THE ULTRASTRUCTURE OF ASBESTOS BODIES FROM GUINEA-PIG LUNGS

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WHEN asbestos fibres are inhaled and lodge in lung tissue many of them become coated with a thick layer of gelatinous material, and the coated fibres are called Asbestos Bodies. It is generally accepted, that the function of the body coating is to protect the tissue from the harmful effects of asbestos dust. Asbestos bodies were probably first observed by Fahr and Feigel (1914) who described strange " crystals " in lung sections from a case of asbestosis. Later Cooke (1924) also reported " curious bodies " in asbestotic lungs, but because of their beaded nature he thought they might be fungi. Stewart and Haddow (1929) believed that the bodies were associated with the disease and coined the term " asbestosis bodies ". This term was used universally for many years, but it is now realised that the presence of these bodies in the lung or sputum is only an indication of exposure to asbestos dust and is not diagnostic of the disease itself. For this reason the term " asbestos body " has been substituted for " asbestosis body ". Gloyne (1932) demonstrated that each body contained a fibre of asbestos and suggested that the capsule contained iron.

The exact chemical nature of the asbestos body capsule material has been the subject of a considerable amount of speculation and study. McDonald (1927) believed that the coating was primarily a silicic gel formed by the decomposition of asbestos, and Gardner and Cummings (1931) suggested that it was ferrisilicate gel. Koppenhöfer (1935) thought that the asbestos body coating was produced from silicic acid formed from the asbestos fibre after the iron and magnesium had been leached out. In contrast Cooke (1929) suggested that the body coating was iron containing protein material derived from damaged blood cells and Gloyne agreed with this view. The iron-protein nature of the capsule was confirmed by Beger (1933) and Sundius and Bygden (1937). Simpson and Strachan (1931) suggested that the body protein was laid down on the asbestos by cells in contact with the fibre and Beattie (1961) expanded this idea suggesting that the body protein was collagen laid down on the asbestos fibre by fibroblasts. Beattie was able to show the presence of proline and hydroxy-proline in hydrolysates of asbestos bodies, which supported the collagen theory, but was unable to determine the exact quantities present. Recently Leach (personal communication) made a complete amino acid analysis of the asbestos body protein and showed that the quantities of proline and hydroxyproline were much too small for the coating to be collagen.

Gloyne (1932) observed that in lungs from persons who had undergone long periods of dust exposure many of the asbestos bodies present showed signs of

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erosion in the protein coating, and that some of them had become segmented so that the capsule appeared like " a string of beads ". It was assumed that these changes were the result of the ageing of the bodies after long periods in the lung. It was noted that many of the segmented bodies had fragmented at right angles to their length, and that when this happened the fractures occurred at the places of segmentation and included the asbestos fibre as well as the capsule. It was also reported that after the breaking up of the asbestos bodies the fragments were engulfed by phagocytes and the protein coating at least was dissolved away. These observations were confirmed by Beattie (1961) who also demonstrated that segmentation alone does not reduce the mechanical strength of the body as initially the segments are very closely opposed.

Beattie suggested that when segmented bodies become engulfed in macrophages the cleavage planes open out and the asbestos dust becomes exposed at the bottom of each cleft. When this occurs the mechanical strength of the body is affected and breakage occurs where the dust is exposed.

In previous work (Davis, 1963*a*) and (Holt, Mills and Young, 1964) it was reported that initial experiments, in which rats and guinea-pigs were dusted with chrysotile, produced considerable pathological changes in the lung tissues but no asbestos bodies. More recently the dusting technique has been modified to increase the proportion of long fibre dust and in subsequent experiments guinea-pigs dusted for only 100 hr. have developed many asbestos bodies in their lungs in as little as 2 months. The results of light microscope studies on this material are being published separately. The present paper describes the observations made when some of the guinea-pig lung material containing asbestos bodies was examined in the electron microscope.

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 which he described (Holt and Young, 1960). The guinea-pigs remained in the tunnel for 6 weeks, during which time the dusting apparatus was running for 18 hr. a day. After this time they were killed at intervals, the last 16 months after removal from the dust tunnel. 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.

OBSERVATIONS

During this study partly or completely formed asbestos bodies were found in three sites only, in alveolar macrophages (Plate 1), in fibroblasts or embedded among collagen fibres in areas of fibrosis. It has been previously shown (Davis, 1963*b*) that in experimental asbestosis, dust-carrying macrophages are converted to fibroblasts, and that during fibrosis fibroblast cytoplasm is lost leaving any dust they contained embedded in collagen. It therefore seems fairly certain that asbestos body production is an intracellular process and that asbestos bodies are only found free after the breakdown of the cells in which they are formed. It is true that a light microscope examination of the guinea-pig lungs showed quite a number of bodies that appeared to be extra-cellular or with only one end inside a cell, but under the electron microscope such bodies were usually seen to be covered

by very thin layers of macrophage cytoplasm. However, although asbestos body production is an intracellular process it does not appear to require any structural modification of the cell cytoplasm. Both macrophages and fibroblasts that contained asbestos bodies were entirely normal in other respects, and neither the structure nor the distribution of the cytoplasmic organelles was altered in any way. This is well illustrated in Fig. 1 which shows a lung macrophage containing an asbestos body about 8 μ. long. Mitochondria are present and evenly distributed and their structure is normal. A small area of endoplasmic reticulum is also present which suggests that the cell will soon undergo conversion to a fibroblast. The fact that the cell is still phagocytic is indicated by the elongated cytoplasmic processes on its surface as well as the presence of foreign body inclusions in the cytoplasm.

The earliest sign of asbestos body production in lung macrophages was the accumulation around the chrysotile fibres of small densely staining granules

EXPLANATION OF PLATES

FIG. 1.—A lung macrophage containing an asbestos body about 8 μ. in length. For most of its length the body coating consists of a single layer of dense granular material, but at each end layers of less dense material are being laid down (arrowed). Apart from the presence of the asbestos body the macrophage appears quite normal. ×16,500.

FIG. 2.—A single chrysotile crystal from a guinea-pig lung macrophage. Small dense granules approximately 60 Å in diameter have started to congregate around the crystal. ×132,000.

FIG. 3.—Chrysotile crystals on which known ferritin material has been absorbed in vitro (arrowed). It can be seen that in the electron microscope ferritin appears as small dense granules approximately 60 Å in diameter. ×132,000.

