THE ULTRASTRUCTURE OF ASBESTOS BODIES FROM HUMAN LUNG

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In an earlier paper (Davis, 1964) the previous work on the structure and formation of asbestos bodies was summarised, and the results of an electron-microscope study of the structure of asbestos bodies in guinea-pigs was reported. It was found that the basic element of the asbestos body coating material consisted of ferritin granules with a particle size of approximately 60 Å. Sometimes only a single coating was present, but sometimes bodies with several coats were found in which the density of the ferritin granules varied giving the bodies a layered appearance. When this occurred the outermost coating of the layered bodies was often found to consist of fine fibrils rather than granules. Only a few " segmented " bodies were found in the guinea-pig material and it was demonstrated that these had been formed by the irregular deposition of the ferritin material rather than by the breakup of previously smooth bodies as had been reported from studies on human material. In addition to the normal elongated asbestos bodies with a neat core of asbestos fibre, a new type of structure was also found in which odd particles of dust were found scattered throughout irregularly shaped ferritin deposits.

In November 1961 it was possible to obtain some biopsy material from a worker who had worked in the disintegration department of an asbestos factory from 1937 until 1958 apart from a seven-year break for military service from 1939 until 1946. He had been exposed mainly to amosite dust. As this material contained large numbers of asbestos bodies the present study was undertaken to see how the ultrastructure of human asbestos bodies compared with that seen in the guinea-pigs.

MATERIALS AND METHODS

The lung tissue used in this study was obtained at an operation for right lower lobectomy, and the tissue was placed in fixative within 3 min. of the blood supply being cut off. Fixation and embedding were by the methods described previously (Davis, 1959). Owing to the large amounts of asbestos dust in the tissue, section cutting was extremely difficult, and it was found that if sections of normal thickness were cut most of the dust and asbestos bodies were torn from the plastic. It was therefore decided to standardise on a section thickness of about 1000 Å. This allowed the tissue to be examined with the electron microscope but of necessity resulted in some loss of resolution at high magnifications.

OBSERVATIONS

In this electron-microscope study of asbestos bodies from human lung the bodies were found in exactly the same sites as in guinea-pigs, that is, intracellularly in macrophages or fibroblasts, or embedded among the collagen fibres in areas of

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fibrosis, where they would have been deposited by disintegrating fibroblasts. The various types of asbestos body described in this paper appeared equally distributed among these sites, and no particular variety was peculiar to one location. Except for those bodies embedded in collagen, none were found that were entirely extracellular although occasionally one end of a long body was found protruding from a cell. The deposition of the body coat therefore appears to be a function of the cell cytoplasm and asbestos dust must have been phagocytosed for this deposition to take place.

The asbestos body coating was found to consist mainly of ferritin granules approximately 60 Å in diameter as was the case in guinea-pigs. The initial deposit may be sparse (Fig. 1), but a thick coating is usually built up (Fig. 2), and the diameter of human asbestos bodies can be as much as 5 μ. Layering of the body coating was found to be much less frequent than in guinea-pigs, and when it was found the density of the ferritin in the different layers varied only slightly (Figs 2 and 3). Low density layers containing relatively few ferritin granules were not found at all in human asbestos bodies. Two structural variations in the body coating were, however, occasionally seen in human material. The first was a clumping of the ferritin material in the innermost layer of some bodies (Figs 2 and 3). This clumping appears to be most marked close to the asbestos fibre. The second variation was the inclusion of fine needle like particles among the ferritin granules (Fig. 4). These needles vary from 50 Å to 250 Å in diameter and are from 0·50 μ. to 0·5 μ. in length. They appear scattered fairly evenly throughout the body coat but almost invariably have their long axis parallel to the surface of the body. An examination of amosite dust particles in the lung macrophages of this specimen (Figs 5 and 6), shows frequent signs that this type of asbestos can break down to form elongated particles of similar size and it seems likely that if amosite dust is breaking up in a cell in which an asbestos body is also forming the needle-like fragments are attracted to the body in the same way as ferritin and become incorporated along with the ferritin granules in the body coat.

In addition to normal asbestos bodies the irregular bodies with no definite core of asbestos dust that were reported from guinea-pigs have also been found in the human material. Their structure is similar to that reported from guinea-pigs and they consist of irregular masses of ferritin granules in which are embedded haphazardly arranged particles of asbestos dust together with any other foreign body inclusions present in the macrophages (Fig. 7). The needle-like particles described in the previous paragraph can be found in these irregular bodies as well as in normal ones.

