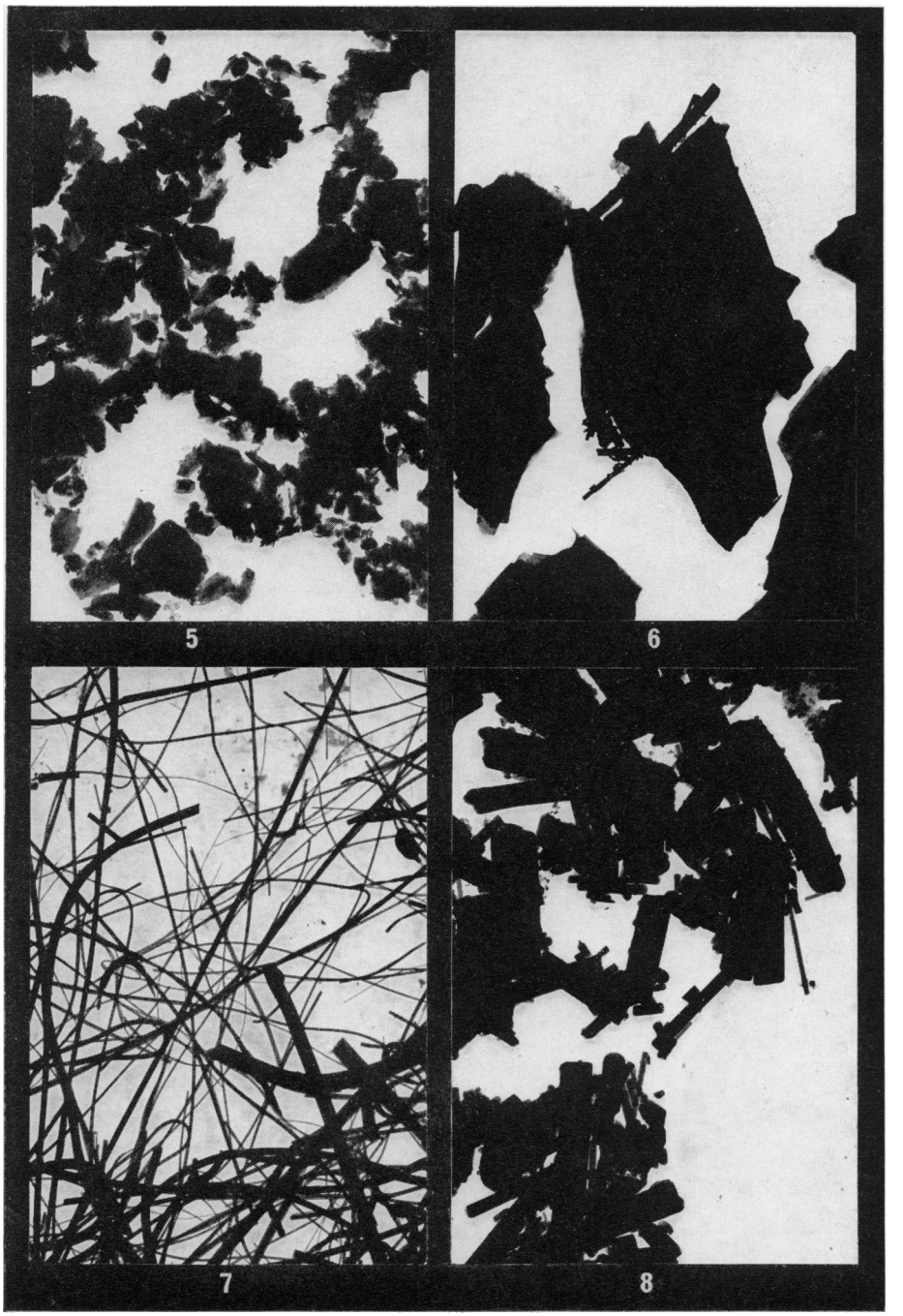
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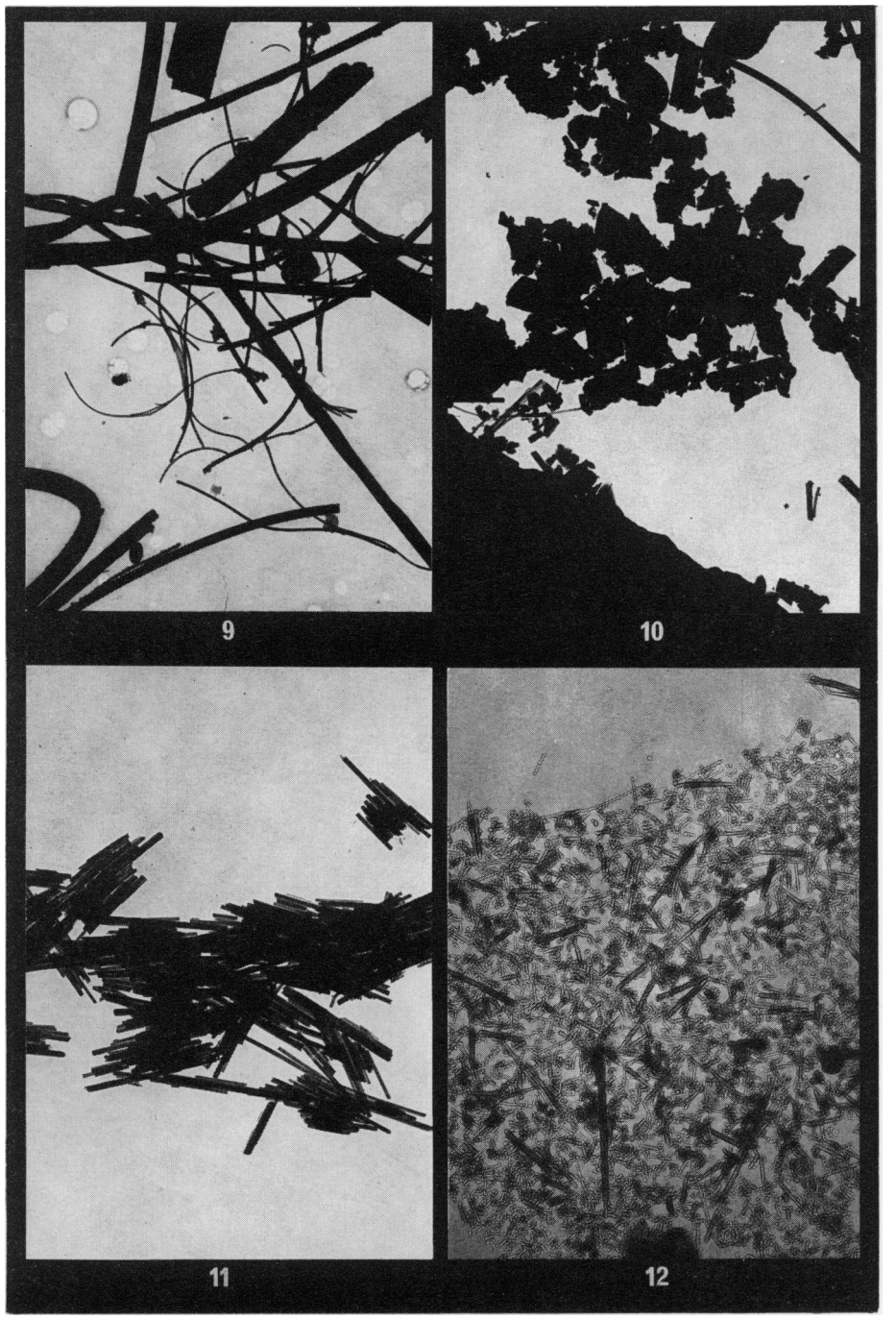