FIG. 4.—Chrysotile crystals from a guinea-pig lung macrophage, shown during an early stage of asbestos body formation. The coating material consists of small granules approximately 60 Å in diameter. ×73,300.

FIG. 5.—An asbestos body from a guinea-pig lung macrophage. The coating material of this still contains dense granules approximately 60 Å in diameter, but these granules are more loosely packed than is usual. In this case it appears that much of the body coat consists of amorphous material that does not contain iron or any other heavy metal and therefore does not appear very dense in the electron microscope. ×85,000.

FIG. 6.—A transverse section of an asbestos body from a guinea-pig lung macrophage. A thick layer of granular coating material is present and the diameter of the body is approximately 2 μ. ×47,000.

FIG. 7.—A longitudinal section of an asbestos body from a guinea-pig lung macrophage. The body coating shows an early stage of layering. At two points (arrowed) layers of little density exist, but are already being overlaid by another dense layer. ×48,000.

FIG. 8 and 9.—Transverse sections of asbestos bodies from guinea-pig lung fibroblasts. Several layers of coating material of varying thickness and density are present around the central cores of dust. These two Figures are included together to show that the thickness and density of the various layers varies considerably from body to body. In these two bodies the outermost layer is very irregular and consists not of granules but of fine filaments approximately 50 Å in diameter. ×32,250.

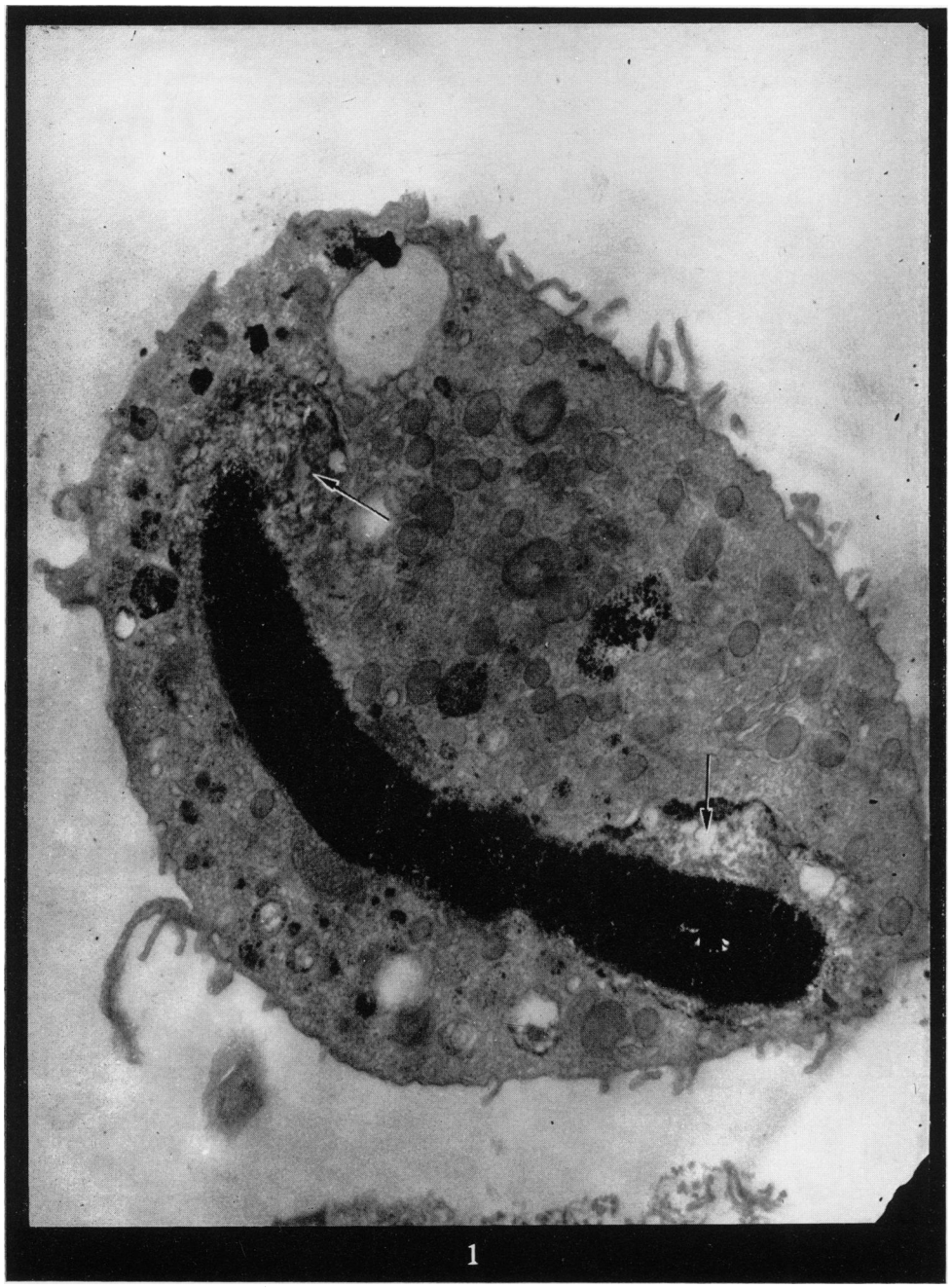
FIG. 10.—A transverse section of an asbestos body from a guinea-pig lung fibroblast. In this case most of the body coat consists of fine fibrils approximately 50 Å in diameter. ×100,000.

FIG. 11.—A transverse section of an asbestos body from a guinea-pig lung fibroblast. In this case the dust is initially surrounded by a thick layer of material that contains very few dense granules, outside of which is a dense layer of fine fibrillar material. ×73,300.

FIG. 12.—This plate shows a long chrysotile crystal (arrowed) that is uncoated at one end but is covered by a thick globule of granular material at the other. ×35,200.

FIG. 13.—A longitudinal section of a chrysotile fibre which is covered by three separate globules of granular material. These globules produce the appearance of a segmented asbestos body. ×44,000.

FIG. 14.—An irregularly shaped inclusion body from a guinea-pig lung macrophage. Much of the material of the body consists of dense granules approximately 60 Å in diameter, But other larger and less distinct structures are present among the granules and at one point (arrowed) a chrysotile fibre is present. ×30,000.