In the study of guinea-pig asbestos bodies it was reported that segmented bodies were only rarely found and that when they were present they were formed by the uneven deposition of ferritin material rather than the erosion or fracture of a previously smooth layer of body coating. In the human material the picture is much more complicated. In some cases the segmentation still appears to be produced by uneven ferritin deposition, but often the segmentation does result from the splitting of a previously smooth coat and does seem to be part of a process by which the asbestos bodies break up. Fig. 3 shows a segmented asbestos body whose coating consists of several layers. Of these the innermost coat is not present in a number of places, and only the outermost one is present over the whole length of the body that appears in this section. This outer coating is of uniform thickness over the whole body and shows no signs of erosion or splitting. From this evidence

it seems likely that the segmented appearance is due to the irregular deposition of the initial coating. In Fig. 8, however, an asbestos body is shown with a single smooth coating of fairly even thickness in which thin cracks or fissures have developed. These cracks extend right through the coating in most places and although initially narrow they can widen as shown in Figs 9 and 10 causing the breakup of the body. It is obvious from these Figures that the splits in the body coat do not always correspond from one side of the body to the other and unevenly shaped segments often occur. It was not possible to study the final disintegration of the asbestos body in this material as it was seldom possible to determine whether a small area of body material in any cell was indeed a fragment of disintegrating body or merely an area of a large intact body, that had been sectioned tangentially.

DISCUSSION

The findings in this study that in most respects the structures seen in human asbestos bodies are very similar to those produced experimentally in guinea-pigs and described previously is very encouraging, as it means that any future results obtained from experimental animals on the chemical mechanisms that control body formation are likely to have a direct bearing on the human problem. There were however, some differences to be noted between asbestos bodies from guinea-

EXPLANATION OF PLATES

FIG. 1.—A chrysotile crystal from a human lung macrophage. The deposition on this crystal of dense granular material has commenced, and the surrounding cytoplasm also shows large numbers of similar granules. Granule diameters are approximately 60 Å. ×132,000.

FIG. 2.—A transverse section from an asbestos body from a human lung macrophage. The coating of granular material has been deposited in 2 layers, and the innermost layer shows some clumping of the granules. ×47,000.

FIG. 3.—An asbestos body from a human lung fibroblast. The coating material of this body consists of at least 3 layers. The body has a segmented appearance, but an examination of the different layers shows that only the outermost one (arrowed) is present over the whole body. This suggests that the segmented appearance is due to the deposition of the internal coating as a series of globules. It can be seen that during the life of this asbestos body there has been some clumping of the granules of the innermost layer. ×30,000.