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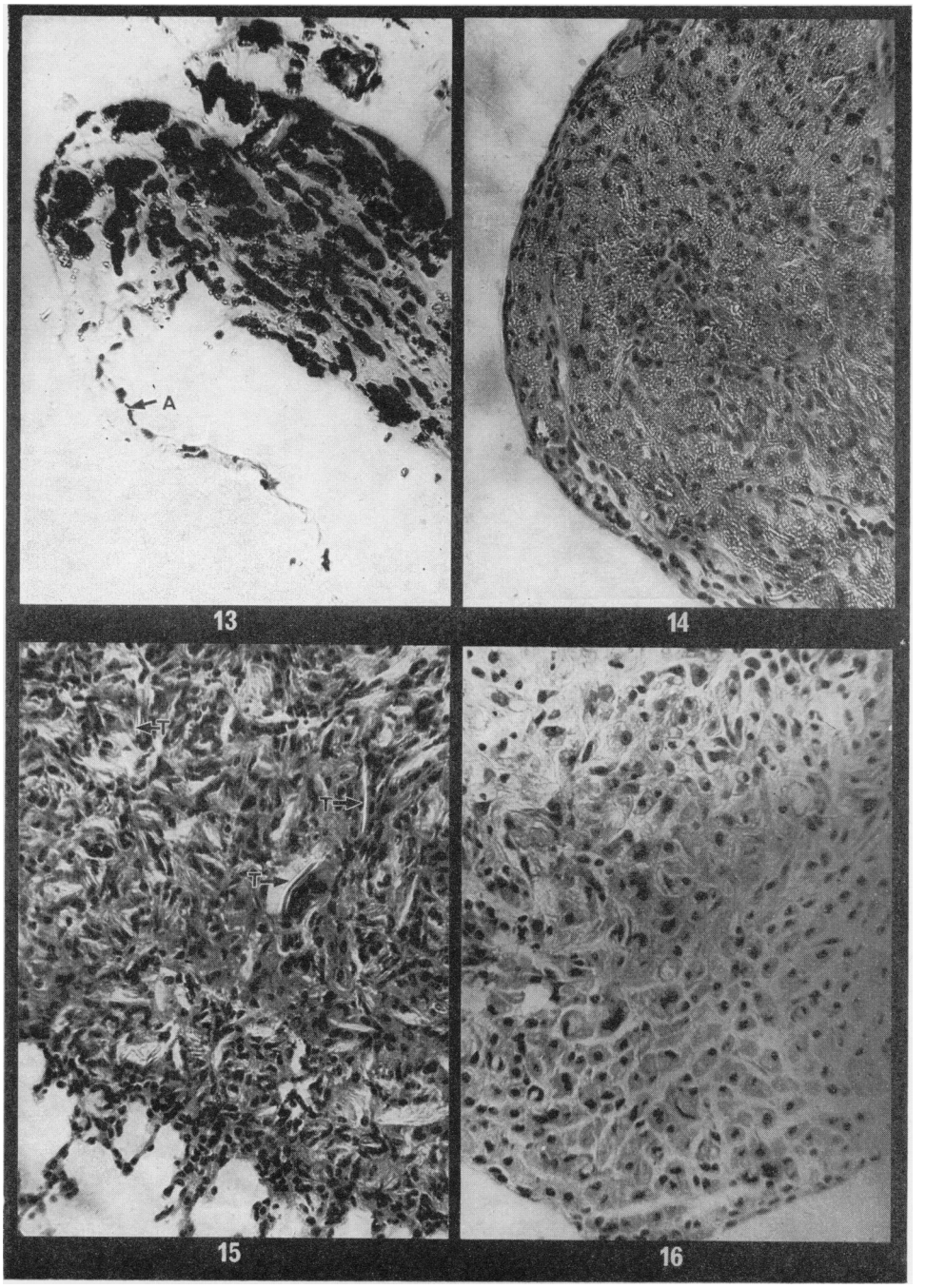
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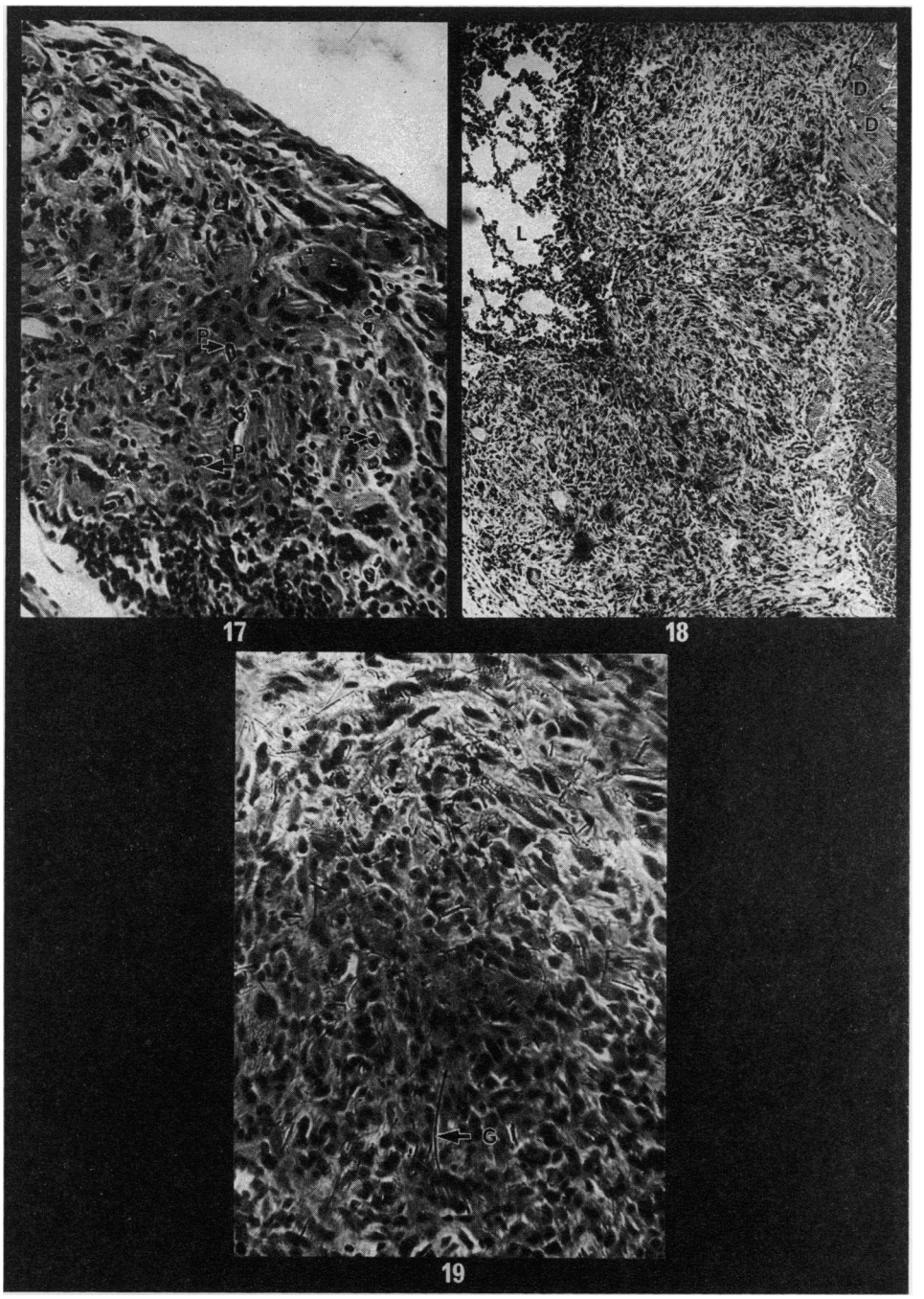
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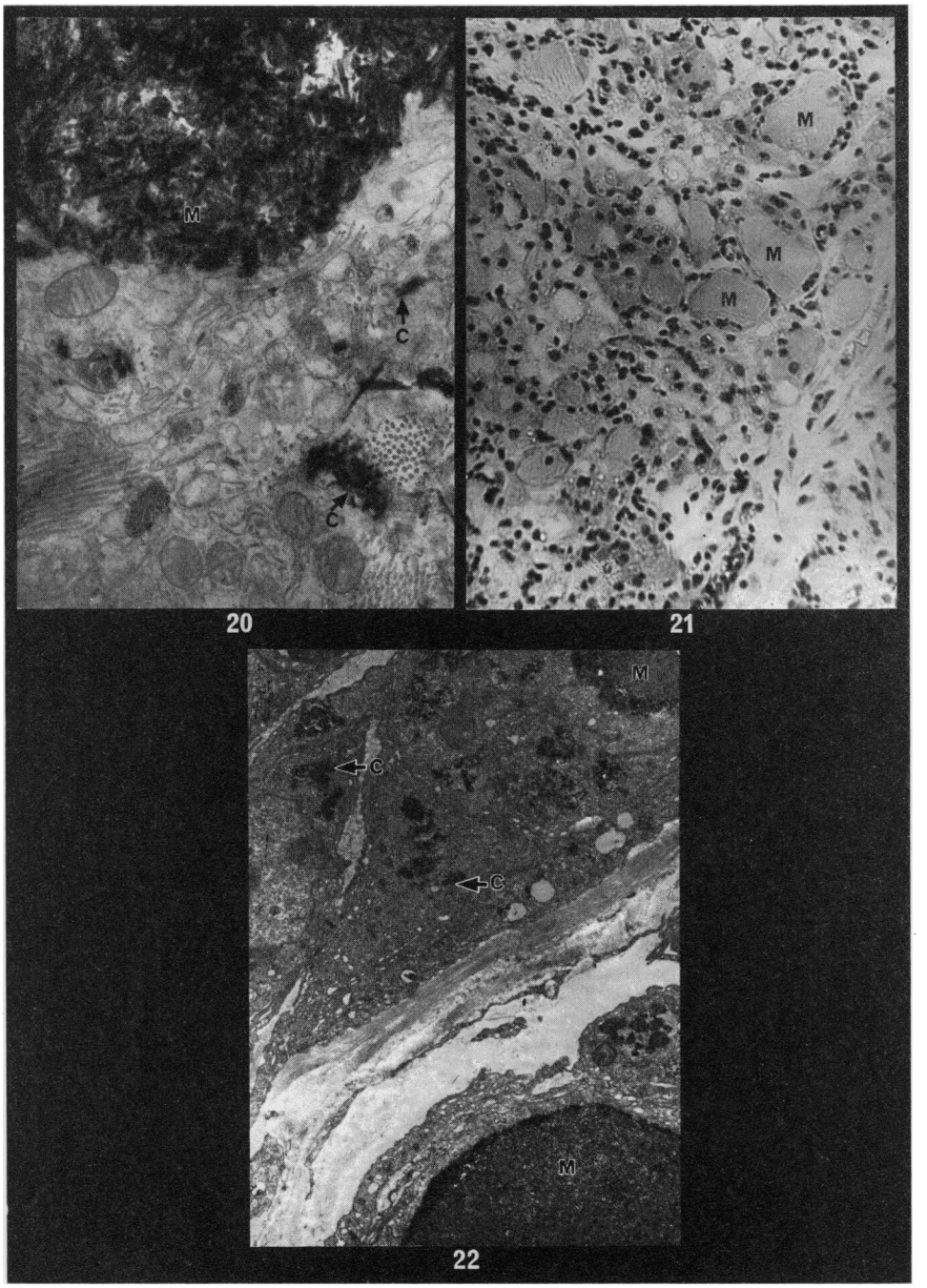
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too large to be phagocytosed by single macrophages, but could easily have been enclosed by giant cells. The macrophage response, was, however, too small for giant cells to form and those macrophages that did penetrate the dust only phagocytosed an occasional small particle. Around the outside of the dust masses a thin capsule of fibroblasts was present from about a week after injection, and this capsule was usually connected to thin strands of connective tissue which anchored the whole lesion to one place in the chest cavity (Fig. 13). These strands were, however, very pliable and at autopsy the lesions could be moved freely. The granulomata were never firmly attached to any organ and formed no adhesions. The granuloma capsules contained some collagen by 2 weeks after injection and were usually completely fibrosed by 6–8 weeks. Where cells had penetrated the dust masses these were eventually replaced by small amount of collagen, but this process usually took several months.