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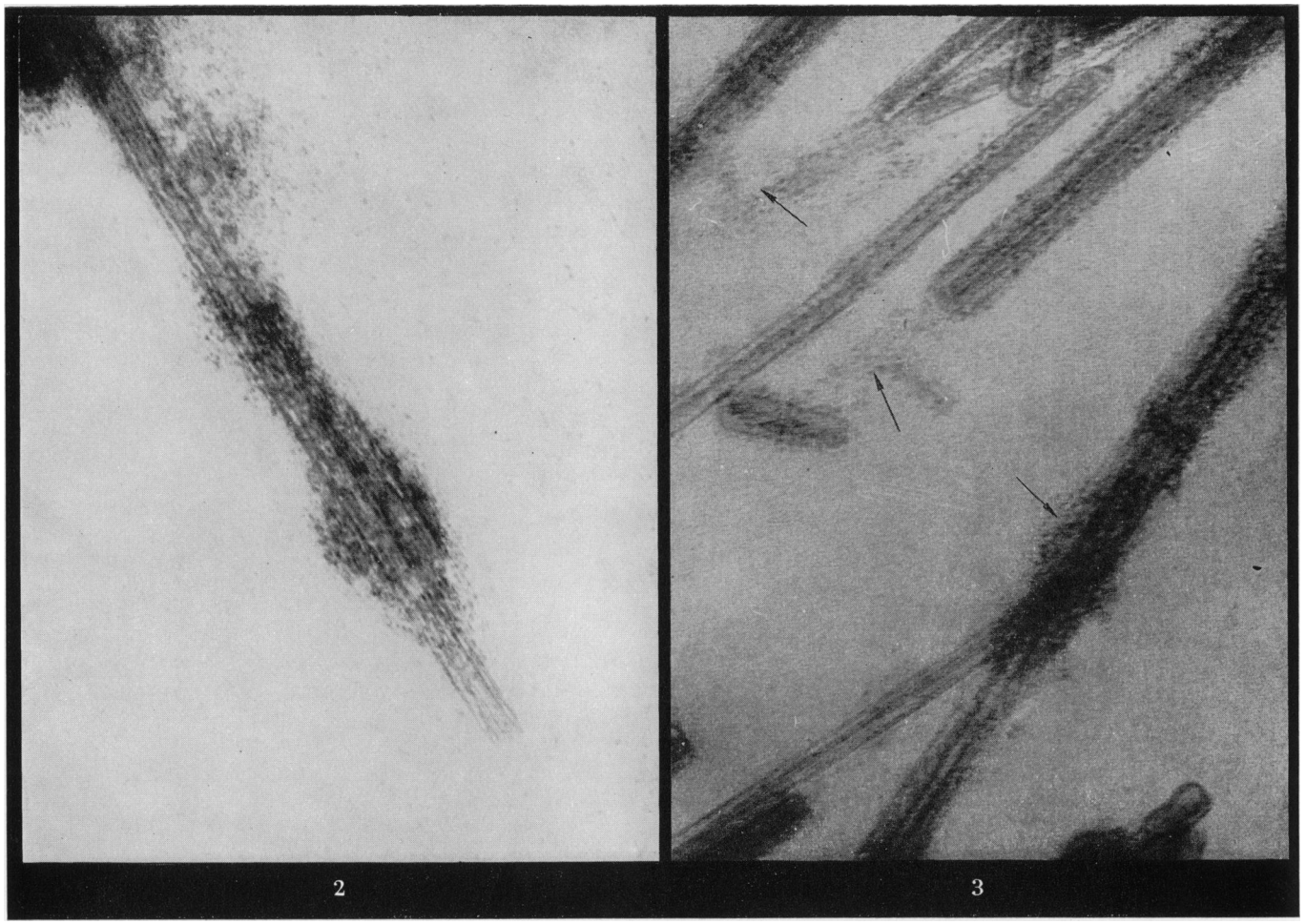

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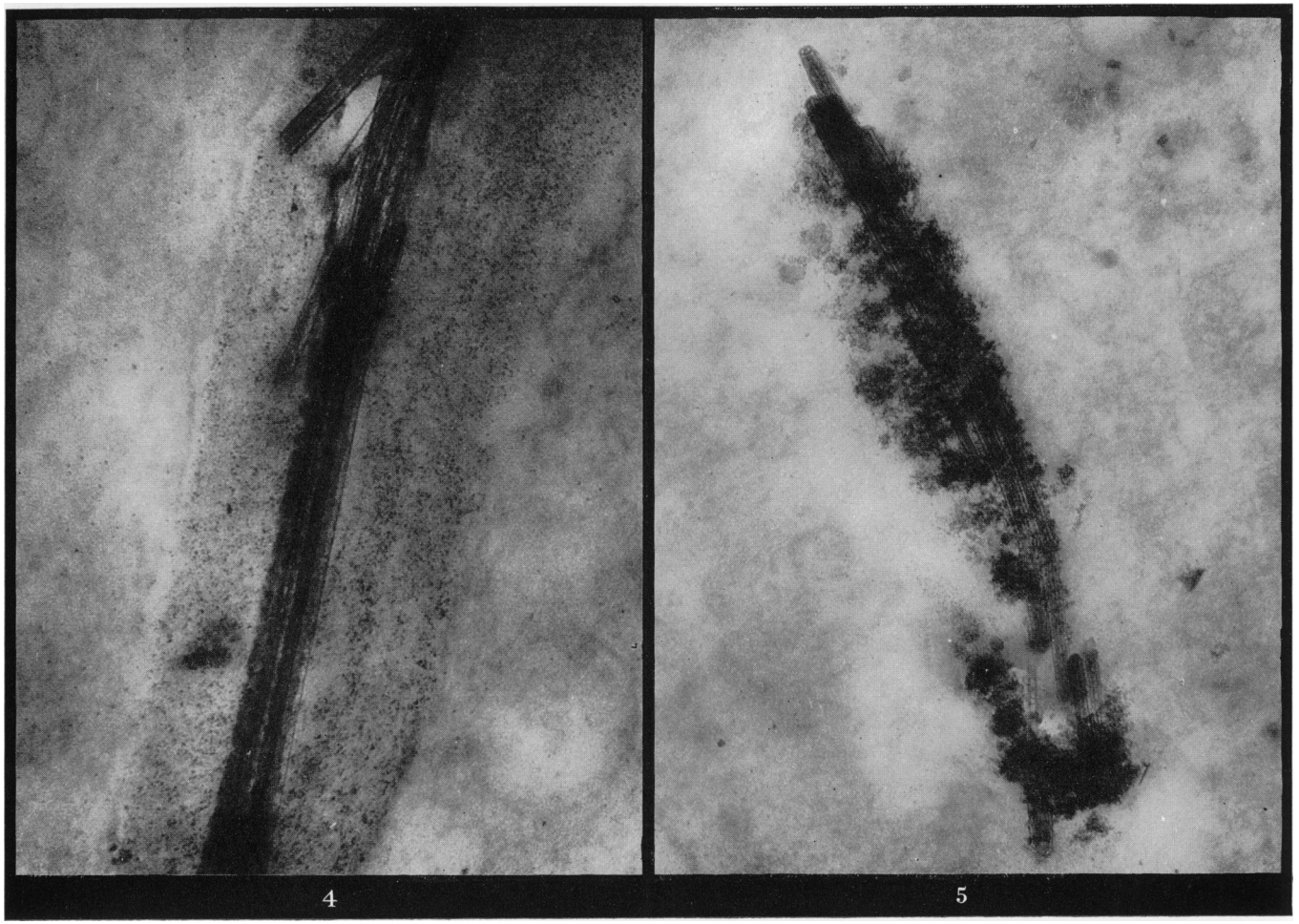

