THE ULTRASTRUCTURAL CHANGES THAT OCCUR DURING THE TRANSFORMATION OF LUNG MACROPHAGES TO GIANT CELLS AND FIBROBLASTS IN EXPERIMENTAL ASBESTOSIS

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THE possibility of the formation of specialised connective tissue cells from mononuclear blood cells and other undifferentiated cell types has produced considerable controversy in the past, and the limits of these conversions and the pathways involved are still not well understood. It is, however, generally accepted that macrophages and later fibroblasts can be produced from mononuclear blood cells (Carrel, 1926; Maximov, 1927; Chevremont and Chevremont-Comhaire, 1945), and that foreign body giant cells are produced by the fusion of macrophages (Haythorn, 1929).

Detailed electron-microscope studies of the ultrastructural changes that accompany such transformations have not so far been forthcoming, due no doubt to the fact that as living material cannot be used one cell cannot be followed throughout the transformation process. Even in systems where transformations are known to occur it is not possible to be sure that any cell examined is in fact involved.

In a study of the effect of chrysotile asbestos dust on rat and guinea-pig lungs (Davis, 1963) it was found that although during the first few days of dusting the dust was found only in the lung macrophages, at later stages in the experiments it was found in large quantities in both giant cells and fibroblasts. It was fairly certain therefore that the lung macrophages were acting as stem cells for the production of both giant cells and fibroblasts, and also that the chrysotile dust could be used as a marker to spot the intermediate stages.

The structure of the alveolar macrophages was studied by Karrer (1958 and 1960) who reported that these cells were very irregularly shaped. Occasionally elongated processes were seen on the cell surface but no typical microvilli were present. The cytoplasm was found to contain many small vacuoles among which occasional elements of granular endoplasmic reticulum were present. Ferritin granules were scattered throughout the cytoplasm, and phagocytosed inclusions were common. These took the form of either circular laminated bodies or granular masses bounded by a single or double membrane. Karrer considered that the phagocytosis of foreign material was achieved by the formation of large leaf-like pseudopodia on the macrophage surface which could fold back upon the cell and entrap foreign material in the resulting impocketings.

The ultrastructure of fibroblasts engaged in collagen production has been studied by many workers including Wasserman (1954), Jackson (1954, 1956, 1957), and Porter and Pappas (1959). The structures reported from different types of fibroblasts are quite uniform, and in general it may be said that fibroblasts are spindle-shaped cells that contain many granular elements of the endo-

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plasmic reticulum (α cytomembranes). Large numbers of very fine filaments are often found in the cell cytoplasm among the α cytomembranes and it is suggested that these may be collagen precursors. In most cases no foreign cytoplasmic inclusions have been reported in the fibroblasts examined. Ross and Benditt (1962) in studies of experimental wound healing in guinea-pig skin studied the ultrastructure of both the macrophages and fibroblasts found in the wounds. They found that fibroblast structure conformed exactly with that described previously, and that the cells contained many well developed α cytomembranes. They also found that macrophage cytology was similar to that described by Karrer for lung macrophages except that the wound macrophages contained fewer foreign body inclusions. These workers suspected that in their experiments fibroblasts were produced from macrophages because "myelin" inclusions similar to those common in macrophages could occasionally be found in fibroblasts, and also because some macrophages could be found which contained many α cytomembranes.

So far there have been few electron-microscope observations on the formation of giant cells although Ham and Leeson in 1961 suggested that they might be formed from macrophages by an interdigitation of the cell membranes. In 1959 Gusek and Naumann reported some further electron-microscope studies of giant cells from tuberculosis but no photographs were published.

MATERIALS AND METHODS

The animals used in this study were supplied by Dr. P. F. Holt of Reading University, and were treated by him with chrysotile dust in the apparatus described in 1960 (Holt and Young). The rats remained in the dusting tunnel for 95 days, during which time the apparatus was operating on fifty days for 18 hours a day. The guinea-pigs remained in the tunnel for 77 days, during which time the dust generator was running for 51 days for 18 hours a day. Animals were killed at intervals from three days after the start of dusting. The last rats and guinea-pigs were killed 35 and 31 weeks after the start of dusting, that is to say, after they had been removed from the dust tunnel for 22 and 20 weeks respectively.

In the earliest experiments some animals were treated with very fine dust in which the maximum particle size found in the electron microscope was about 2 μ while the rest were treated with a coarser dust in which some fibres as long as 20 μ were present. It was, however, found from both light and electron microscope studies that the pathological changes produced in the lungs by the two types of dust were identical and, in later experiments, only the coarse dust was used. All tissues were prepared for electron microscopy by the method described previously (Davis, 1959), except that in most cases only half the material was stained with phosphotungstic acid (PTA). This procedure has been adopted because it has been found that the dense staining of some cell components by PTA can, in some cases, mask fine structural details. In this study PTA stained tissue was used to obtain low power photographs in which the additional contrast is very useful. High power pictures were, in general, obtained with unstained material, except where stated in the text.

In these studies the cutting of ultrathin sections was made very difficult by the presence of large amounts of chrysotile dust in the lung tissue. The individual crystal diameters range from 200–400 Å and it was found that in any sections much under 1000 Å thick, most of the dust had been torn out of the plastic. As this process made the material impossible to study, it was decided to standardize on a section thickness of 1000 Å. This allowed examination of the tissue but, of necessity, resulted in some loss of resolution at high magnifications.

OBSERVATIONS

In the present study the ultrastructure of the lung macrophages found in control animals conformed exactly with that described by Karrer in 1958.