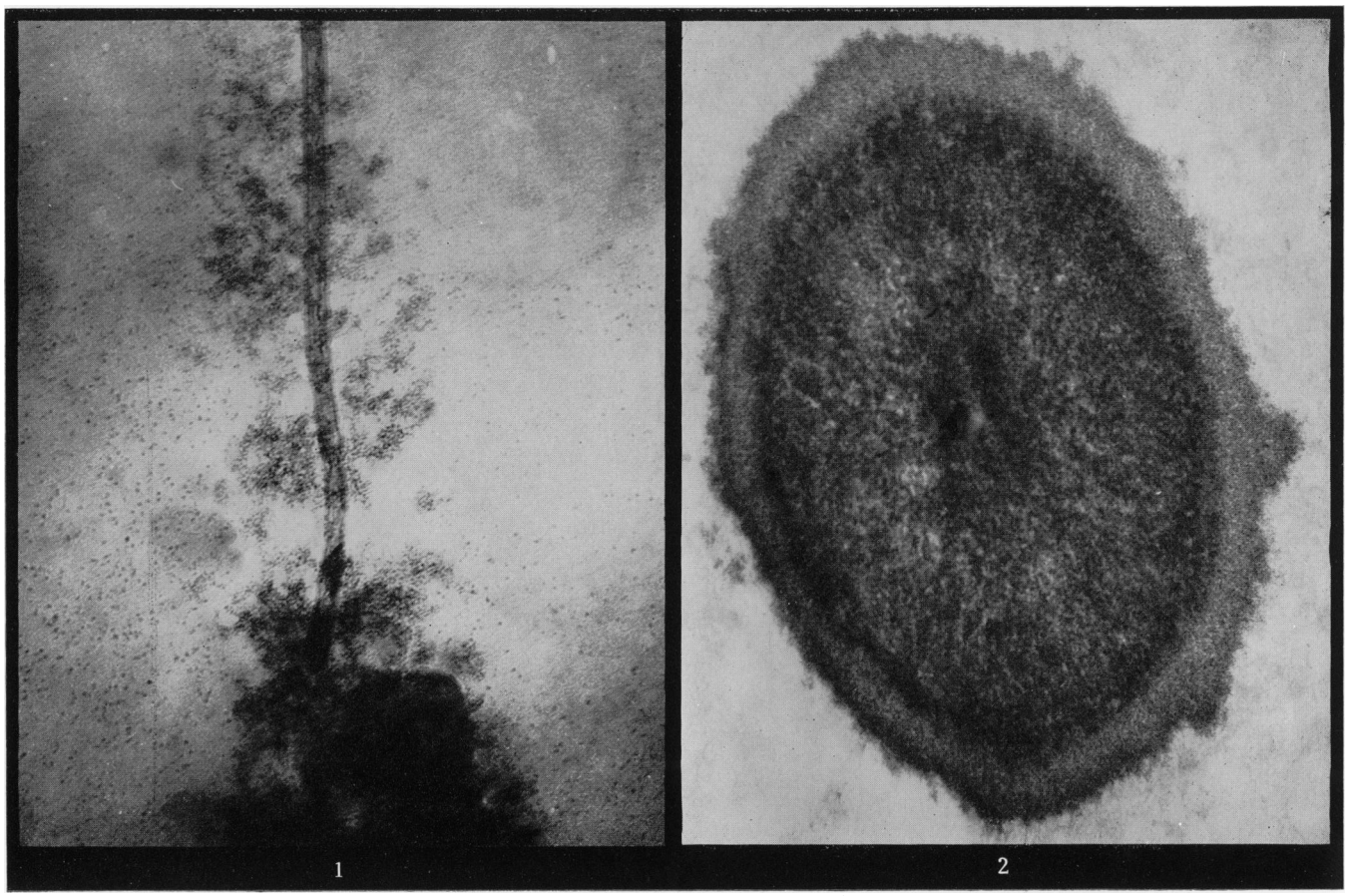
FIG. 4.—This plate shows an area of the coating material from a human asbestos body. Most of the coating consists of small granules approximately 60 Å in diameter, but many small needle-like particles are embedded among the granules. The smallest of these needles appears to have a diameter of only 50–60 Å. ×115,500.

FIG. 5.—An asbestos crystal in an asbestos body from human lung. The crystal shows the transverse fractures (arrowed) that are commonly found in amphibole dust from lung tissues. It does, however, show signs of longitudinal splitting to form fine needles of only about 50 Å diameter, and this has not previously been reported from any asbestos type. ×100,000.

FIG. 6.—This plate shows an elongated area of material that is breaking up into fine needles. It is not possible to recognise this material with certainty but it is in the centre of a clump of definite crystals of one of the amphibole asbestos types. ×100,000.

FIG. 7.—A large irregular inclusion in a human lung macrophage. Much of the material consists of dense granules approximately 60 Å in diameter, but many particles of both amphibole and chrysotile asbestos are included along with other foreign material including carbon particles. Large numbers of the fine needles shown in Figs 4, 5 and 6 are also present in the inclusion (arrowed). ×66,000.

FIGS 8, 9 and 10.—These plates are longitudinal sections of asbestos bodies from human lung tissue. The coating material of all 3 consists of a single thick layer of granular material. These coatings show varying degrees of transverse splitting, which gives the bodies a segmented appearance. ×38,000.