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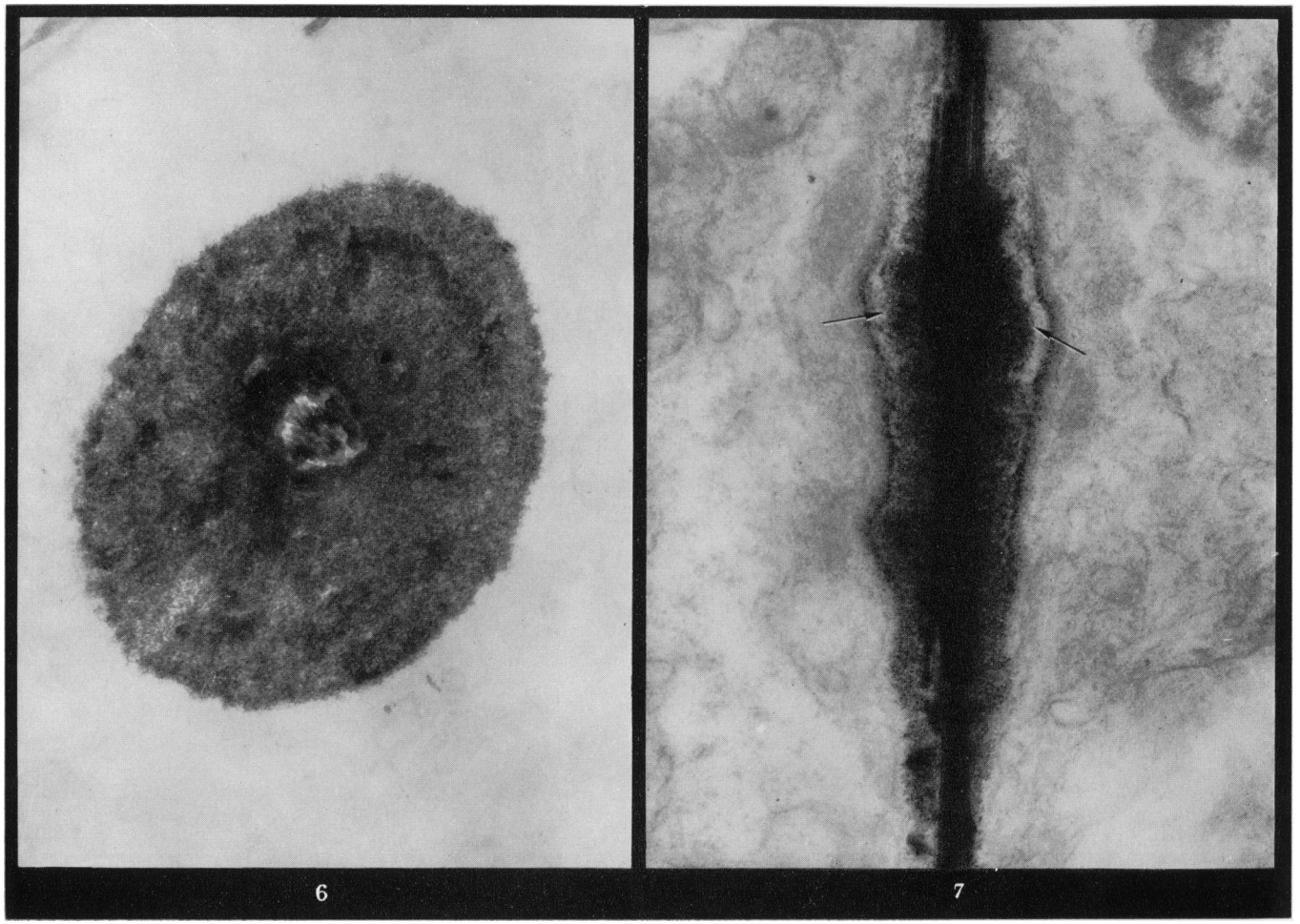