Macrophages were found scattered fairly evenly throughout the lung and their plasma membranes were comparatively smooth, showing only occasional cytoplasmic processes. After as little as three days dusting, however, the picture had changed considerably, and large numbers of macrophages had accumulated around the terminal bronchioles where the dust concentrations were highest. Phagocytosis of the dust proceeds rapidly and some macrophages were found containing very large amounts indeed. In the early stages of dust uptake the macrophage plasma membrane remains comparatively smooth (Fig. 1), but after only a few days of dusting many macrophages can be found whose surfaces are covered with large numbers of long processes (Fig. 3).

While not all dust containing macrophages seen in this study have shown these cytoplasmic processes, no such processes have been found in macrophages that contained no dust. The formation of cytoplasmic processes on the macro-

EXPLANATION OF PLATES

FIG. 1.—An alveolar macrophage from guinea-pig lung dusted with chrysotile dust for three days. The cell contains several particles of dust (arrowed) as well as laminated foreign body inclusions (F). At this stage the cell membrane has remained comparatively smooth although one or two elongated cytoplasmic processes have developed. ×9,000.

FIG. 2.—In this plate are included seven lung macrophages (m) which are becoming joined together in the early stages of giant cell formation. All the cells have large numbers of elongated cytoplasmic processes on their surface membranes and these are beginning to intertwine, binding the cells together. The two central cells are already closely bound, while the others are only at the point of initial contact. Particles of chrysotile dust are arrowed. The material was from a rat lung dusted for three days. ×4,500.

FIG. 3.—An alveolar macrophage from guinea-pig lung, dusted with chrysotile dust for three days. Many elongated cytoplasmic processes have developed on the cell membrane. Particles of chrysotile dust are arrowed. ×7,200.

FIG. 4.—Elongated cytoplasmic processes on the surface of a rat lung macrophage from an animal dusted for seven weeks with chrysotile asbestos dust. In this photograph the longest process is approximately 2 μ long and the average diameter of the process is 900–1,200 Å. ×31,200.

FIG. 5.—An area of interdigitation between the cytoplasmic processes of two lung macrophages from a rat lung dusted with chrysotile dust for seven weeks. The cells are only bound together loosely. ×27,500.

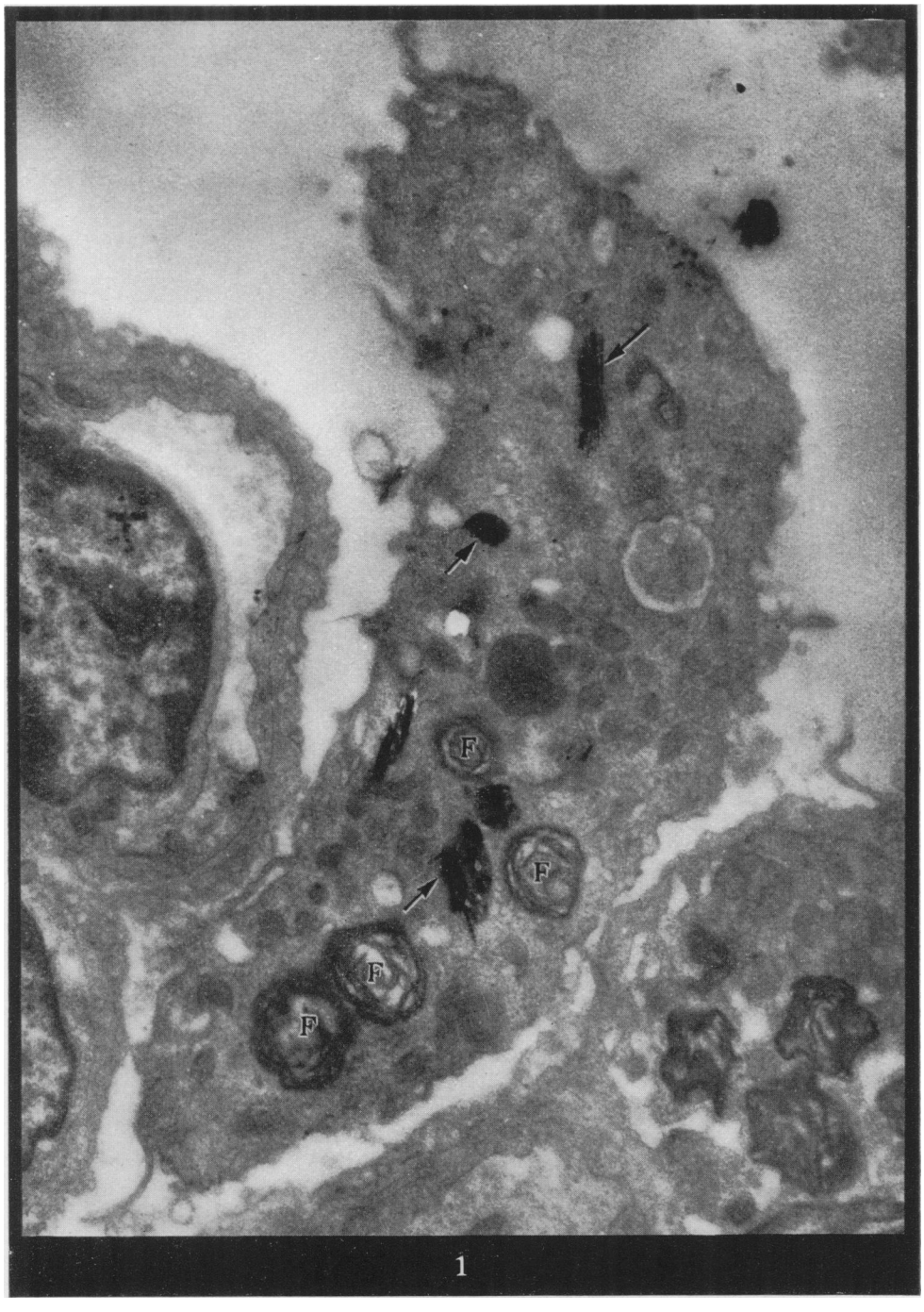
FIG. 6.—An area of interdigitation between the cytoplasmic processes of two lung macrophages from a rat lung dusted with chrysotile dust for 7 weeks. The processes are quite short, averaging only about 1μ in length and the two cells are bound closely together. ×25,000.