Chlorite, ground glass fibre (both thick and thin), ground silica fibre and ground insulation fibres all produced very similar lesions. In these the dust particles were also grouped into compact masses, but the degree of cell penetration was much greater than with chromite or magnetite (Fig. 14). Most of the cells that initially penetrated the dust were macrophages, and these phagocytosed some of the smallest dust particles. Many particles were too large, however, and since no giant cells were seen in these lesions the largest pieces of dust remained extracellular. A thin capsule of fibrous tissue had formed around the granulomata by 2 weeks after injection, and this often connected with thin strands of supporting connective tissue. The lesions were, however, freely movable and no adhesions were formed. The capsule fibrosed within a few weeks of dust injection and the granulomata cells were gradually replaced by collagen over a period of 6–8 months. The amount of collagen finally produced in these granulomata was much greater than that found with chromite and magnetite, and in old lesions the dust was firmly encased in a network of fibrous tissue.

Talc, serpentine, pyroxine, olivine, forsterite, and finely ground brucite formed another group with considerable similarity between the lesions. With these minerals the dust became much less closely packed and the cellular response was extremely good. Large cellular granulomata were produced, and those dust particles visible with the light microscope were widely spaced (Figs. 15, 16 and 17). Macrophages phagocytosed small dust particles and with all these minerals some giant cells were formed in the lesions. As with the previous two groups, the granulomata were quickly encapsulated by a thin layer of fibrous tissue, and some of them remained mobile within the chest cavity supported by thin strands of connective tissue. These included granulomata produced in response to serpentine, olivine, forsterite and brucite. With talc, however, and occasionally with pyroxene, some granulomata were formed very firmly attached to the surface of the lungs or other chest contents. In the case of talc some granulomata produced an occasional tenuous adhesion between the lung lobes. A little collagen was quickly produced in the surface layers of all these granulomata, and throughout the lesions many of the cells were replaced by collagen over a period of 6–8 months. Since the granulomata in this group were much larger in relation to the dust dosage than those of the two previous groups the amount of collagen produced in response to the dust was proportionally much greater. One complicating factor in this group was found with brucite. Initially brucite granulomata were as cellular as the others and the early stages of fibrosis certainly occurred. From 6 months