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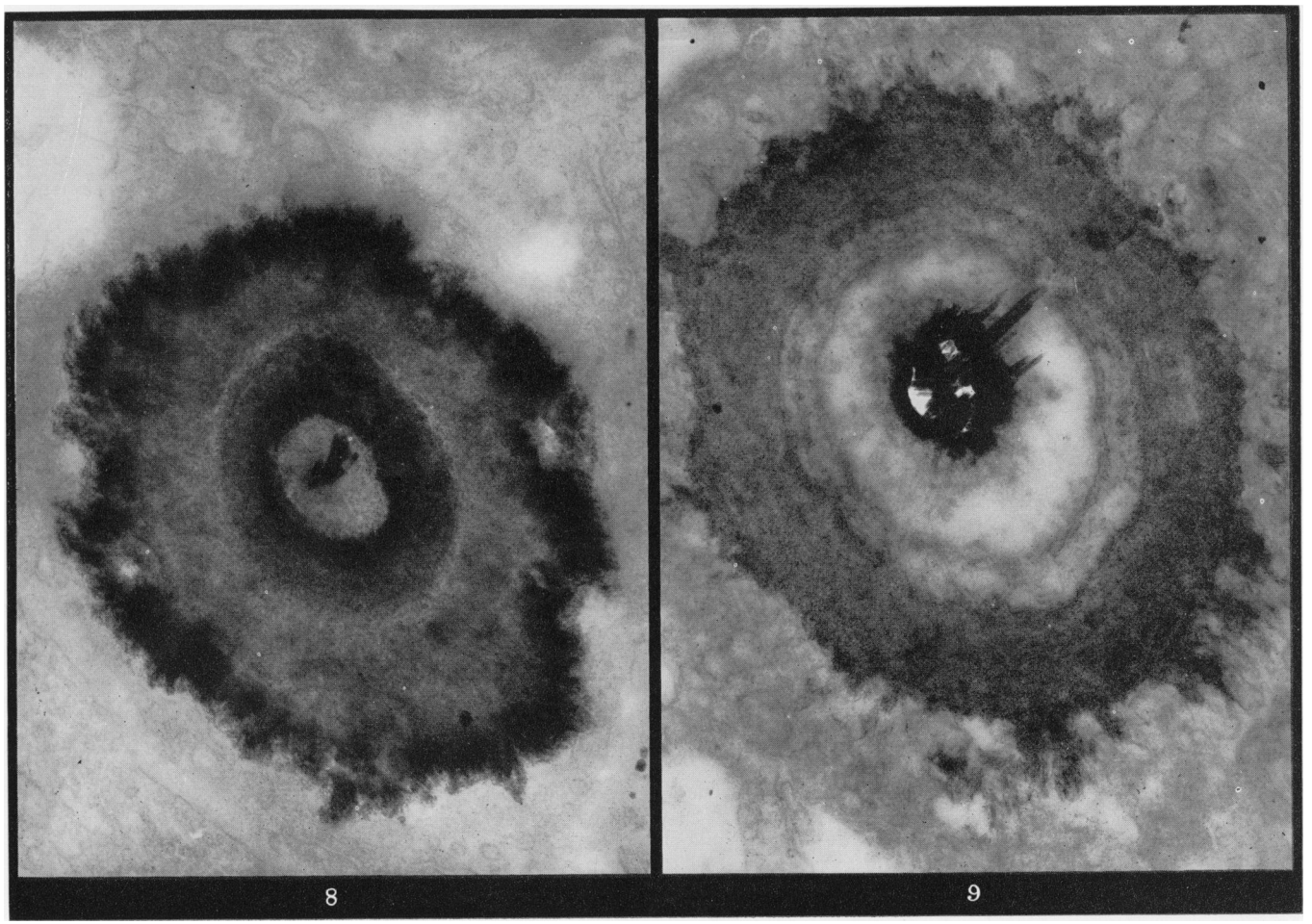

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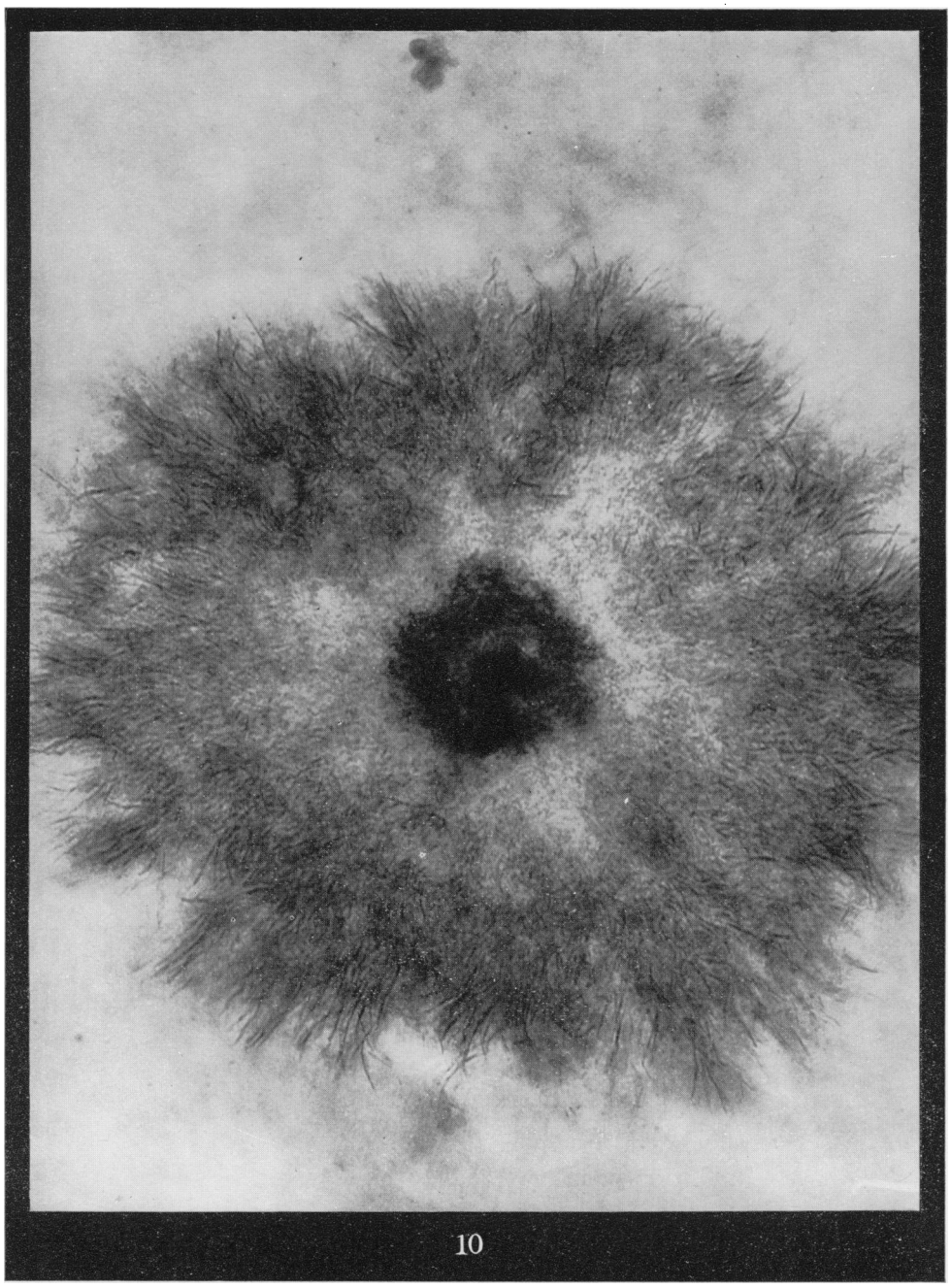