FIG. 7.—An area of cytoplasm from a guinea-pig lung macrophage after seven weeks' dusting. Layers of membranous sacs can be seen below the cell surface. These sacs have the same diameter (approx. 800–1,200 Å) as the elongated cytoplasmic processes seen during giant cell formation and are probably associated with these. ×18,000.

FIG. 8.—Part of a binucleate fibroblast from an area of fibrosis in a guinea-pig lung dusted for seven weeks with chrysotile dust. The granulated endoplasmic reticulum is well developed and all the foreign organic inclusions normally found in lung macrophages have disappeared. The fact that the cell was once phagocytic is indicated by the presence of clusters of chrysotile crystals (c). ×21,000.

FIG. 9.—A small giant cell formed near to a terminal bronchiole in guinea-pig lung dusted for 7 weeks with chrysotile dust. The constituent macrophages of this cell are at this point converting to fibroblasts and at two points a considerable amount of collagen (c) has already been formed. Inset is shown a small area of the same cell at much higher magnification and it can be seen that collagen fibres (arrowed) are laid down between the cell units while they are still closely opposed. Main photograph ×3,300. Inset ×22,000.

FIG. 10.—An area of fibrosis from rat lung after 16 weeks dusting with chrysotile dust. This fibrosis is situated close to a terminal bronchiole and as it is nodular in form it is fairly certain that it has been produced from what was formerly a giant cell. Parts of several fibroblasts are present among the bundles of collagen fibres and chrysotile fibres are present in all these cells. At some points (arrowed) where part of the cytoplasm has been consumed during collagen production, the dust has been partly liberated from the cell and is embedded in the collagen fibres. ×8,200.



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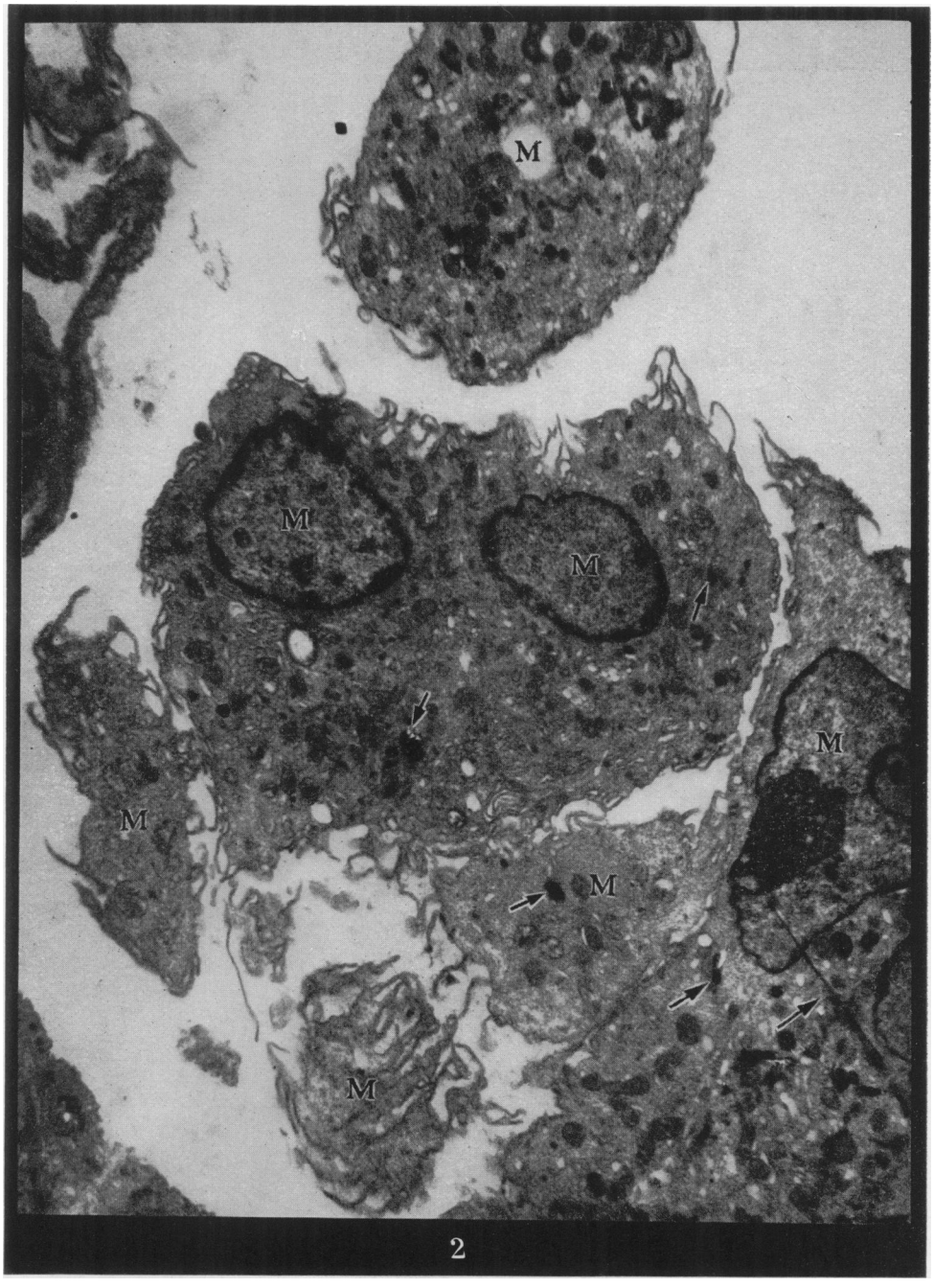