onwards, however, the brucite lesions appeared to become reduced in size and in some of the oldest animals they could not be found at all. Since brucite would be expected to have a relatively high solubility in tissue fluids, it was assumed that the dust was dissolving with a consequent reduction in the size of the lesions.

The long fibre group of dusts, glass, silica, long brucite, and the insulation fibres, produced the largest granulomata of all. These dust fibres remained widely separated in the tissues, and were quickly surrounded by very large numbers of macrophages some of which combined to form giant cells (Figs. 18 and 19). Small dust fibres were phagocytosed by single macrophages and the large ones if not all completely enclosed within giant cells were all closely surrounded by macrophages. These granulomata did not usually become encapsulated, but often filled the space between the various organs of the chest cavity, binding them together with firm adhesions (Fig. 18). A small amount of collagen was present in the granulation tissue from about 2 weeks after injection. After this time collagen production was gradual but continuous, until in old animals the lung lobes were often found firmly bound to the chest wall by masses of old fibrous tissue that still contained dust fibres. In this group brucite once again showed some differences from other minerals. The initial brucite lesions were similar to others in the group and formed firm adhesions. The lesions in old animals, examined 12 months or more after injection, were, however, smaller than those seen during the first few weeks of the experiment. The amount of collagen produced was less than might have been expected from the size of early lesions, and the dust which was initially clearly visible, was more difficult to find in old animals.