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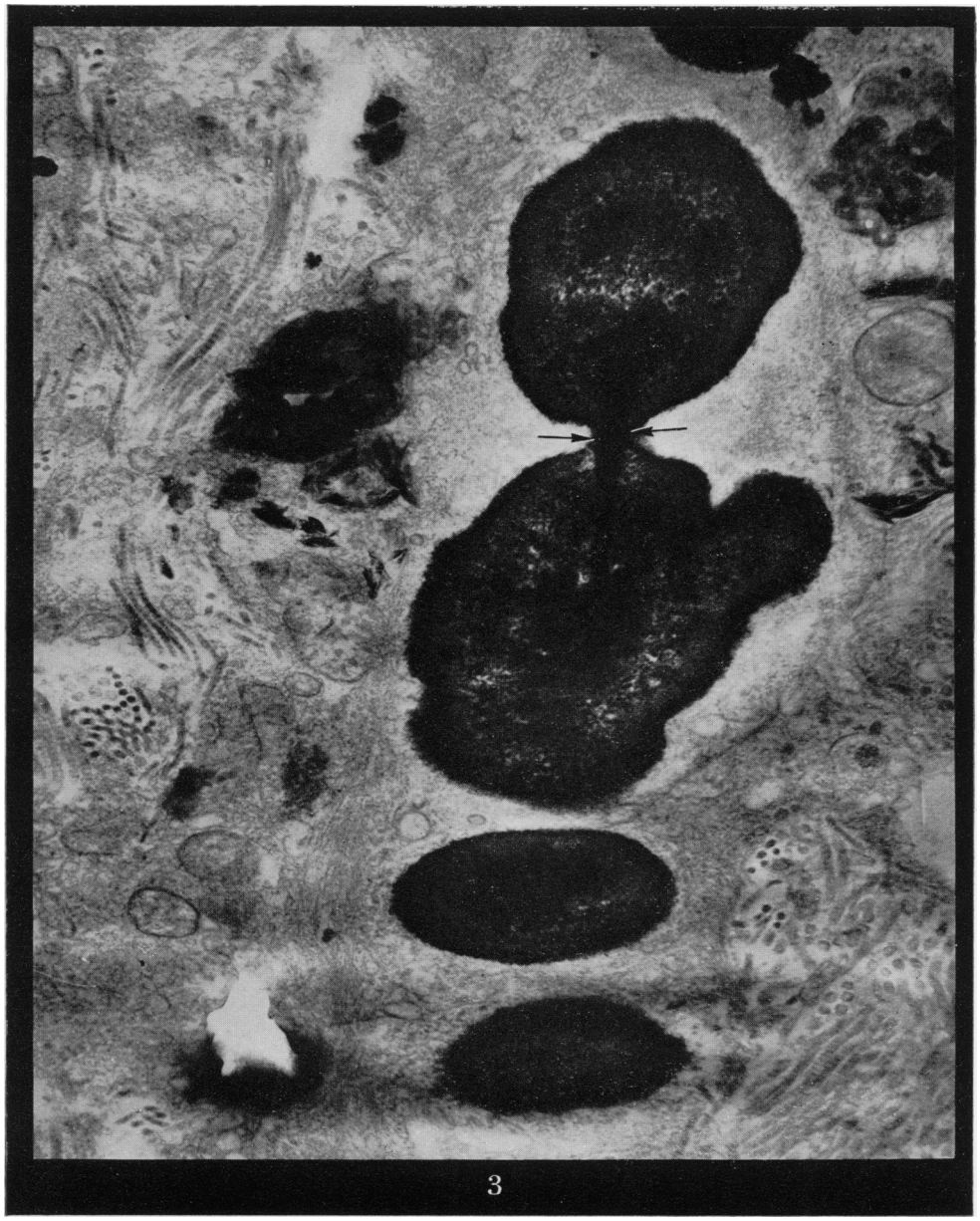