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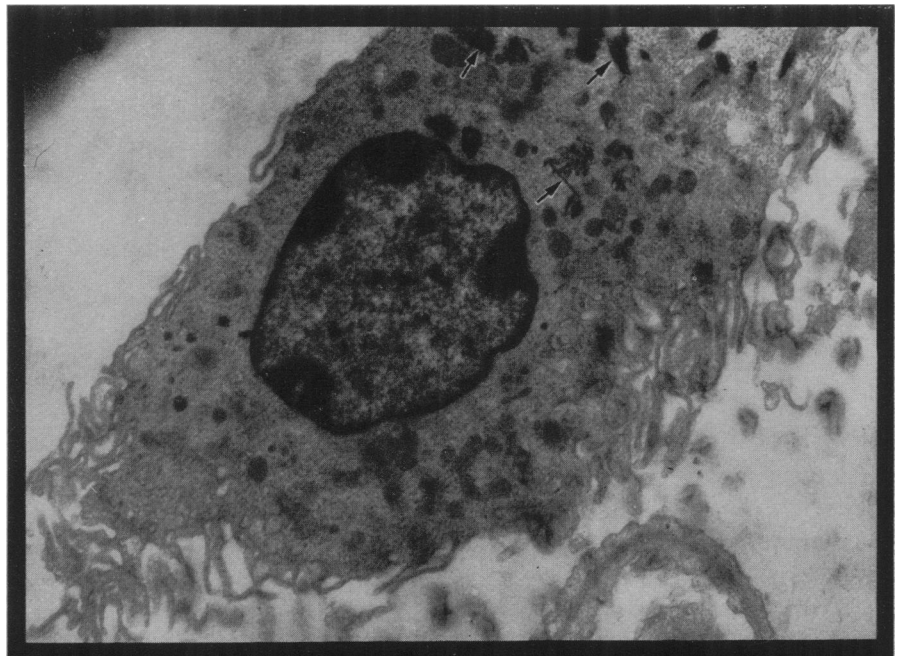

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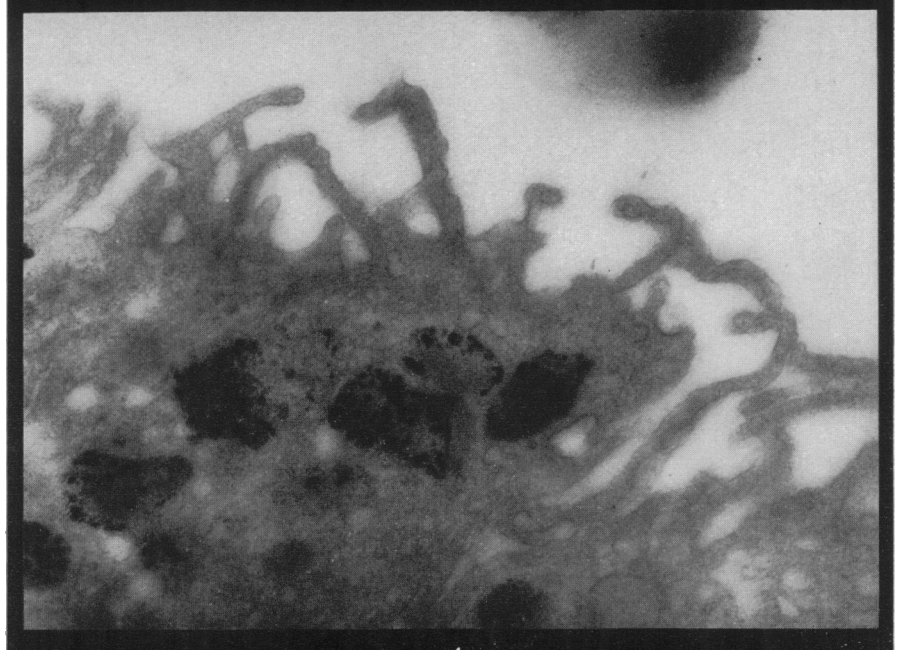

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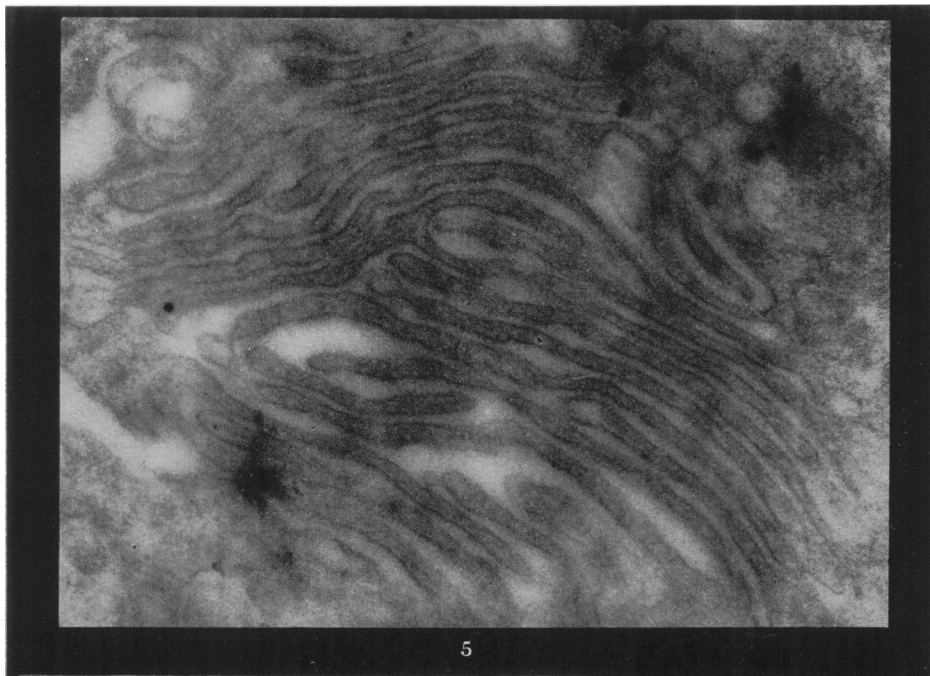

