ASBESTOS-BODY FORMATION IN THE LUNGS OF RATS AND GUINEA-PIGS AFTER INHALATION OF ANTHOPHYLLITE

SUSAN K. BOTHAM AND P. F. HOLT

Department of Chemistry, University of Reading, Berkshire

PLATES CXXVI–CXXX

ALTHOUGH asbestosis was briefly described in this country by Murray (1907), the first clear reference to asbestos bodies was not made until 7 yr later, when Fahr (1914), in a demonstration of specimens and photomicrographs of an asbestos worker's lungs, showed the presence of a large number of crystals and stated that similar structures had been seen by Marchand and Riesel.

Marchand (1906) had described yellow needle-shaped structures in sections from human lungs, and had further suggested that some of these needles appeared jointed, the individual swellings being connected by a colourless substance. He also stated that with potassium ferrocyanide and hydrochloric acid all these formations became intensely blue, behaving like the so-called haemosiderin.

In England, Cooke (1924) described the microscopic appearance of sections of an asbestos worker's lung without mentioning asbestos bodies; but the second report of the same case includes an account by McDonald (1927) of the histology of pulmonary asbestosis, in which he mentioned the large, irregular, black fragments noted by Cooke (1924), but went on to describe the more numerous smaller curious bodies, and gave a figure. Photomicrographs of the structures were published later by Beintker (1931) and Beger (1933).

Speculation on the chemical composition of asbestos bodies began with McDonald and with Gloyne (1932). Cooke (1929) reported that the coating was not crystalline, since Bragg, using isolated asbestos body coating, had found no crystal structure. Sundius and Bygdén (1937–38) demonstrated that the asbestos body contained both organic material (mainly protein) and inorganic material (mainly iron), and they found that the asbestos fibre represented only about 5 per cent. of the sample they examined.

Cooke (1924) at first believed that asbestos bodies were micro-organisms. Others thought that they were silica gel or silicates (McDonald; Beintker; Timmermans, 1931; Koppenhöfer, 1935), a ferrosilicate gel (Gardner and Cummings, 1931), or an adsorption complex of silica gel, albumin and ferric hydroxide (Beger), or were formed by adsorption on asbestos fibres of constituents of blood released from capillaries mechanically damaged by fibres (Sundius and Bygdén). Simson and Strachan (1931) believed that a coating was deposited on asbestos fibres by phagocytes. Beattie (1961) suggested that the coating was mainly collagen laid down on asbestos fibres by fibroblasts. Blount, Holt and Leach (1966) estimated 20 amino acid residues in the protein of the coating of human asbestos bodies; the values for hydroxyproline, glycine, leucine and phenylalanine indicated that the protein could not be principally collagen.

The presence of iron has always been clearly demonstrated by Perls' staining technique, but there was doubt as to whether the iron was derived from the asbestos or from the tissue. The electron-microscope studies of Davis (1964) indicated that the asbestos body coating contained ferritin. Holt, Mills and Young (1967) suggested that the coating was probably

Received 2 July 1971; *accepted* 31 Dec. 1971.

produced from macrophages that had engulfed the fibre, but gave no explanation for the high iron content. Botham and Holt (1968) observed that one of the first effects of asbestos fibres in the lung was to cause diapedesis of erythrocytes through the walls of capillaries in the vicinity of the fibres. They claimed that haemoglobin or haemoglobin derivatives from these erythrocytes were ingested by macrophages, and that ferroprotein material from the cytoplasm could be deposited on a fibre when a macrophage had also ingested an asbestos fibre. Subsequent morphological changes produced asbestos bodies of typical appearance, and the bodies followed a developmental sequence through a beaded form that eventually fragmented.

The formation of giant cells in the proximity of asbestos fibres was one of the earliest observations (Cooke, 1924). Davis (1963) has shown that they are formed from macrophages by a process of interdigitation. Several macrophages may be associated with long asbestos fibres, and these cells may fuse to form a giant cell that incorporates the fibres.

Vorwald, Durkan and Pratt (1951), working in Gardner's laboratory at Saranac Lake, administered asbestos to several species of animal by inhalation and intratracheal injection. They reported that asbestos bodies developed abundantly in guinea-pigs as they do in man, that a few asbestos fibres developed an atypical coating in cats, rabbits and mice, that asbestos bodies were rarely seen in rats and that none could be found in dogs. This observation has been confirmed by other workers, but no explanation has been offered.

The morphological changes in the development of asbestos bodies were studied (Botham and Holt) by causing guinea-pigs to inhale asbestos dust for a short period, then killing the animals, some immediately, others after various intervals of up to 270 days, and examining their lungs histologically. The present paper describes a similar, but short-term, experiment carried out with rats. Sections of the rats' lungs were compared with those of the guinea-pigs.