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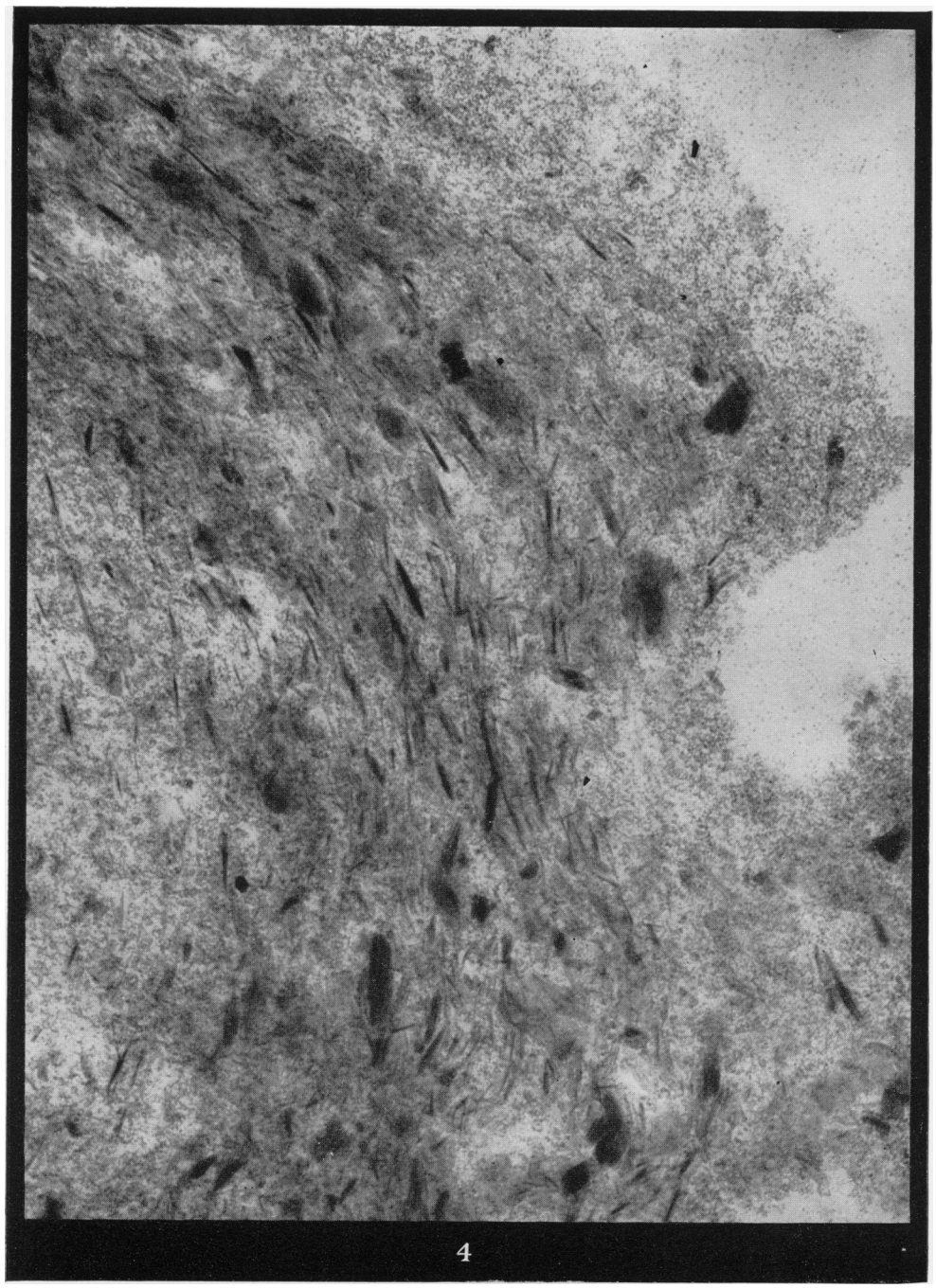

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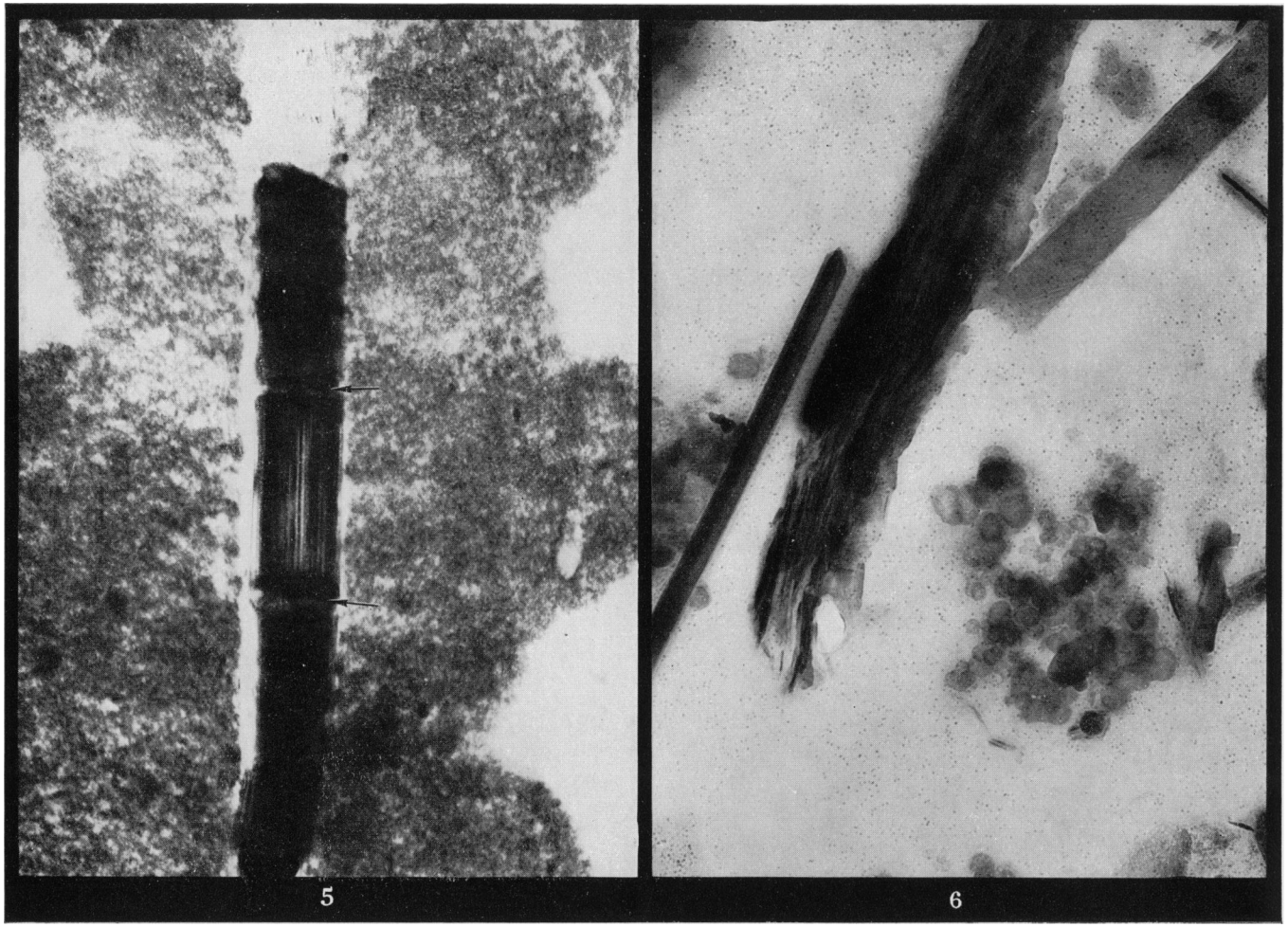