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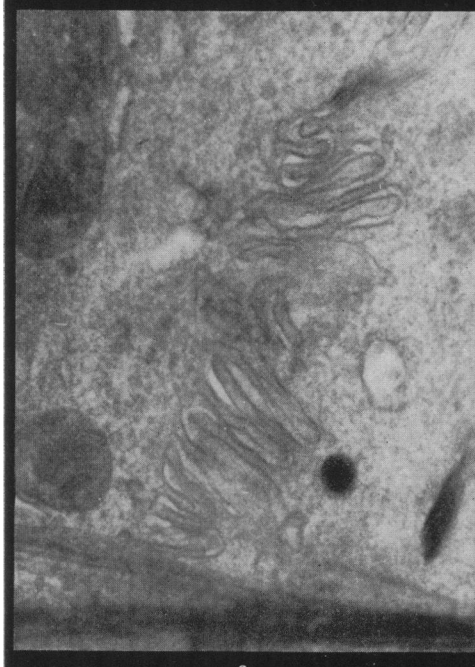

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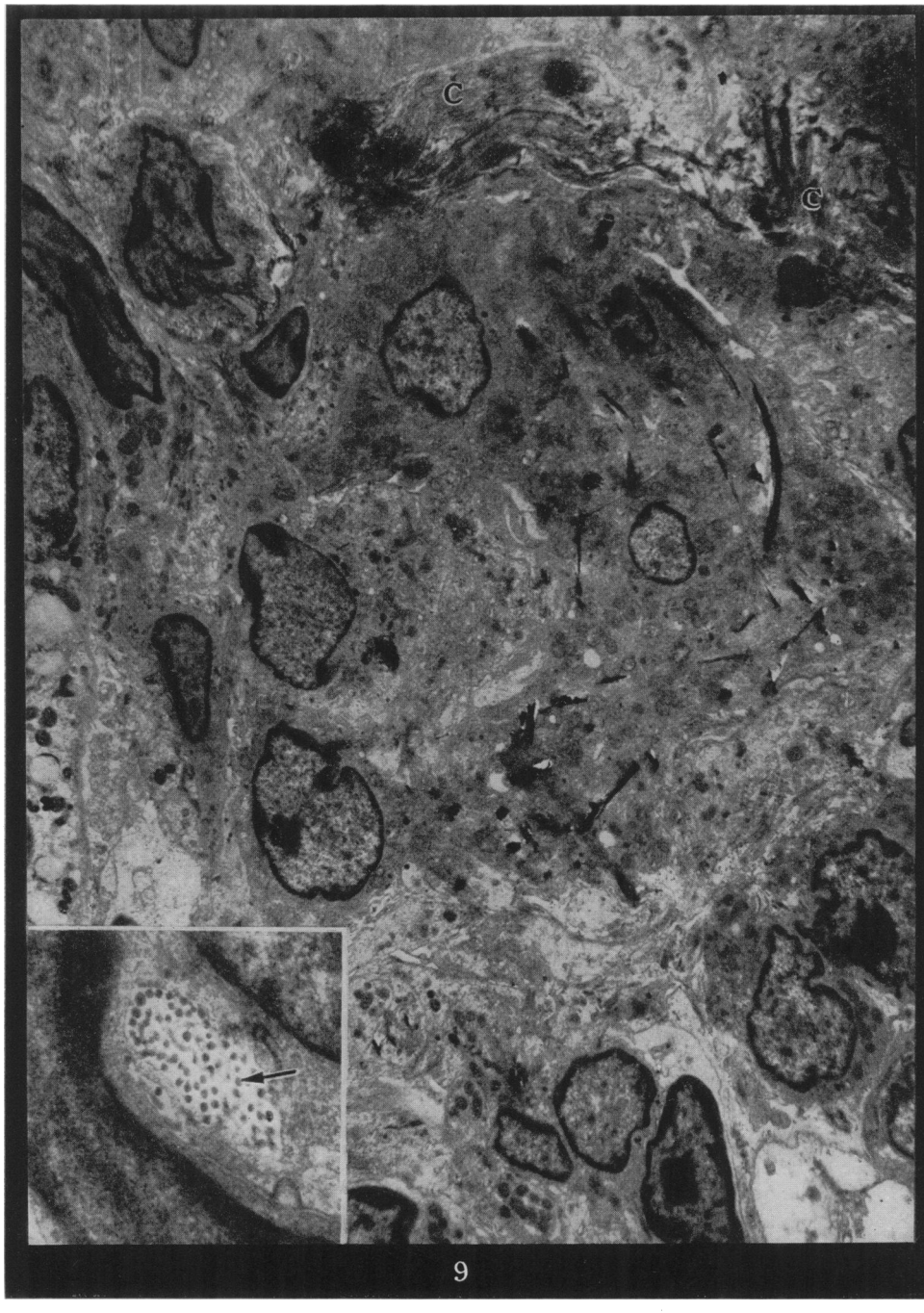
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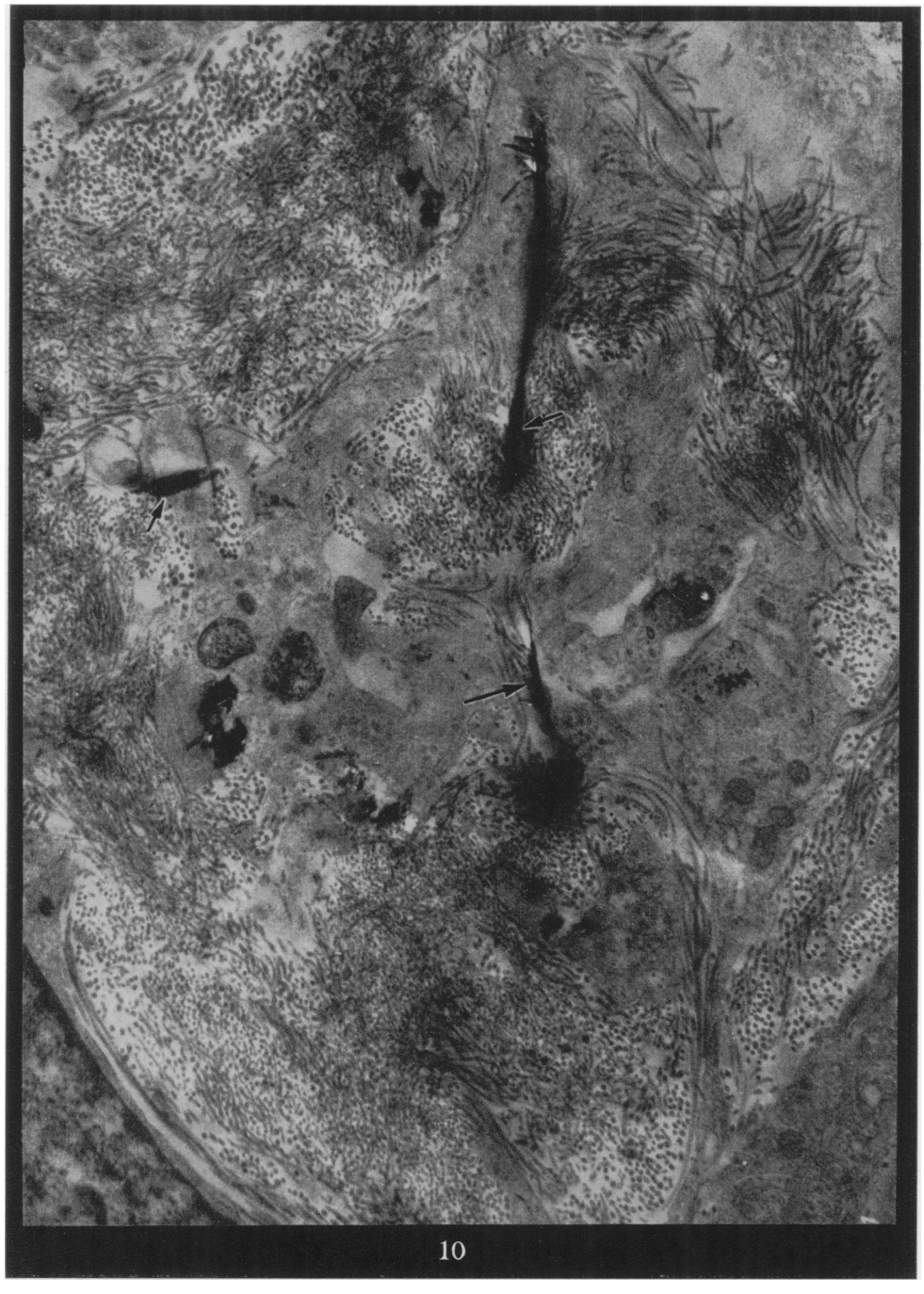

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phage cell membranes, and subsequent giant cell production, occurred equally well in animals given only small particle dust as in animals given dust of a larger particle size. These cytoplasmic processes can be up to 20 μ long but have a largely uniform diameter of about 800–1200 Å (Figs. 2, 3 and 4). Occasionally one of these processes gives a circular profile in cross-section but this is rare and an elongated profile is far more usual. It therefore seems likely that the processes are flattened and leaf-like, having greatly varying widths but being of remarkably consistent thickness. The method of formation of the cytoplasmic processes on the surface of dust containing macrophages is difficult to determine although layers of flattened sacks bounded by a single smooth membrane are often seen in the cytoplasm of cells with these processes (Fig. 7). A possible interpretation of this phenomenon was given by Karrer (1960) who suggested that the phagocytosis of foreign material by lung macrophages resulted from flattened leaf-like processes on the cell surface, folding back on to the cell and engulfing foreign particles in flattened vacuoles. It was shown that if this process was repeated at one site on the cell surface, layers of flattened vacuoles were produced each containing foreign material. This gave a picture very similar to that seen in Fig. 7, except for one important point. When layers of flattened sacks were seen in dust-containing cells they seldom contained dust, and it therefore seems unlikely that they were formed solely for the process of phagocytosis. It is possible to assume that under the influence of chemical stimuli resulting from the presence of dust in the surrounding tissues, the macrophage plasma membrane produces large quantities of leaf-like ruffles and that these tend to fold back on the cell surface even if they fail to make contact with any foreign material. On some occasions, of course, dust would be trapped by the cell processes, but the fact that no flattened dust containing sacks have been found could result from the sharp dust particles piercing the sack membrane and escaping into the cell cytoplasm where they are usually found.