Materials and methods

A group of ten white rats, each weighing about 400 g, was exposed in the dusting tunnel described by Holt *et al.* to a cloud of Finnish anthophyllite. In another experiment nine guinea-pigs, each weighing about 500 g, were exposed to the same dust. In both experiments an animal was removed from the tunnel and killed after 3 hr of anthophyllite inhalation, and a second guinea-pig was taken after a further 3 hr. The remaining nine rats and seven guinea-pigs were exposed to asbestos for a total of 24 hr. The animals were kept in cages in the dusting tunnel throughout the dusting periods, which were continuous. An animal from each experiment was killed at the end of the dusting period; the remainder were returned to a normal atmosphere with the control animals and killed at intervals over the following month, as shown in the table, these intervals being calculated from the beginning of the exposure to anthophyllite.

The animals were killed with coal gas and the contents of each thoracic cavity were fixed in buffered neutral 4 per cent. formol-saline. Paraffin-wax sections from the lungs were stained with Perls' stain, and with Perls' stain followed by Harris' haematoxylin and eosin. In addition, unstained sections and those treated only with Perls' stain were examined by phase-contrast microscopy. Photomicrographs of the sections were taken with a Leitz Ortholux microscope fitted with an Orthomat camera.

Results

Terminal bronchioles

The debris in the terminal bronchioles of the rats (fig. 1) was, in general, similar to that seen in guinea-pigs (figs. 2–4) killed at a corresponding time after exposure to anthophyllite. The majority of the extracellular fibres were cleared in both species by 2 wk after dust exposure and, although intracellular fibres

were seen slightly earlier in the rat (after 3 hours' exposure; in the guinea-pig after 24 hours' exposure), clearance continued in both species throughout the month, a reduction in the number of free phagocytes occurring by a week after dusting.

In the guinea-pigs killed from 5 days onwards, a far greater proportion of these phagocytes were giant cells formed from macrophages (figs. 2 and 3), whereas in the rats giant cells became a feature of the bronchiolar debris only after 2 wk. Phagocytes containing both fibres and Perls-positive material appeared in the terminal bronchioles as early as 2 days after the beginning of dusting in the rats, but in the guinea-pigs these cells were not found until 7 days after dusting began.

TABLE

History of experimental animals

Time of exposure to anthophyllite dust (hr)	Survival time (from beginning of dusting to killing)	Rat no.	Guinea-pig no.
3	3 hr	1	1
6	6 hr	...	2
	24 hr	2	3
	2 days	3	4
	3 days	4	5
	4 days	5	...
24	5 days	6	6
	7 days	7	7
	14 days	8	8
	21 days	9	...
	28 days	10	9

Control rats were killed at 0 hr and 7 days; control guinea-pigs at 0 hr, 21 days and 28 days.

Respiratory bronchioles

The situation in the respiratory bronchioles of rats and guinea-pigs was very similar; extracellular fibres were cleared rapidly in the 1st wk after dusting, whilst clearance of fibres in macrophages and giant cells took rather longer. Fewer free phagocytes were seen in animals killed a week after dusting. Again more giant cells were seen in guinea-pigs.

Red blood cells, although seen in respiratory bronchioles of the younger rats, were more conspicuous in those animals killed later than 2 days after dusting. After a further week the numbers of red cells declined in both rats and guinea-pigs.

Alveoli

The spread of fibres into the alveoli was similar in both species. In the lungs of rat no. 1, killed 3 hr after the beginning of dusting, asbestos fibres had reached alveoli near respiratory bronchioles but not the fine bronchioles and alveoli in subpleural regions. In rat no. 2, which was exposed to anthophyllite for 24 hr, fibres had again reached the alveoli in most peribronchiolar areas, but had

reached only the respiratory bronchioles supplying the subpleural regions and were not seen in subpleural alveoli. In rat no. 3, killed a day after 24 hours' anthophyllite exposure, fibres were seen in the subpleural alveoli.

Ingestion of fibres by alveolar phagocytes began immediately in both species. Desquamation of these phagocytes was more common in the rats (figs. 5 and 6); more examples of fibres only partly ingested were found in the younger animals. The rat phagocytes generally contained fewer fibres than those of the guinea-pig, where by 2 wk after dusting a greater proportion of phagocytes containing fibres were giant cells that had developed from macrophages (fig. 7).

