73-257 K

DEVELOPMENT OF ASBESTOS BODIES ON AMOSITE, CHRYSOTILE AND CROCIDOLITE FIBRES IN GUINEA-PIG LUNGS

SUSAN K. BOTHAM AND P. F. HOLT

Department of Chemistry, University of Reading, Berkshire

PLATES LXI–LXIII

IN a previous paper (Botham and Holt, 1968) the sequence of events that lead to the formation of asbestos bodies after the inhalation of anthophyllite fibres into the guinea-pig lung was described.

This sequence was deduced from experiments in which animals were exposed to dust for a short period at the beginning of each experiment so that when they were killed the time during which the fibres had been in the lung was accurately known, and the results were not complicated by the presence of recently inhaled fibres. Anthophyllite was the first dust used in the detailed studies, as preliminary tests had shown that these fibres were more easily observed in histological sections, since being less flexible than chrysotile they tended to lie in a single focal plane and thus longer fibres were seen.

Stages in the development of anthophyllite asbestos bodies were differentiated. The first observed result of the inhalation of the asbestos was the diapedesis of erythrocytes from alveolar capillaries and the subsequent haemolysis of these cells, or their ingestion by macrophages, or both. Afterwards both granular and diffuse Perls-positive material was observed in macrophages, and when such cells also contained asbestos fibres this material had often begun to accumulate upon the longest fibre. Morphological changes in the coating around the fibre followed and led first to the formation of corrugated and then of beaded structures that eventually fragmented.

This paper reports observations from similar experiments on the clearance of other types of asbestos fibres after their inhalation.

METHODS

The experiments were conducted by the methods previously described (Botham and Holt) in which a group of guinea-pigs inhaled anthophyllite and were killed after the intervals shown in the table. Three groups of albino male guinea-pigs were exposed in a dust tunnel for 24 hr to an atmosphere in which fine amosite, chrysotile or crocidolite dust was suspended. Animals were killed at intervals over the following 18 mth, as indicated in the table. Two animals died in each of the groups receiving amosite and chrysotile and three in the group that received crocidolite; these were discarded. Paraffin-wax sections from the lungs of the remaining 21 animals were stained with Perls' stain and examined by phase-contrast microscopy. Control guinea-pigs that had received no dust were also killed and sections of their lungs were examined.

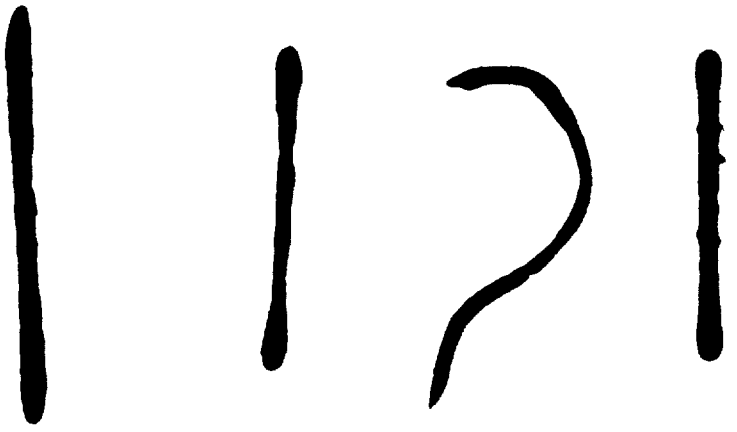
RESULTS

Changes in the lungs associated with the fibres of each dust are described under the following headings: (*a*) cartilaginous and terminal bronchioles, (*b*)

Received 12 Nov. 1970; *accepted* 23 Feb. 1971.

79-107

ASBESTOS BODIES IN GUINEA-PIG LUNGS



Amosite Anthophyllite Chrysotile Crocidolite

FIG. 1.—Equivalent early forms of asbestos bodies showing smooth even coating covering the whole fibre. Perls' method. Phase contrast. × 2280.