When several macrophages with elongated cytoplasmic processes occur in close association, the processes interdigitate and the individual macrophages are bound loosely together in the early stages of giant cell formation (Fig. 2). Eventually the interdigitated processes appear to contract and the macrophages are bound more and more closely together (Figs. 5 and 6). Finally the cell walls between two united elements of a giant cell would appear to break down as some parts of giant cells appear as single multinucleate cells with no signs of interdigitated membranes between the nuclei. While the individual macrophages are still loosely knit the cell cytoplasm contains large numbers of foreign inclusions of the types reported by Karrer (1960). When the cellular elements of the giant cell become closely bound together, however, it is noticeable that cytoplasmic inclusions apart from asbestos dust have largely disappeared. The process of giant cell formation would appear to be gradual and continuous at any one site and new macrophages seem to be added to the outside of the cell even once the central regions are very closely knit. It is usual, therefore, for the outer regions of giant cells to show more cytoplasmic inclusions than the more internal parts. In large giant cells the central regions often show few cytoplasmic structures at all, and in stained preparations these areas take up very little PTA stain. It may be that both fixative and stain have difficulty in penetrating these large cytoplasmic masses, but it is also possible that the central regions of large giant cells have become caseous and necrotic.

In the present series of experiments three main lesions were seen in the lungs of dusted animals (Davis, 1963). These were nodular giant cell formations usually found in the walls of terminal bronchi, interstitial pneumonia in which the alveolar septa were infiltrated with plasma cells, eosinophil leucocytes and dust containing macrophages, and general lung consolidation in which the alveoli were filled with desquamated epithelial cells, eosinophil leucocytes, plasma cells and dust containing macrophages. In animals that had been dusted for some time fibrosis was found to some extent in all of these sites, and in each case most of the fibroblasts contained large amounts of chrysotile dust, showing that they had been formed from the dust-containing macrophages. In giant cell and interstitial lesions, all the fibroblasts appeared to contain dust, but in areas of general consolidation quite a number of fibroblasts were found in which dust could not be demonstrated. The transformation of macrophages to fibroblasts which begins to occur as little as fourteen days after the start of dusting can be traced fairly easily. In the early stages of dusting the cytoplasm of almost all macrophages is made up largely of small vacuoles and granulated elements of the endoplasmic reticulum are rare. In dust carrying macrophages, however, α cytomembranes begin to appear among the cytoplasmic vacuoles. Eventually large numbers of these membranes are produced and the cytoplasmic vacuoles disappear. Foreign organic inclusions are retained in the cytoplasm during the early stages of α cytomembrane production, but most of them have disappeared by the time the endoplasmic reticulum is well developed, and only occasionally is one of these inclusions found in a fibroblast engaged in collagen production. The chrysotile dust is, however, retained throughout the process and is found free in the fibroblast cytoplasm. Ferritin is also retained by active fibroblasts but is now only found scattered free in the cytoplasm, and is no longer concentrated in cytosomes.

The conversion of giant cell elements to fibroblasts follows exactly the same pattern as in free macrophages, and if the giant cell is comparatively small all its constituent macrophages may produce collagen. The central regions of large giant cells do not, however, seem capable of this conversion. In some cases, therefore, giant cells are completely replaced by small fibrous nodules (Figs. 9 and 10), while in others the central part of the giant cell remains in its original form while its outer regions form layers of active fibroblasts. When the macrophage elements of a giant cell start collagen production, the interdigitated cytoplasmic processes retract and collagen is laid down in the resulting intercellular spaces (Fig. 9). Whenever collagen production was found in these experiments, it was noticeable that the fibroblast cytoplasm was consumed in the process, and in many cases only small remnants of the cells could be seen among the bundles of collagen. When this occurs any asbestos dust contained in the cytoplasm is liberated and is found free among the collagen fibres. Both these points are illustrated in Fig. 10.

DISCUSSION

This study of giant cell formation in experimental asbestosis lesions of rat and guinea-pig lung has shown that the giant cells are formed by the interdigitation of elongated cytoplasmic processes on the macrophage plasma membranes. A similar aggregation and interdigitation is reported by Ham and Leeson (1961) from a study of giant cell formation around injected agar in the subcutaneous

tissue of a rabbit, and it would therefore seem likely that foreign body giant cells are produced by similar methods regardless of the site of their formation.