Perls-positive material

Escape of red cells into the alveoli was a constant feature in both rats (fig. 5) and guinea-pigs (fig. 8) killed within 28 days of dusting but, although this diapedesis was more severe earlier in the rats, it continued for longer in the guinea-pigs. Intracellular Perls-positive granules were found in all the rats, but were very scarce (fig. 9) in comparison with those in guinea-pigs killed at corresponding times. In the guinea-pigs, cells containing these granules were found in the interalveolar septa throughout the lungs, but in all the rats they were found only in small groups in isolated areas that apparently showed no constant relation to any other feature of the lung.

The numbers of these cells containing Perls-positive material increased with increase in survival time until 14 days after anthophyllite exposure, and then decreased. In all rats, except the first, some macrophages that contained Perls-positive granules also contained anthophyllite. Not all the macrophages that contained anthophyllite contained Perls-positive material also and sometimes these three types of macrophage, either with fibres or Perls-positive material or both, were found close together in an interalveolar septum. This situation was also found in the guinea-pigs, where there were, however, fewer macrophages that contained only fibres. Although some phagocytes in the rats killed later than 4 days after the beginning of dusting were found with both unstained fibres and deeply stained blue granules in a paler cytoplasm, the number and size of these granules and the intensity of the colour they gave with Perls' technique did not approach that seen in the guinea-pigs.

By the 5th day after anthophyllite inhalation, a thin coating, stained pale blue by Perls' technique, had been deposited on some intracellular fibres in guinea-pigs' lungs (fig. 8). This intracellular accumulation around a fibre of material containing iron appeared to be an early stage in the formation of an asbestos body. No comparable stage was seen in rats, even in those killed later than 5 days after exposure to anthophyllite, although in two cases, at 14 and 28 days after dusting, intracellular blue-stained material was very close to a fibre in the same cell (fig. 10); possibly these structures could later have given rise to the atypical asbestos bodies referred to by Vorwald, Durkan and Pratt. In a previous experiment, even in rats killed 18 mth after 100 hours' exposure to anthophyllite, only a very few structures with Perls-positive material associated with part of an asbestos fibre were found.

ANTHOPHYLLITE ASBESTOS-BODY FORMATION

FIG. 1.—Terminal bronchiole of rat 5 killed 4 days after first inhaling anthophyllite. The bronchiolar debris contains extracellular fibres and macrophages enclosing short fibres. Perls' method, haematoxylin and eosin (PHE). ×645.

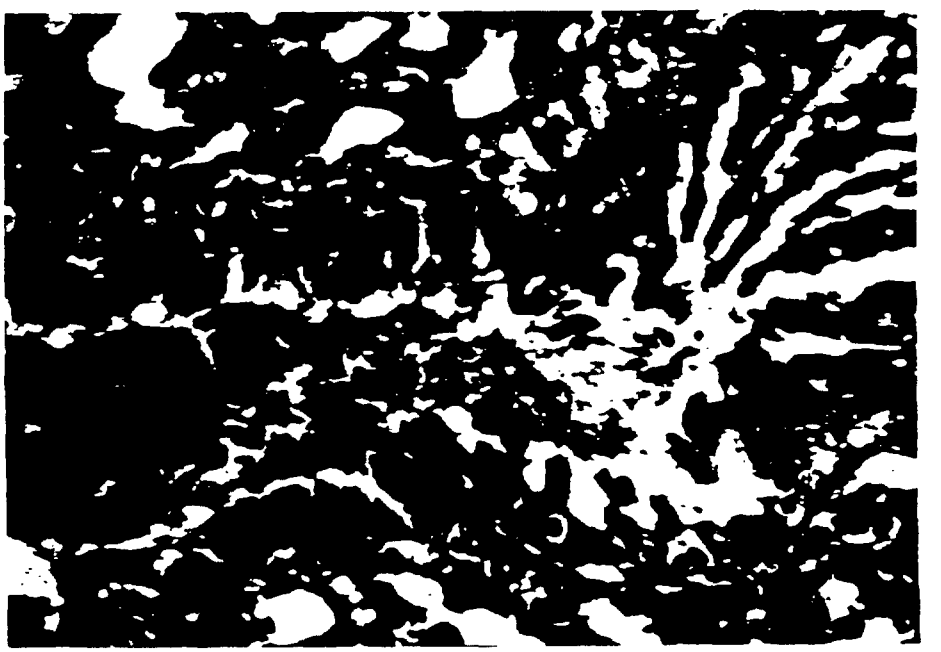

FIG. 2.—Terminal bronchiole of guinea-pig 6, killed 5 days after first inhaling anthophyllite, showing bronchiolar debris. PHE. ×390.