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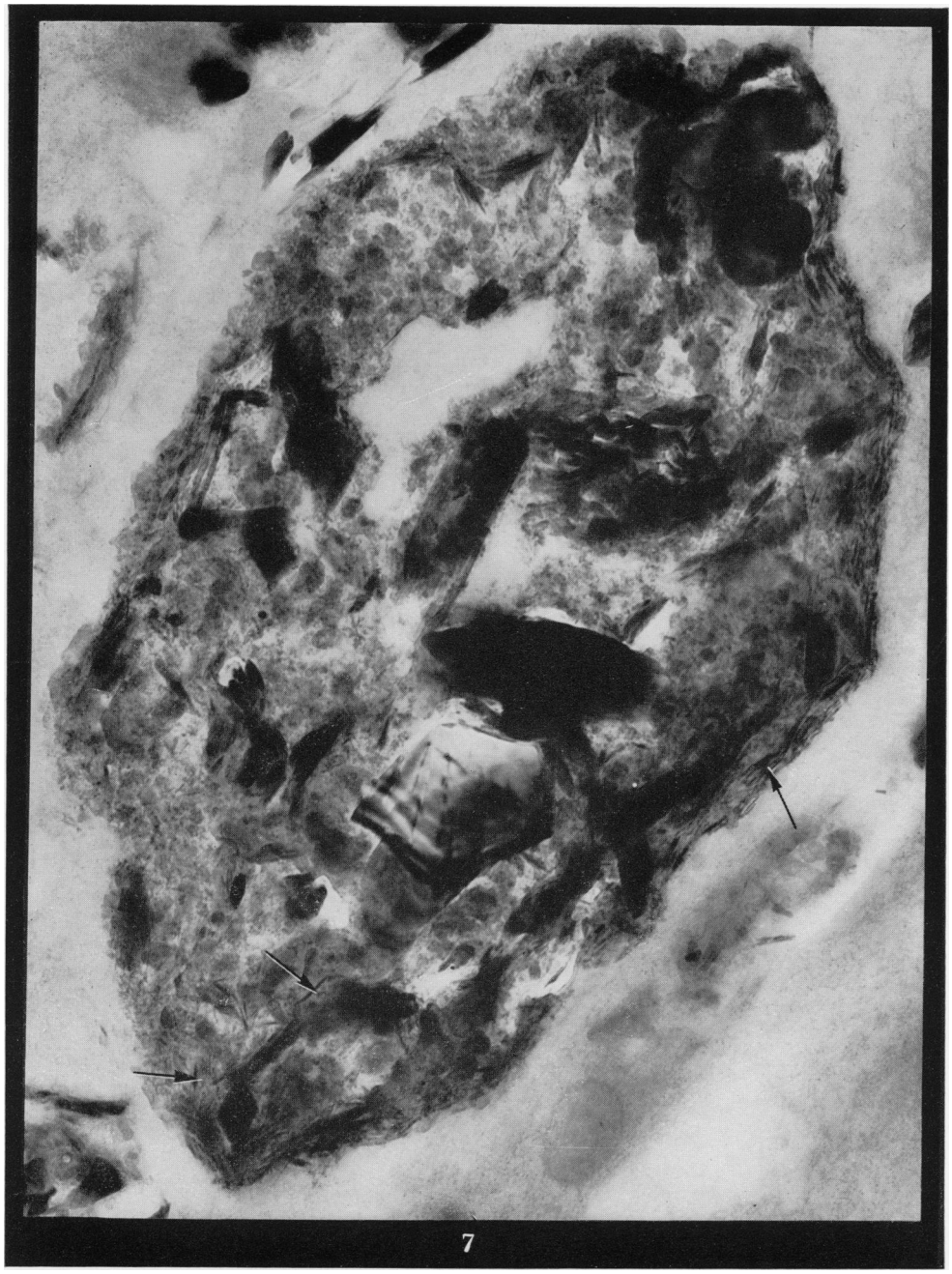