Apart from producing new information on the method of giant cell formation and demonstrating two possible fates of giant cells once they are formed, the present series of experiments has produced information which conflicts somewhat with the normally accepted theories on the reasons for giant cell formation. Haythorn (1929) said : " There is no longer any question that the chief function of giant cells is one of defensive phagocytosis ", and this idea is still generally accepted. It is assumed that giant cell formation allows the phagocytosis of large particles that are too large to be dealt with by a single macrophage. This is, however, not necessarily the case. In the present study two types of chrysotile dust were used in the initial experiments. In one, long fibres up to 20 μ in length were present, but in the other the dust was finely ground and the longest particles were approximately 2–3 μ in length. Giant cell formation occurred equally with both types of dust although single macrophages could phagocytose large numbers of the 2–3 μ particles. It was, in fact, found that in many cases single macrophages could engulf the 20 μ particles as well. It would thus seem that giant cell formation is a response to chronic irritation in general and that they are not formed specifically for the phagocytosis of large particles. From this study it even seems doubtful that they are phagocytic at all. All those that were examined were found to contain asbestos dust, it is true, but all the individual macrophages that took part in giant cell formation contained large amounts of dust already. At the time of giant cell formation, these macrophages also contained numbers of phagocytosed organic inclusions. If giant cells retained their phagocytic properties, it would be expected that the amount of dust and organic debris they contained would eventually be markedly greater than that of the macrophages at the time of giant cell formation, but this is not the case. The content of dust in giant cells is usually about the same as that of free macrophages, but more important the number of organic inclusions they contain is definitely less than that found in free macrophages. In the central regions of even small giant cells, organic inclusions are rarely found at all. It seems likely that whereas asbestos dust is difficult to dispose of, and remains in the cell cytoplasm for quite a long time, the organic inclusions are easily digested by the macrophage or giant cell cytoplasm. If the cells remained phagocytic, more organic inclusions would be ingested and the number in the cell at any one time would remain roughly constant. As, however, these inclusions are lost from the cytoplasm, it seems that the cell is no longer capable of phagocytosis. From these considerations, it appears likely that the reasons for giant cell formation are as follows. Macrophages are attracted in large numbers to any area of chronic irritation caused by foreign bodies regardless of the particle size of the irritant bodies. The chemical environment found in such areas causes the development of the elongated cytoplasmic processes on the macrophage plasma membranes, and these intertwine with those on neighbouring macrophages binding the cells together. Those macrophages that remain on the surface of a giant cell probably retain some phagocytic properties on their outer surfaces, but the central regions are completely non-phagocytic. When it is considered that large numbers of macrophages go to make up a giant cell it would appear that the phagocytic properties of the tissue are reduced and not increased by giant cell formation. Inevitably when macrophages are able to approach a large

foreign body from all sides, they will unite on its surface and a giant cell will be formed around the irritant material. Although this cannot be described as phagocytosis, it may well serve a useful purpose by walling up the foreign body and so protecting the surrounding tissues from further irritation. In summary it seems likely that in areas of chronic irritation the formation of giant cells results from damage to the macrophages by the irritant forces and represents a reduction in the phagocytic potential of the tissue.

In the present series of experiments, the use of phagocytosed asbestos dust as an intracellular marker has made it possible to trace not only conversions of macrophages to giant cells, but also the conversion of macrophages to fibroblasts. The fact that very few fibroblasts were found that did not contain dust makes it seem possible that this conversion might be directly stimulated by the chemical action of the dust on the macrophage cytoplasm. In most cases of pathological fibrosis, collagen production is probably stimulated by an environment of general tissue damage, but it seems quite likely that direct intracellular damage by certain types of phagocytosed dust could be just as effective and perhaps more effective in stimulating macrophages to collagen production. The fact that the initial effects of dusting on the lung macrophage is a tendency to giant cell production and that only later do macrophage-fibroblast conversions take place may suggest that two processes are involved, but it is also possible that they are both steps in the same process as it has been shown that many of the component macrophages of giant cells do later become fibroblasts. It is hoped to study this problem of the chemical control of macrophage conversion to giant cells and fibroblasts further, using tissue cultures of lung macrophages.

SUMMARY

In an experimental study of the effects of chrysotile asbestos dust on rat and guinea-pig lungs, the formation of giant cells from individual lung macrophages was observed, and also the transformation of lung macrophages to fibroblasts. It was found that after only a few days dusting, the surfaces of dust-carrying macrophages showed large numbers of elongated processes, and where several such macrophages occurred together, the processes interdigitated, binding the cells together to form a giant cell. Fibrosis frequently occurred in the lungs used in these experiments, and it was found that most of the fibroblasts in these areas contained chrysotile dust. As this dust was found only in macrophages and giant cells apart from fibroblasts, the transformation of dust-carrying macrophages to fibroblasts was indicated, and it was possible to demonstrate the various stages in this process. It was also found that some of the macrophages making up giant cells were capable of being converted into fibroblasts. Small giant cells appeared capable of total conversion to fibrous tissue, but in large ones only the macrophages on the surface were capable of transformation to fibroblasts.

REFERENCES

CARREL, A.—(1926) *J. exp. Med.*, **44**, 285.
CHEVREMONT, M. AND CHEVREMONT-COMHAIRE, S.—(1945) *Acta anat.*, **1**, 98
DAVIS, J. M. G.—(1959) *Nature*, **183**, 200.—(1963) *Brit. J. exp. Path.*, **44**, 454.
GUSEK, W. AND NAUMANN, P.—(1959) *Verh. dtsch. path. Ges.*, **43**, 254.

HAM, W. A. AND LEESON, T. S.—(1961) ' Histology ', 4th ed. London (Pitman), p. 253.
HAYTHORN, S. R.—(1929) *Arch. Path. (Lab. Med.)*, **7**, 651.
HOLT, P. F. AND YOUNG, D. K.—(1960) *Ann. occup. Hyg.*, **2**, 249.
JACKSON, F. S.—(1954) *Proc. roy. Soc.* B, **142**, 536.—(1956) *Ibid.*, **144**, 556.—(1957) *Ibid.*, **146**, 270.
KARRER, H. E.—(1958) *J. biophys. biochem. Cytol.*, **4**, 693.—(1960) *Ibid.*, **7**, 357.
MAXIMOW, A.—(1927) *Proc. Soc. exp. Biol., N.Y.*, **24**, 570.
PORTER, K. R. AND PAPPAS, G. D.—(1959) *J. biophys. biochem. Cytol.*, **5**, 153.
ROSS, R. AND BENDITT, E. P.—(1962) *J. Cell Biol.*, **12**, 533.
WASSERMAN, F.—(1954) *Amer. J. Anat.*, **94**, 399.