ANTHOPHYLLITE ASBESTOS-BODY FORMATION

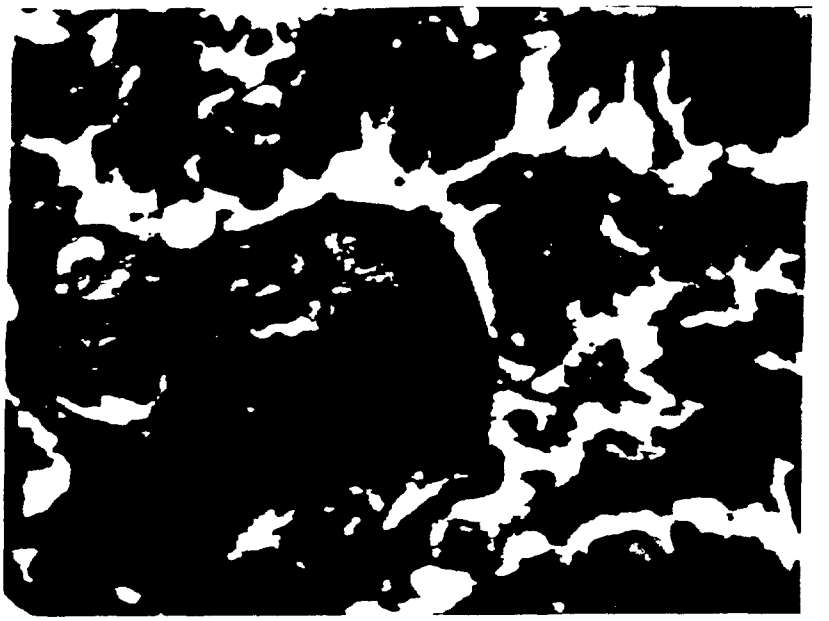

FIG. 3.—Higher-power view of giant cells in fig 2. The cells have peripheral nuclei and contain fibres. PHE. × 750.

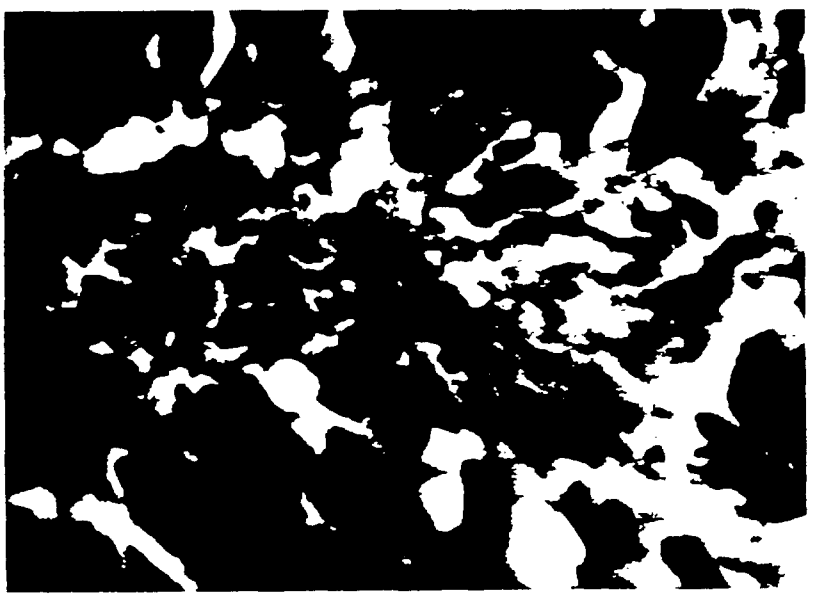

FIG. 4.—Higher-power view of part of bronchiolar debris in fig. 2: extracellular fibres, and red blood cells and intracellular fibres in desquamated macrophages. PHE. × 750.

ANTHOPHYLLITE ASBESTOS-BODY FORMATION

FIG. 5.—Detached macrophages containing Perls-positive material (A) and red blood cells (B) free in alveoli of rat 7, killed 7 days after first inhaling anthophyllite. Perls' method (P). Phase contrast. × 1430.