The last group of dusts studied consisted of varieties of chrysotile asbestos. The results obtained in mice with a standard sample of chrysotile have already been published (Davis, 1970). Large cellular granulomata were produced in response to this dust and these consisted mainly of macrophages and giant cells. These granulomata from the first formed firm adhesions between the chest contents. Some collagen had been produced by 2 weeks after dust injection and the collagen content quickly increased until by 12–18 months some granulomata had been converted completely to old fibrous tissue. These lesions were in fact very similar to those found with the other " long fibre " dusts except that finely divided chrysotile is invisible with the light microscope and could only be seen in the tissues by electron-microscopy. These electron-microscope studies showed that all the dust present in the granulomata was very quickly engulfed by cells. Small particles were phagocytosed by single macrophages and large fibres were enclosed by the fusion of macrophages to form giant cells.

With the sample of short fibre chrysotile, which consisted of crystals 1 μm or less in length, much smaller granulomata were produced and these were usually encapsulated and did not form adhesions. Macrophages were much more widely spaced in these lesions than in " long fibre " granulomata and giant cells were not produced. Some collagen was quickly formed in the capsule, and later the macrophages within the granulomata were replaced by collagen. Since fewer cells were present to start with, however, the final collagen content of these lesions was much lower than that found with long fibre chrysotile. Electron-microscope examination of the short fibre lesions showed that while some of the dust had been phagocytosed by macrophages, much of it had been compacted into quite large masses that had not been penetrated by cells (Fig. 20). These masses were usually closely sur-

rounded by macrophages, but there was no sign of macrophage fusion to form giant cells.

With synthetic chrysotile, granulomata were also produced but these were even smaller than those produced in response to " short fibre " chrysotile. They never formed adhesions but were usually anchored by very fine strands of connective tissue. These granulomata contained even fewer macrophages than " short fibre " chrysotile lesions, and no giant cells were formed. The granulomata did, however, contain some very large eosinophilic masses often 200–300 μm in diameter that were usually surrounded by a thin coating of macrophages (Fig. 21). In old animals the lesions did contain a thin network of collagen but very little in comparison to that produced in response to other chrysotile types. Electron-microscope examination showed that the large eosinophilic areas were made up of compacted masses of synthetic chrysotile (Fig. 22). Those macrophages present in the granulomata had taken up some of the dust, but for the most part they merely surrounded the large masses in a thin layer. There were no signs of macrophage fusion.

DISCUSSION

Intrapleural injection of a variety of mineral dusts into mice has shown that in this site at least, all foreign material will stimulate the production of a certain amount of collagen. Both the chemistry of the foreign material and particularly its shape do, however, produce considerable variation in the amount of collagen produced per milligram of dust injected, and it is important to consider these aspects in more detail. It is obvious from these experiments that the final degree of fibrosis produced in the pleural cavity is exactly paralleled by the initial accumulation of cells around the injected material. Long fibre dusts quickly result in large cellular granulomata and these are eventually replaced by large amounts of fibrous tissue. However, some granular dusts like magnetite and chromite attract very few cells indeed and the eventual production of collagen is minimal. The reasons for these differences are of great interest. As far as granular dusts are concerned it would appear that chemistry is more important than shape. Figures 1 to 5 show that there was no significant difference in particle shape or size distribution between the samples of chromite, magnetite, pyroxene, olivine and serpentine although the first two produced little tissue response while the last three resulted in marked though localized fibrosis.