Amosite Anthophyllite Chrysotile Crocidolite

FIG. 2.—Equivalent forms of asbestos bodies with coatings showing the beginning of corrugation. Perls' method. Phase contrast. × 2280.

Perls-positive coating. Although sometimes they have thicker ends and some are partly beaded, only a few asbestos bodies are fragmenting.

Chrysotile

(*a*) *Cartilaginous and terminal bronchioles.* From 1 mth onwards after dusting, a progressively higher proportion of cartilaginous and terminal bronchioles are empty, but the composition of the debris seen in remaining bronchioles shows gradual changes. One month after dusting, occasional red cells are found in the debris, but subsequently they are only seen in areas of vascular congestion in the animal killed at 9 mth after dusting. From 2 mth onwards after dusting, eosinophils are occasionally found in these bronchioles.

Short fibres (less than 10 μm) are found in macrophages, and occasional giant cells in the bronchiolar mucus. Such intracellular fibres are rare by 9 mth after dusting and are not seen thereafter. Fibres thinly or partly coated with Perls-positive material, however, show a progressive increase, the first being seen at 2 mth after dusting and recognisable intracellular asbestos bodies being noted at 12 mth and over. Intracellular Perls-positive granules are seen at 2 mth after dusting, but are not found after a further 10 mth.

(*b*) *Respiratory bronchioles and alveoli.* Occasional plugs composed of fibrin with entrapped red cells and eosinophils are seen at the junction of some terminal and respiratory bronchioles in the first guinea-pig killed. At this stage most respiratory bronchioles are empty, but their lumina are often reduced because of collapse of alveoli and thickening of interalveolar septa. The alveoli, too, are generally empty, though with increasing age of the guinea-pigs a progressive reduction in size of some alveoli is seen due to thickening of their interalveolar septa, a change also observed in control animals. Isolated red cells appear in the alveolar spaces in the first animal, where there are also rare extracellular fibres. In all subsequent animals the only fibres seen are in macrophages or giant cells in the alveolar walls, or occasionally in detached cells that lie free in the alveoli. The detached cells are common in the first two animals killed, but, though occasionally found up to 6 mth after dusting, are very rarely seen in guinea-pigs killed later. They also contain Perls-positive diffuse, or granular material, or both, but coated fibres (i.e., asbestos bodies) are found only in cells free in alveoli at 9 mth after dusting.

(*c*) *Asbestos bodies and reaction to Perls' staining technique.* One month after dusting, both fibres and asbestos bodies are distributed throughout the lungs, including subpleural regions. Perls-positive granules are seen in macrophages and giant cells that also contain fibres, and a Perls-positive coating has formed on many fibres. In some cases this coating is very thin, in others it appears incomplete and banded; occasionally a coating that has begun to corrugate and form beads is seen.

In the guinea-pig killed 1 mth later, smooth and partly beaded forms are more numerous and there are some completely beaded asbestos bodies. Three months after dust exposure the first fragmenting chrysotile asbestos body is found. By 6 mth after dusting there are proportionally fewer uncoated fibres:

Rate of development

Similar forms of asbestos bodies were observed with the different types of asbestos and it would seem impossible to distinguish them on a morphological basis, but as with anthophyllite, it was possible to classify them structurally into five groups, namely: (1) a smooth, even coating covering the whole fibre (fig. 1); (2) the coating showing the beginning of corrugation (fig. 2); (3) indentations in the coating extending to the fibre, forming beads over part of the body (fig. 3); (4) the asbestos fibre completely enclosed in small beads (fig. 4); (5) fragmentation due to fracture of the fibre between beads (fig. 5).