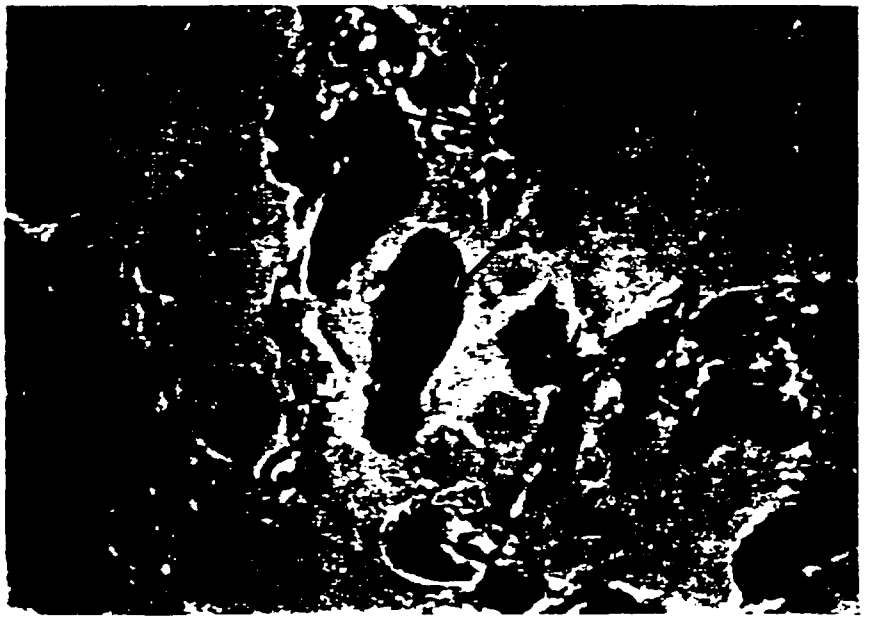

FIG. 6.—Detached macrophages (arrow A) containing fibres and Perls-positive material shed into alveolus of rat 7, killed 7 days after first inhaling anthophyllite. P. Phase contrast. × 1430.

ANTHOPHYLLITE ASBESTOS-BODY FORMATION

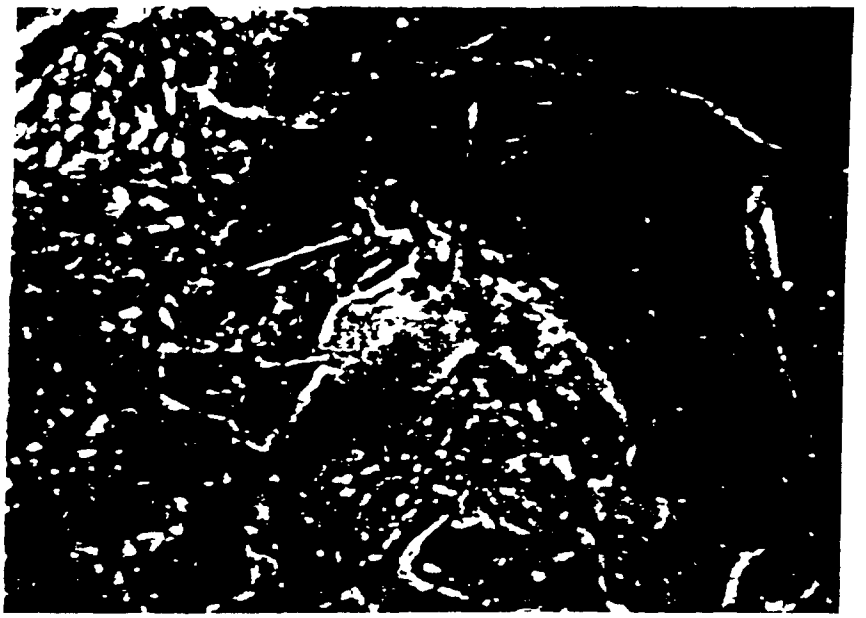

FIG. 7.—Giant cells containing fibres (A) and Perls-positive material (B) in an interalveolar septum of guinea-pig 6, killed 5 days after first inhaling anthophyllite. P. Phase contrast. × 1450.

FIG. 8.—Fibres in interalveolar septum of guinea-pig 8, killed 14 days after first inhaling anthophyllite. The longest fibre of the group has a smooth Perls-positive coating forming a young asbestos body (A). Other fibres are uncoated. Perls-positive granules (B) in an adjacent cell are not associated with a fibre. A red blood cell (C) is seen free in an alveolus. P. Phase contrast. × 1710.