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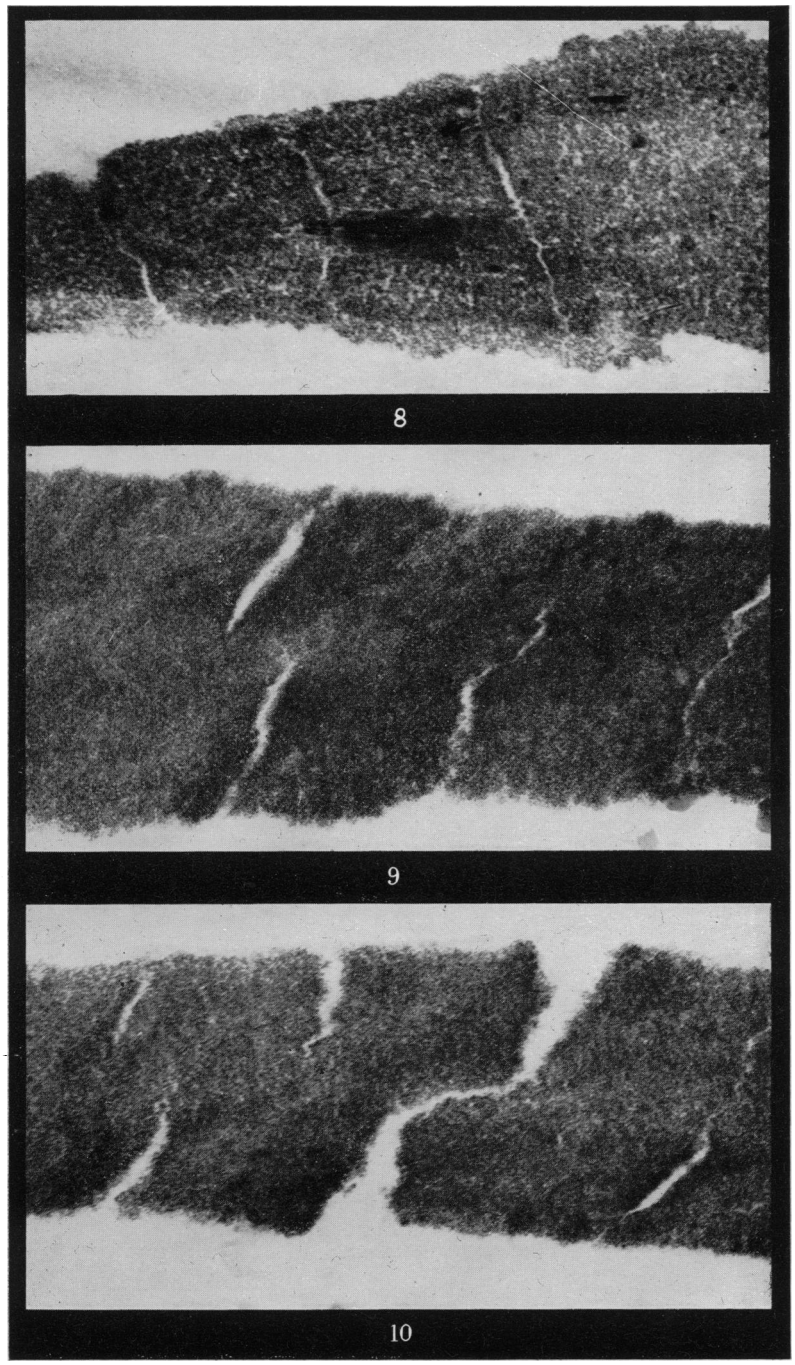

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pigs and human material, the chief of which was the scarcity of layering in the coating of human asbestos bodies. It may simply be that the conditions affecting ferritin deposition on the asbestos dust are more stable in humans than in guinea-pigs, but it must also be remembered that whereas the guinea-pig bodies were at most a few months old, those from human material may have been up to 20 years old, and it may be that any layering in an asbestos body becomes less marked with ageing. The fibrous structure reported in the outermost layer of some guinea-pig asbestos bodies was also absent from human material. Here too the reason may be that the fibrils are only clearly defined in young bodies and become less distinct with increasing age. Alternatively the outer fibrous coating may be transient and present only a short time during the formation of the asbestos body. This could explain why it was not present on all the layered guinea-pig bodies as well as being absent from the human material.