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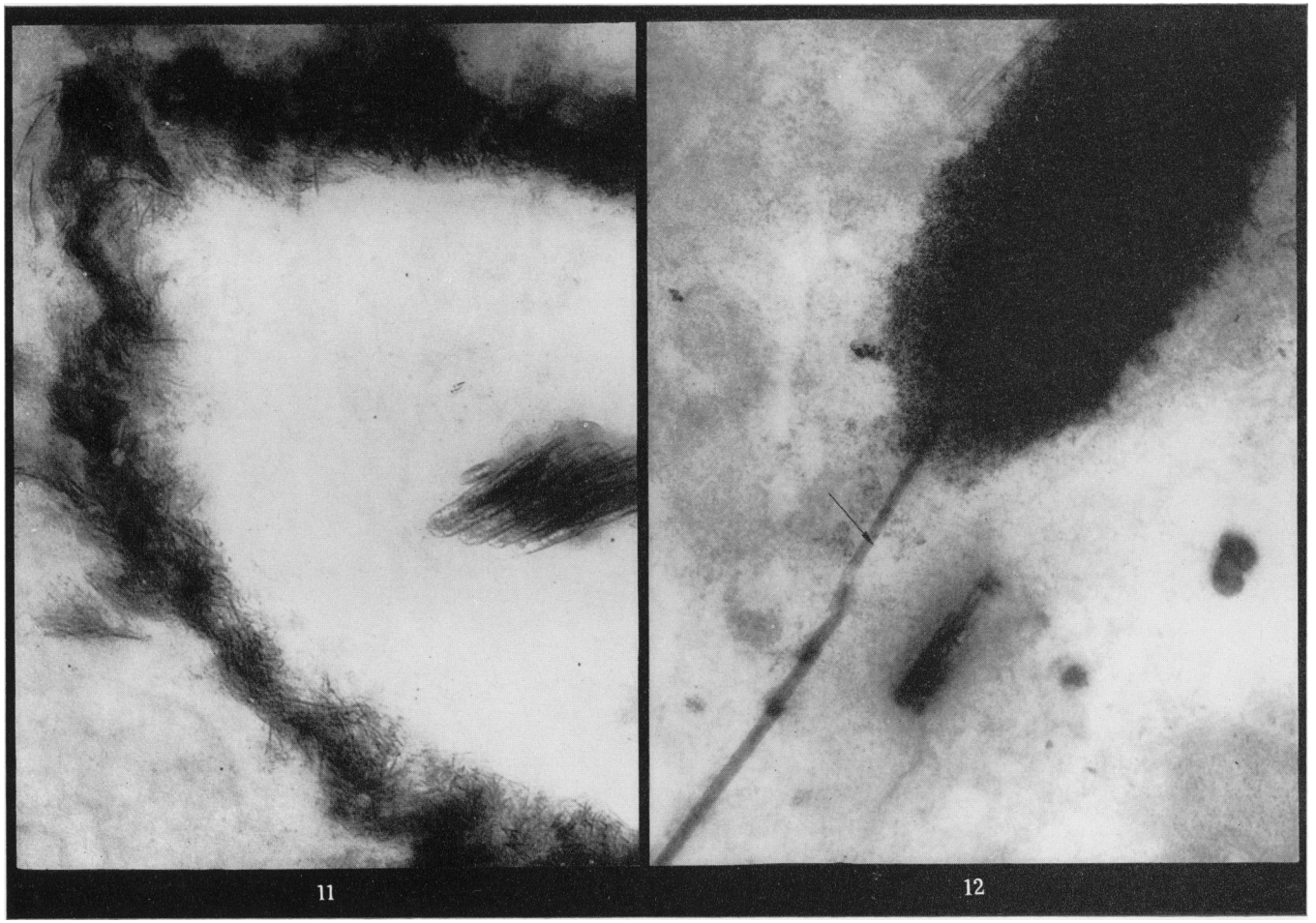