ANTHOPHYLLITE ASBESTOS-BODY FORMATION

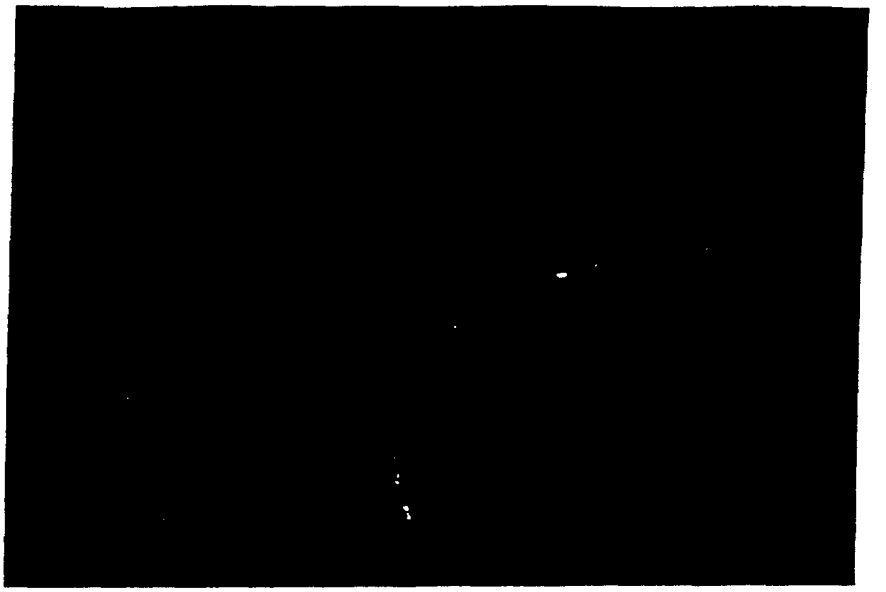

FIG. 9.—Intracellular fibres (A) in interalveolar septum of rat 8, killed 14 days after first inhaling anthophyllite. The fibres are unstained, although there is a very small area of Perls-positive material in the cell. Two red blood cells (arrow B) are seen free in an alveolus. P. Phase contrast. ×1710.

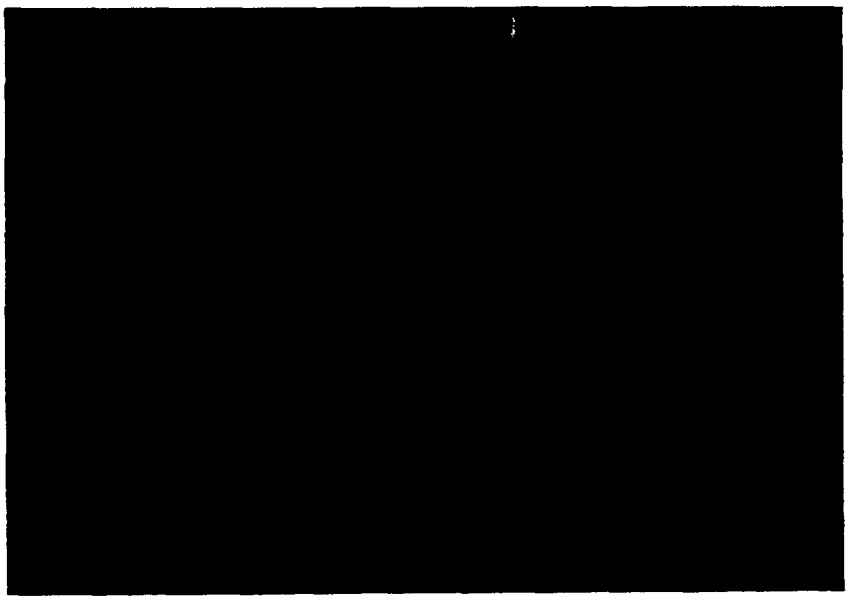

FIG. 10.—Isolated example of intracellular Perls-positive material (A) localised near an asbestos fibre (B) in rat 8, killed 14 days after first inhaling anthophyllite. Unlike the example shown in fig. 8, there is no tendency towards asbestos-body formation. P. Phase contrast. ×1710.

Controls

In the control rats and guinea-pigs there was slight thickening of some interalveolar septa and occasional desquamation of epithelial cells. Such cells, and rare examples of red blood cells, were seen in the terminal bronchioles and in a few subpleural alveoli, where some capillaries appeared slightly congested. Although no asbestos fibres were found, isolated cells contained cytoplasm that stained faintly blue.

Discussion

In a previous report (Botham and Holt, 1968) it was pointed out that more fibres had reached the alveoli in a guinea-pig killed 3 hr after the end of a dusting period of 3 hr than in an animal killed immediately after the dusting period. This movement of fibres from the respiratory bronchioles to the alveoli was also observed in the rats. In rat no. 2 fibres were not seen in the subpleural alveoli, but in rat no. 3, which was killed a day later, they were, although both rats had received 24 hours' exposure to anthophyllite. In both species fibres were ingested by alveolar macrophages, some of which were shed from the alveoli and were found in the bronchiolar debris (fig. 4), whilst others remained in the interalveolar septa (figs. 7 and 9).