That particle size and shape may be very important in dust pathology is shown by the experiments using long fibre dusts. When these dusts are finely ground and sieved, they produce small distinct lesions which although cellular contain fewer cells than the serpentine groups, and they never produce adhesions. Samples specially prepared to contain only long fibres produce much larger and more cellular lesions, which almost invariably produce adhesions. An examination of old lesions of both types also shows that long fibre samples result in much more fibrosis than short fibre specimens of the same mineral. Attempts to elucidate the exact cause of these difference must be based on speculation. However, the most likely reason seems to be that haphazardly arranged long fibres will take up much more room than they would if broken into short lengths. Assuming, therefore, that the cellular response will continue until all the dust is enclosed, the widely spaced fibres will produce much larger lesions than the closely packed short

ones. Moreover, a network of long fibres would have many more spaces for macrophages to penetrate and this could also explain the much greater cellularity of long fibre granulomata. Masses of compacted short fibres would be difficult for cells to penetrate and the ratio of cells to dust particles would be smaller. Chrysotile dust appears to conform to these ideas very well. Standard chrysotile contains many long fibres and a small dust sample takes up a very large volume. This dust produces very large granulomata. Chrysotile broken by ultrasonic vibration to an average crystal length of 1 μm takes up less space and produces small granulomata. Synthetic chrysotile which consists of very short crystal lengths mixed with amorphous material takes up even less space and produces very small granulomata. This dust is compacted into large masses in the tissues and macrophages appear unable to penetrate these masses.

Regardless of the differences in size and cell content between granulomata produced in response to long and short fibre dusts, the greatest point of difference is that long fibre dusts are the only ones to produce adhesions. The reason for this is also probably based on mechanical considerations. Short fibre dusts are easily aggregated into compact masses in the pleural cavity, and are probably encapsulated before they are able to damage the surface of any organs. Masses of long fibre dusts are much less easily compacted and surrounded by cells. It seems likely that the constant movements of the pleural cavity with respiration would result in the repeated penetration of sharp long fibres through any coating of macrophages, and these would cause considerable irritation. This irritation of the pleural surfaces would itself result in the accumulation of inflammatory cells and the granulomata would eventually be firmly bound to any surface with which they came into contact. Talc was the only " non-fibrous " dust to produce adhesions, but this did contain some long plates of dust which could have acted in the same way as long fibres.

The results obtained in the pleural cavity appear quite definite, and show interesting variations in tissue reaction between the various dust types. From the clinical point of view, however, it is the reaction of lung tissue to dust that is important, and this dust will arrive in the lung by the inhalation of widely dispersed particles rather than by the injection of compacted dust masses. It is important, therefore, to consider whether the findings from the present series of experiments give any indication on how the same dusts would behave after inhalation. It has been suggested that the lung is much less susceptible to fibrosis than other tissues of the body, and this may well be true. Some dusts do, however, cause lung fibrosis, and it is difficult to imagine that lung tissue would react in the way that would change the relative fibrogenicity of a series of dusts if the dusts remained in their initial site of deposition. The susceptibility of dusts to the lung clearance mechanisms is probably the most important factor deciding on whether a particular sample will cause fibrosis. Long fibres of any dust once they have reached the alveoli will be difficult to move, while short fibres, or non-fibrous dust that can easily be phagocytosed by a single macrophage will be cleared much more easily. It is important to find out if long fibre dusts, fibrogenic in the pleural cavity are still fibrogenic in the lung and whether short fibre dusts also fibrogenic in the pleural cavity are less damaging to lung tissue because the particles are attacked by macrophages and removed. No doubt the ability to clear any particular dust sample varies with the individual, and it should be noted that occasional cases of pneumoconiosis have been reported due to such dusts as talc

(Tronzano, 1966); although large amounts of talc are handled industrially, and most workers seem able to deal with the dust effectively.

If the above suggestions are true then all long fibre dusts so far tested are potentially pathogenic to lung tissue. Fortunately, however, fibres with a diameter of more than about 3 μm do not reach the alveoli so that many industrially used fibres are completely safe, and this applies to the insulation fibres used in the present series of experiments. It is possible, however, that if industrial use is found for any fibrous materials with a diameter of much less than 3 μm then these materials will need to be handled with care. At present a series of inhalation experiments is being planned in which a series of long fibre mineral dusts will be tested for their fibrogenic effect on lung tissue.

This study was undertaken as part of the programme of research sponsored by the British Asbestosis Research Council,

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