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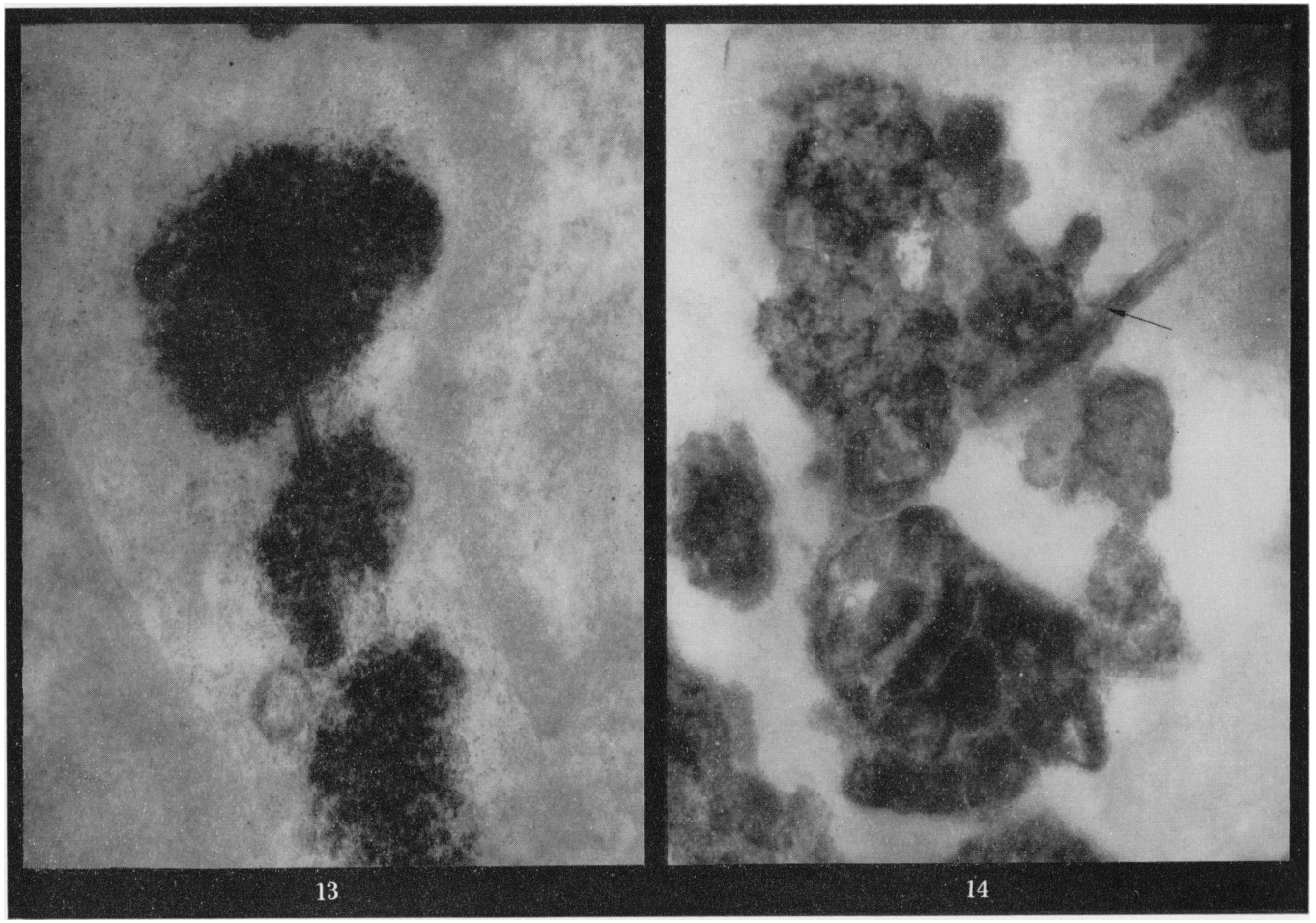

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approximately 60 Å in diameter (Figs. 2 and 4). Similar granules have previously been described in lung macrophages and other cells by a number of workers including Karrer (1958), Richter (1958) and Lindner (1958). They have been generally assumed to be ferritin particles, although Lindner suggests that there could be some confusion between ferritin and haemosiderin granules. In order to test the hypothesis that this granular material was ferritin Blount (personal communication) who was studying the adsorption of various protein materials on to asbestos fibres included a ferritin preparation in his experiments. The chemical results of these studies will be published separately, but some of the chrysotile used in these experiments was embedded in Araldite and after sectioning was examined in the electron microscope. As shown in Fig. 3 the known ferritin granules are of very similar size and density to the granules that accumulate around the chrysotile dust in guinea-pig lung macrophages, and it therefore seems very likely indeed that the basic asbestos body coating material is ferritin or at least contains a very large proportion of ferritin.

The density of the ferritin deposition on chrysotile dust was found to vary considerably. As shown in Fig. 6 the granules are often packed very closely together, but in some cases the dust becomes surrounded by a material that contains relatively few ferritin granules (Fig. 5). In fact the ferritin density in the asbestos body coat can vary considerably during its formation and this gives the body a layered appearance (Figs. 7, 8 and 9). When this occurs neither the number nor the thickness of the layers is at all consistent and the least dense layers have few if any ferritin granules. The chemical nature of these lighter layers is uncertain but they probably consist of protein material that contains no iron and therefore has little contrast in the electron microscope.

In most cases the only structures visible in the guinea-pig asbestos body coats were granules approximately 60 Å in diameter, but in a few cases the outermost coat of a layered body consisted of very fine fibres approximately 50–60 Å in diameter and up to 0·2 μ. long. These fibres are usually arranged radially, but whereas the granulated coats are always deposited in a very symmetrical fashion giving a circular profile in transverse section, the fibrous outer layers are irregular and give the body a very ragged appearance. These points are illustrated in Figs. 9, 10 and 11. In Fig. 9 the fibrous outer coat has been deposited on a many layered body, but in Fig. 10 it forms the bulk of the body protein. In Fig. 11 there is no granular ferritin at all in the body and the dust is surrounded by a layer of clear material on which the fibrous outer coat has been deposited. It is a point of interest that all asbestos bodies that had a fibrous outer coating were found in fibroblasts rather than in macrophages. The total number of such bodies found in this study was, however, small (only 21 in fact), and their location in fibroblasts may have been coincidence. Where only a single coating of ferritin is present the diameter of the asbestos bodies is comparatively uniform, ranging between 1 μ. and 2 μ. Where layering is present, however, the total body coating can be much thicker and some have been found with a diameter of more than 5 μ.

During the examination of the guinea-pig material used in this study it became obvious that the deposition of ferritin or other material on chrysotile crystals to form asbestos bodies was by no means an inevitable occurrence. Many uncoated fibres were found in the oldest animals which must have been in their lungs for at least 15 months. In contrast some bodies were found after as little as 8 weeks dusting, so that body formation is not merely a matter of the dust remaining in the

lung tissues for long periods of time. Even when coating occurs it is by no means uniform along the whole length of the fibre. This is shown in Fig. 12 where a thick globular deposition of ferritin is present at one end of a chrysotile fibre, while the other end is entirely uncoated. It would appear, however, that any parts of an asbestos fibre not coated initially may be coated later and this deposition of isolated globules of ferritin along the length of a fibre gives the appearance of segmentation (Fig. 13).

The asbestos body is usually described as an elongated structure consisting of an asbestos fibre surrounded by a neat protective protein coating, but in this study some structures have been found in lung macrophages that are made up of the main constituents of asbestos bodies without the usual neat arrangement (Fig. 14). These structures consist basically of irregularly shaped clusters of ferritin granules in which are embedded small scattered crystals of chrysotile dust as well as any other foreign inclusions that are present in the macrophage cytoplasm. The majority of these irregular bodies are less than 1 μ. in diameter, but occasionally one has been found as large as 3 μ.