TABLE

Correlation of morphology of 100 asbestos bodies from each guinea-pig with time between dusting and death

Dust inhaled	Survival time (mth) after dusting began	Percentage (O) of asbestos body of form					$\chi^2 = \Sigma\frac{(O-E)^2}{E}$
		1	2	3	4	5	
Amosite	1	93	7	0	0	0	334·9
	2	87	11	2	0	0	284·7
	3	53	21	26	0	0	96·3
	6	61	20	12	3	4	114·5
	9	37	31	20	7	5	40·2
	12	39	22	21	9	9	30·4
	18	39	32	15	7	7	43·4
Anthophyllite	1	96	4	0	0	0	361·6
	2*	73	18	6	3	0	184·9
	3	66	21	10	1	2	145·1
	6	64	23	12	1	0	138·5
	9	19	21	28	13	19	5·8
	12	27	26	16	16	15	7·1
	18	23	22	26	12	17	6·1
Chrysotile	1	89	11	0	0	0	302·1
	2	76	17	7	0	0	205·7
	3	44	22	24	9	1	53·9
	6	38	29	15	11	7	33·9
	9	26	20	19	21	14	3·7
	12	13	17	13	33	24	14·0
	18	3	5	15	56	21	91·7
Crocidolite	1	91	8	1	0	0	317·3
	2	57	26	14	3	0	106·5
	3	67	17	12	1	3	146·6
	6	44	36	15	2	3	73·5
	9	32	35	15	8	10	31·9
	12	43	19	23	9	6	42·8
	18	32	27	17	16	8	18·0

* Count made on animal from another experiment with 100 hr dusting.

formed preferentially on the longest fibre of a group in a macrophage. This was also found with amosite, but with chrysotile and crocidolite there were examples where this was not so, and also where more than one asbestos body had developed in a cell.

A further modification, not previously seen with anthophyllite, was the development of forked chrysotile asbestos bodies. Also, even after 1 mth many chrysotile asbestos bodies were curved, whilst a few of those formed on crocidolite showed slight curvature. By 18 mth it was virtually impossible to find a straight chrysotile asbestos body, since as most were beaded the flexible fibre was exposed.

The statistical treatment of the asbestos body counts from each animal showed that even taking a 0·1 per cent. significance level, the forms of asbestos body were not generally randomly distributed; the majority of the calculated values of χ^2 (cf. the table) were significantly greater than the value of 18·47 required for a random distribution. For example, the forms of asbestos body did not have an equal chance of occurring in any of the first four animals killed in each experiment. This implied that some forms predominated, and the observed values show that these were the smooth-coated and corrugated forms. Of these two forms the corrugated showed a gradual increase in numbers at the expense of the smooth-coated with increasing time after dusting. It appeared that asbestos bodies formed on amosite, chrysotile and crocidolite fibres followed the same developmental sequence as that described for anthophyllite (Botham and Holt, 1968). Equivalent morphological forms of asbestos body formed on the four types of asbestos fibres at each stage of development are shown in figs. 1–5.

In the animals killed at 6 mth, more beaded asbestos bodies had been observed than in younger animals, and the four guinea-pigs killed at 9 mth after dusting showed a complete range of the different forms of asbestos body. This was particularly emphasised by the χ^2 values for the chrysotile- and anthophyllite-treated animals, where each form had an equal chance of occurring.

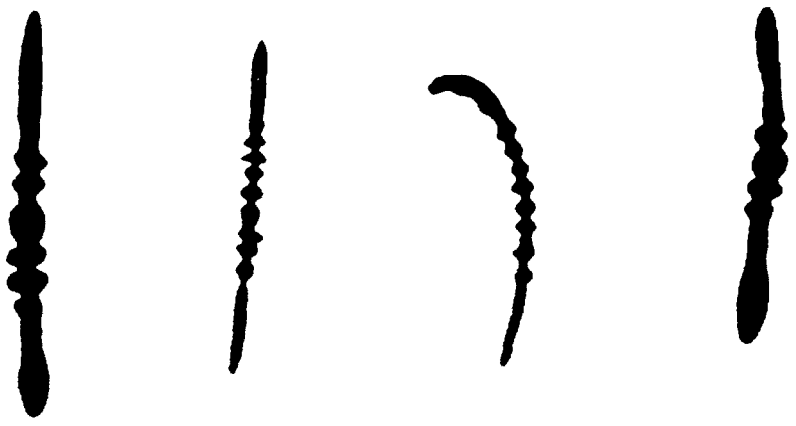
In the final chrysotile-treated animals, however, the χ^2 test showed that the different forms did not have an equal chance of occurring, and from the microscopic observations it could be seen that the majority of asbestos bodies were completely beaded or fragmenting. Thus the development and fragmentation of a chrysotile asbestos body were faster than with the other types of asbestos.