The finding of tiny needles embedded in asbestos body coating which appeared to be produced by the breakdown of amosite crystals is of interest as it had previously been assumed that chrysotile with a crystal diameter of 250–400 Å had a much smaller diameter than the basic unit of the amphibole asbestos types. The finding of amosite needles of as little as 50 Å in diameter would therefore be new. Dr. R. Gaze (personal communication) thinks that Fig. 6 shows not amosite but an atypical form of chrysotile breakdown and this is theoretically possible as the specimen did contain a small amount of chrysotile dust. Against the idea, however, is the fact that the transverse fractures of the dust shown in Fig. 6 are common in amosite and crocidolite that have been in the lung for some time, but neither these fractures nor the fine needles were found at all in a fairly extensive examination of chrysotile dust in guinea-pig lungs (Davis, 1963). A study of the effect of amosite dust on guinea-pig lung is now in progress and although the full results will be published later it is pertinent at this point to state that fine needles of similar size and shape to those under discussion have frequently been found in the guinea-pig lung macrophages that contained fragmented amosite dust.

The present study of human asbestos bodies has solved the difficulty that arose with the finding of segmented bodies in guinea-pig material after only a few months dusting (Davis, 1964). This contradicted the generally accepted idea that segmentation in asbestos bodies was a sign of ageing, and part of a process by which asbestos bodies broke up. It now appears that two distinct processes can produce a segmented appearance in asbestos bodies which would probably be indistinguishable with a light microscope. The first of these processes and the one reported from guinea-pig material is a differential deposition of the body coating, and the latter is a true splitting of a previously even coat which does appear to be associated with the breakup of the bodies. The presence of two such types of segmentation was suggested by Beger in 1933, but does not seem to have found favour with later workers. The fact that two forms of segmentation do occur means that light microscope reports that segmented bodies are present in a specimen cannot be taken as a definite indication that the bodies are either old or breaking up. They may equally well have been in the lung for only a few months. This could explain Beattie's (1961) finding that asbestos body segmentation did not affect the mechanical strength of the enclosed asbestos fibre. If the bodies examined owed their segmentation to uneven deposition of the coating material then no structural change would be expected in the dust. Where true

segmentation is present however, and the splits in the body coating go right through to the dust one would certainly expect the dust core to be weakened. It has not, however been possible to see any structural signs of such weakening in early segmented bodies, and in any case signs of dust breakup would not be conclusive. In this study many uncoated amosite fibres were seen to be breaking up, and some of these fibres undoubtedly became coated later, resulting in fragmented dust being found in smooth and unsegmented bodies.

SUMMARY

In a study of asbestos bodies from human lung tissue it was found that the body structure was very similar to that previously found in asbestos bodies from guinea-pig lungs. The coating material was found to consist mainly of fine granules approximately 60 Å in diameter, and it is suggested that these are ferritin material. The coats of some bodies contained layers of varying density, but this was much less commonly found than it had been in guinea-pig material. Some human bodies contained fine needle-like particles approximately 60 Å in diameter embedded in the coating material, and it is suggested that these may be finely divided amosite asbestos. From the evidence of this study it would appear that the well known segmentation of asbestos bodies can be of two types. In one the segmented appearance is due to the deposition of the body coating in separate globules, and in the other it is due to the splitting of a previously smooth coat. In addition to the traditional asbestos body consisting of a neat core of asbestos fibre surrounded by coating material a new structure was found that consisted of an irregularly shaped aggregate of ferritin in which were embedded small scattered particles of asbestos dust as well as other foreign material such as carbon particles.

REFERENCES

BEATTIE, J.—(1961) ' Inhaled particles and vapours '. Oxford (Pergamon Press), p. 434.
BEGER, P. J.—(1933) *Virchows Arch.*, **290**, 280.
DAVIS, J. M. G.—(1959) *Nature, Lond.*, **183**, 200.—(1963) *Brit. J. exp. Path.*, **44**, 454.—(1964) *Ibid.*, **45**, 634.