An accurate assessment of the numbers of the various types of asbestos body found in this study is not possible as relatively little material can be examined with the electron microscope and the number of bodies found was too few for statistical analysis. In general, however, it may be said that the majority of the bodies had a single coating layer of granular ferritin material. Less than a quarter of the bodies examined had a layered structure and " segmented " bodies were comparatively rare. The irregular bodies were quite common although as they are very small they were not easy to find except at high magnifications.

DISCUSSION

The discovery that ferritin is likely to form one of the basic components of the asbestos body coating fits in well with many of the previous suggestions on the chemical nature of this coating material. Ferritin is an iron protein aggregate and this explains the finding of both iron and protein material by many workers. Previously the presence of iron in the asbestos body coating had been difficult to explain. It was suggested by some workers that the iron was derived from the asbestos dust itself, but Gloyne (1932) showed that this was not the case. In any event although some of the asbestos types do contain iron, chrysotile does not and chrysotile based bodies still contain iron in their coating material. Cooke (1929) suggested that the iron was derived from the haemoglobin material of damaged blood cells, but the amount of iron in haemoglobin is very much less than that found in asbestos bodies.

The iron content of asbestos bodies reported by different workers has varied considerably. Thus Leach (personal communication) found only 6 per cent of iron whereas Cooke (1935) gave a figure of 16 per cent and Sundius and Bygden (1937) found that the iron content in their asbestos body sample was as high as 28 per cent. The iron content of ferritin is variable, which could account for the different figures but its maximum is 23 per cent (Granick, 1946). This may mean that the figure given by Sundius and Bygden (1937) is inaccurate or that some iron containing material other than ferritin is present in the body coating.

The fact that much of the body material is ferritin does not at first sight fit in with Beattie's (1961) observation that young asbestos bodies are often quite

transparent and only later show the brown coloration due to the presence of iron salts. The explanation of this discrepancy is probably in the observation made in this study that whereas most of the asbestos body coats consisted of very densely packed ferritin granules, some layers were found in which granules although usually present were relatively scarce. If such a layer formed the initial deposit or even covered a thin layer of dense ferritin then the body coating might well appear to contain no iron.

The layering of the body material is itself of considerable interest, and may perhaps give some insight into the factors affecting asbestos body formation. One possibility is that once asbestos body formation has started the coating of ferritin and other non iron containing proteins is laid down at a constant rate. In such conditions, if the quantity of ferritin available to the cell varied the ratio of this material to the other proteins in the coat would also vary, and layers of differing ferritin density would result. In this case the order, thickness and density of the layers would be a clear reference to the length and magnitude of the ferritin fluctuation within the lung. It must be remembered, however, that only a comparatively small number of the bodies showed marked layering, and most had a single thick coating of material with a very high ferritin density. It is possible, therefore, that the layering of the body represents not a variation in the amount of ferritin available but a variation in the biochemical conditions within the cell that control the cellular uptake of ferritin or its deposition on the asbestos fibre. What these conditions could be is at present unknown.

In addition to the problem of the variation of thickness and density in the layers of some asbestos bodies, the fact that the outermost layer of some bodies is fibrous and not granular is also of great interest. It may be that under some conditions ferritin may exist in a fibrous form, and that the presence of fibres in the outer layer of an asbestos body is merely another indication of a biological change within the cell. The existence of a fibrous ferritin, has, however, not yet been reported. Another possibility is suggested from the fact that all the bodies with a fibrous outer coating were found in fibroblasts rather than macrophages. A number of workers including Wasserman (1954) and Jackson (1956) have reported fine fibres approximately 100 Å in diameter in active fibroblasts and have suggested that they represent collagen precursors. It may be that if an asbestos body is present in such a cell some of these fibres may become attached to its surface. If this is the case Beattie's (1961) suggestion, that the asbestos body coating consisted of collagen, was very near the truth.

In this study only a few segmented asbestos bodies have been found, but it has been shown that these have been produced by uneven deposition of the ferritin coating and not by the erosion and breakup of a smooth coat as has been previously suggested by several workers. It has been assumed that segmentation of an asbestos body is a sign of ageing and occurs after a number of years in the lung, whereas the segmented guinea-pig bodies have been in the lung for a maximum of 15 months. The reason for this discrepancy is not yet known with certainty, but an examination of some human asbestos bodies from biopsy material is being undertaken and at the time of writing it appears likely that at least two different processes can give the appearance of segmentation in asbestos bodies. These details will be reported more fully later.

The finding of large deposits of ferritin material containing scattered particles of asbestos dust adds a new complication to the study of asbestos bodies, as these

structures would probably appear as irregular bodies in the light microscope. It had previously been supposed that only long dust fibres became coated, and that the dust formed a neat core along the whole length of the body. On consideration, however, it would have been a very subtle process that could distinguish between various particles of asbestos dust by size alone. It would appear that small dust particles can become coated and if several such particles are close together a composite body is formed containing not only asbestos dust and ferritin but also any other foreign material such as carbon particles that happen to be in the neighbourhood. The real mystery remains why many dust particles of all sizes can remain in the lung for long periods of time without receiving any coating material at all.

SUMMARY

The ultrastructure of the asbestos body has been examined in guinea-pigs treated with asbestos dust by the method of Holt and Young (1960).

The first sign of asbestos body formation was the accumulation of dense granules approximately 60 Å in diameter around the asbestos fibre. It is suggested that these granules are ferritin material. Usually the asbestos body coating consists of a single dense layer of ferritin, but sometimes low density layers are present that contain few ferritin granules. Often a whole series of layers is built up, but there is seldom any regularity in the thickness or density of these layers. The outermost zone of these bodies often consists of an irregular layer of radially arranged fibrous material but occasionally almost the whole body coat consists of these fibres which have a diameter of 50–60 Å. A few asbestos bodies were found with a segmented appearance, but it seemed likely that this was due to the deposition of the ferritin as separate globules along the length of the asbestos fibre rather than as a smooth coating. There was no evidence of the breakup of asbestos bodies in guinea-pig lungs. 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 around small scattered particles of asbestos dust. These structures often contained foreign material such as carbon particles in addition to the asbestos dust and ferritin. The significance of these new structures in relation to the classical asbestos bodies is as yet uncertain.

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