One of the earliest changes noticed in the lungs of a guinea-pig killed immediately after it had inhaled anthophyllite, was the presence of erythrocytes in the air spaces (Botham and Holt). Suzuki and Churg (1969) observed similar diapedesis of red blood cells in a hamster's lung as early as 3 days after the intratracheal injection of chrysotile, and occasional erythrophagocytosis by macrophages. In the rat lung, as in the guinea-pig lung, red cells were found in alveoli during the month after dust was inhaled (fig. 5). During this time there was a gradual increase in intracellular granular material that contained iron and was stained by Perls' technique. It was suggested that this Perls-positive material is derived from haemoglobin (Botham and Holt). By the end of the experiments there was much less of this material in the rat than in the lungs of the guinea-pig killed at a month after dusting.

Some macrophages in the lung of guinea-pig no. 4 also contained diffuse iron-containing material that caused the cytoplasm to stain evenly with Perls' reagent. Such cells also occurred in all the rat lungs except those of the first animal, but they were rare and stained far less intensely with Perls' reagent. Botham and Holt considered that asbestos bodies formed through the deposition of this intracellular iron-containing material (? ferritin) on asbestos fibres that had been ingested by the same cell. It is possible that the concentration of this Perls-positive material was seldom high enough in the rat macrophages for deposition on a fibre to commence. Though it seemed that the amount of Perls-positive material formed in the rat lung was less than in the guinea-pig, the clearance of this material in cells removed in the bronchiolar debris began earlier in rats and persisted throughout the month. The clearance of this intracellular material continued for longer than in the guinea-pig; a greater amount of Perls-positive material was thus removed from the rat lung and was not available for asbestos-body formation.

Giant cells that developed from adjacent macrophages in the guinea-pigs' lungs contained fibres (fig. 7). These cells occasionally became detached and were coughed up (they were found in bronchiolar debris by 5 days after the beginning of dusting), but more often they became trapped, so blocking a bronchiole (fig. 3) or filling an alveolus, or they remained in the interalveolar septa. It has been observed (Botham and Holt) that in a cell containing several fibres only one fibre normally becomes coated with Perls-positive material and converted into an asbestos body (fig. 8). When several cells combine to form a giant cell, the total amount of ferruginous material available for coating one of the fibres is increased.

Giant cells have been reported in rats that had inhaled chrysotile asbestos for 14 days (Holt *et al.*, 1967). They occurred in a few focal inflammatory lesions, usually situated in the walls of terminal bronchioles. In the present experiment giant cells were rarely seen in the rats, so that the factors discussed above that lead to retention of Perls-positive material and fibres in guinea-pig lungs do not apply.

The factors influencing the appearance of asbestos bodies in the lungs of guinea-pigs and their absence in the lungs of rats may be summarised. There may be a difference in the amount of ferruginous material produced in the two species. Quantitative experiments would be difficult to devise, however, so this point cannot be proved or disproved. What must affect the production of asbestos bodies, and may be the sole factor, is the difference in the clearance mechanism. In the rat, both the dust and the ferruginous material are removed by macrophages that seldom fuse (figs. 5 and 6). The process is continuous, and there is little evidence that alveolar ducts or bronchioles become blocked by cellular material. In the guinea-pig the fusion of cells carrying asbestos and ferruginous material is common (figs. 2 and 7) and this affects (*a*) the availability of ferruginous material for coating the asbestos fibres and (*b*) the rate of clearance of asbestos and ferruginous material from the lung, both because the giant cells tend to remain in the lung and because they block the clearance mechanism.

Giant cells are a normal feature of the human lung that contains asbestos bodies. Schuster (1931) observed neither asbestos bodies nor giant cells in the lungs of a dog exposed to asbestos. In an experiment that will be reported later, many uncoated fibres were found in peribronchiolar regions in lungs of rats dying at up to 22 mth after 400 hours' exposure to crocidolite. Rarely did one of these fibres have a Perls-positive coating, and then the coating extended over only a part of its length. In almost every case these partly coated fibres were adjacent to a vein. Giant cells were rarely found in these lungs.

Summary

Within a month after inhalation of anthophyllite some anthophyllite asbestos fibres develop into asbestos bodies in the lungs of guinea-pigs, but asbestos bodies are rarely found in rats similarly exposed even allowing a development time of 18 mth. In both species diapedesis of erythrocytes through pulmonary capillary walls occurs soon after the anthophyllite is inhaled and

Perls-positive granules result. From this ferruginous material the asbestos-body coating is produced.