Development of asbestos bodies on anthophyllite was not quite so rapid; by 18 mth the χ^2 values showed that each form still had an equal chance of occurring, although the proportion of partly beaded to fragmenting forms was still greater than with chrysotile.

Only at 18 mth did the χ^2 test show that each form of crocidolite asbestos body had an equal chance of occurring. This supported the observation that though fragmentation started at 2 mth after dusting, by 18 mth there were still many partly beaded forms.

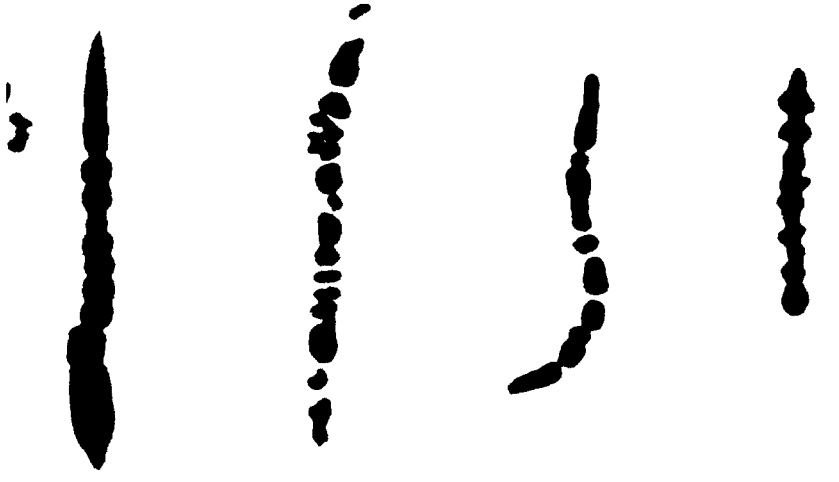
Similarly, observations on the series of amosite-treated animals showed that fragmentation began later, and by the end of the experiment there were

ASBESTOS BODIES IN GUINEA-PIG LUNGS



Amosite Anthophyllite Chrysotile Crocidolite

FIG. 3.—Equivalent intermediate forms of asbestos bodies with indentations in the coating extending to the fibre, forming beads over part of the body. Peris' method. Phase contrast. × 2280.



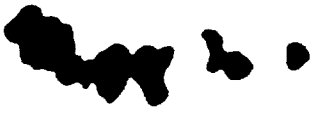
Amosite Anthophyllite Chrysotile Crocidolite

FIG. 4.—Equivalent forms showing the asbestos fibre completely enclosed in small beads. Peris' method. Phase contrast. × 2280.

ASBESTOS BODIES IN GUINEA-PIG LUNGS

Amosite. × 2280.

Anthophyllite. × 1520



Chrysotile. × 2280. ½

Crocidolite. × 2280.

FIG. 5.—Equivalent final forms of asbestos bodies showing fragmentation due to fracture of the fibre between beads. Perls' method. Phase contrast.

These changes were similar to those previously seen following the inhalation of anthophyllite asbestos, but the rates of development differed, the changes being more rapid with chrysotile, slower with crocidolite and slower still with amosite, as determined by changes with time in the percentages of the various stages in the sequence of asbestos body development. There was no apparent morphological difference between asbestos bodies at the same stage of development although formed on different types of asbestos fibres.

This work was supported by the Asbestosis Research Council.

REFERENCE

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