In both species, fibres and ferruginous material are cleared from the lungs by individual macrophages. In the guinea-pig, but seldom in the rat, some macrophages fuse in the alveolar regions to produce giant cells. The giant cells are removed less efficiently than macrophages; some block alveoli or bronchioles, others remain in the interalveolar septa. Thus the asbestos fibres and the ferruginous material required for the production of asbestos bodies are cleared more readily from the rat lung.

Ferruginous material is normally deposited on only one asbestos fibre when several are present in a cell. The fusion of several cells to form a giant cell means that more ferruginous material is available for asbestos-body formation.

This work was supported by the Asbestosis Research Council.

REFERENCES

BEATTIE, J. 1961. The asbestos body. *In* Inhaled particles and vapours, ed. by C. N. Davies. *Oxford*, p. 434.

BEGER, P. J. 1933. Über die Asbestosiskörperchen. *Virchows Arch. path. Anat. Physiol.*, **290**, 280.

BEINTKER, E. 1931. Die Asbestosis der Lungen. *Arch. Gewerbepath. Gewerbehyg.*, **2**, 345.

BLOUNT, M., HOLT, P. F., AND LEACH, A. A. 1966. The protein coating of asbestos bodies. *Biochem. J.*, **101**, 204.

BOTHAM, SUSAN K., AND HOLT, P. F. 1968. The mechanism of formation of asbestos bodies. *J. Path. Bact.*, **96**, 443.

COOKE, W. E. 1924. Fibrosis of the lungs due to the inhalation of asbestos dust. *Br. Med. J.*, **2**, 147.

COOKE, W. E. 1929. Asbestos dust and the curious bodies found in pulmonary asbestosis. *Br. Med. J.*, **2**, 578.

DAVIS, J. M. G. 1963. The ultrastructural changes that occur during the transformation of lung macrophages to giant cells and fibroblasts in experimental asbestosis. *Br. J. Exp. Path.*, **44**, 568.

DAVIS, J. M. G. 1964. The ultrastructure of asbestos bodies from guinea-pig lungs. *Br. J. Exp. Path.*, **45**, 634.

FAHR, T. 1914. Demonstrationen: Preparate und Mikrophotogramme von einem Falle von Pneumoconiose. *Münch. med. Wschr.*, **61**, 625.

GARDNER, L. U., AND CUMMINGS, D. E. 1931. Studies on experimental pneumonokoniosis VI Inhalation of asbestos dust: its effect upon primary tuberculous infection. *J. Ind. Hyg. Toxicol.*, **13**, 65 and 97.

GLOYNE, S. R. 1932. The asbestosis body. *Lancet*, **1**, 1351.

HOLT, P. F., MILLS, J., AND YOUNG, D. K. 1967. The mechanism of production of asbestos bodies from anthophyllite fibres. *J. Path. Bact.*, **93**, 696.

KOPPENHÖFER, G. F. 1935. Untersuchungen zur Pathogenese silikotischer Gewebsveränderungen. *Arch. Gewerbepath. Gewerbehyg.*, **6**, 38.

MCDONALD, S. T. 1927. Histology of pulmonary asbestosis. *Br. Med. J.*, **2**, 1025.

MARCHAND, F. 1906. Ueber eigentümliche Pigmentkristalle aus der Lunge. *Verh. dt. Path. Ges.*, **17**, 886.

MURRAY, H. M. 1907. Report of the Departmental Committee on Compensation for Industrial Diseases. Minutes of Evidence, Appendices, and Index. H.M.S.O. Command no. 3496. p. 127, para. 4077–4102.

SCHUSTER, NORAH H. 1931. Pulmonary asbestosis in a dog. *J. Path. Bact.*, **34**, 751.

SIMSON, F. W., AND STRACHAN, A. S. 1931. Asbestos bodies in the sputum: a study of specimens from fifty workers in an asbestos mill. *J. Path. Bact.*, **34**, 1.

SUNDIUS, N., AND BYGDÉN, A. 1937–38. Der Staubinhalt einer Asbestosislunge und die Beschaffenheit der sogenannten Asbestosiskörperchen. *Arch. Gewerbepath. Gewerbehyg.*, **8**, 26.

SUZUKI, Y., AND CHURG, J. 1969. Formation of the asbestos body. A comparative study with three types of asbestos. *Envir. Res.*, 3, 107.

TIMMERMANS, F. D. 1931. Die Lungenasbestosis und die Asbestosiskörper, ihre Gestalt und Wesenheit. *Zentbl. Gewerbehyg.*, **8**, 280.

VORWALD, A. J., DURKAN, T. M., AND PRATT, P. C. 1951. Experimental studies of asbestosis. *Archs Ind. Hyg.*, 3, 1.