The Protein Coating of Asbestos Bodies

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(Received 13 April 1966)

1. Asbestos bodies were isolated from human lungs and the amino acid composition of the protein content was determined. 2. The hydroxyproline, glycine, leucine and phenylalanine values indicate that the protein in the coating cannot be principally collagen. 3. Albumin can be adsorbed on chrysotile asbestos as a monolayer but more than a monolayer is adsorbed if iron is also adsorbed. 4. Ferritin is adsorbed on chrysotile to give a thick layer. 5. The amino acid composition and adsorption studies are discussed in the light of the suggestions that the protein coating of asbestos is collagen (Beattie, 1961) or ferritin (Davis, 1964).

Asbestos fibres that are inhaled and reach the bronchioles and alveoli may become coated with a thick layer of hyaline material. The resulting structures, called 'curious bodies' by earlier workers and now called 'asbestos bodies', may take several forms; for example, an asbestos fibre 5μ or longer may produce an asbestos body that has a dumb-bell shape or appears as a string of beads. Beger (1933) believed that the 'string of beads' represented a stage in the breakdown of the bodies, but Holt, Mills & Young (1966) have demonstrated beaded bodies in sections of the lungs of guinea pigs only a few weeks after they had inhaled asbestos. Typical asbestos bodies are shown in Plate 1.

Asbestos bodies were first observed in sections of the lungs of persons who had died of asbestosis; their nature has puzzled research workers for nearly half a century. A bibliography of the early literature has been given by Sundius & Bygden (1937). The morphology was described by Beger (1933). By prolonged oxidation with hydrogen peroxide of 250g. of tissue from the lungs of a workman who had inhaled asbestos, Sundius & Bygden (1937) obtained a residue from which they isolated 0.2g. of asbestos bodies, which was sufficient for a simple chemical analysis. They gave the composition of the bodies as: asbestos, 4.6; organic matter, 31 (S, 0.43); iron oxide, 40; P_2O_5 , 6.9; water, 16%. An elemental analysis suggested that the organic material was mainly protein. They agreed with Fahr & Feigl (1914) and Cooke (1935) that the coating of the asbestos body is derived from haemoglobin released from blood vessels damaged mechanically by the ends of the asbestos fibres. Simson (1928), however, had suggested that

the coating was deposited on fibres by phagocytes. The views of McDonald (1927), Gardner & Cummings (1931), Beintker (1931), Timmermanns (1931) and Koppenhöfer (1935) that the coating is colloidal silicic acid or iron and magnesium silicates with adsorbed albumin are not entirely discounted by the analytical value quoted since silicic acid could have been removed in the extraction process used by Sundius & Bygden (1937). Beger (1933) believed that the bodies were essentially ferric oxide impregnated with albumin.

Recent electron micrographs of asbestos bodies (Davis, 1964) have revealed that parts of the coating are opaque to electrons and Davis (1964) believes that they consist of the iron-containing protein ferritin. He observed crystalline deposits of ferritin in the proximity of asbestos bodies. Beattie (1961), however, had reported hydroxyproline in a hydrolysate of asbestos bodies, suggesting that the coating contained collagen and was laid down by fibroblasts. Holt *et al.* (1966) observed that in some, but not all, phagocytes containing small asbestos particles the particles could be stained by the Prussian blue reaction a few days after the dust had been inhaled. This suggested that the type of protein in these very small asbestos bodies might vary.

We have examined the amino acid residues in the protein of asbestos bodies with the object of differentiating between collagen-type and other proteins. We have also studied the adsorption of proteins on chrysotile asbestos and the effects of iron on protein adsorption. Chrysotile is a magnesium silicate, $Mg_3Si_2O_5(OH)_4$.

Small quantities of asbestos bodies were isolated from post-mortem material and, by using the

EXPLANATION OF PLATE 1

Asbestos bodies in lungs of guinea pigs. The two photographs are of paraffin sections, unstained, and phase-contrast microscopy was used. Magnification $\times 1500$.

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micro-techniques of Moore & Stein (1951), the asbestos bodies were analysed with respect to the amino acid residues of the protein. At the beginning of the century no precautions were taken against the inhalation of asbestos in asbestos works; now the atmospheric concentration of asbestos is rigorously controlled and consequently the amount inhaled by a workman is very small. In this case only 15–20mg. of asbestos bodies could be isolated.

The amino acid composition of the protein indicated that the asbestos coating was not primarily collagen. The protein could conceivably be derived either from plasma or from phagocytic cells and an investigation was made into the adsorption of soluble proteins on chrysotile asbestos. The adsorption isotherms of albumin and of ferritin on chrysotile were first studied and subsequently the effects of Fe^{3+} ions on the adsorption of albumin. The results are discussed in the light of the suggestion by Davis (1964) that the protein coating of the asbestos bodies is predominantly ferritin.

MATERIALS AND METHODS

Asbestos bodies. An asbestos body concentrate, prepared by the method of Beattie (1960) from four lungs, was provided by Dr J. M. Davis (Department of Pathology, University of Cambridge). It contained asbestos bodies attached to lung tissue and tissue debris. The asbestos bodies were separated mechanically from adhering tissue by the following method. The sample was placed in a glass bottle containing N-NaOH (50 ml.) saturated with Na_2SO_4 and a number of thick glass rods. The bottle was stoppered and rotated for 25 hr. The asbestos bodies were kept in the liquid for a further 3 days, then separated in the centrifuge. The material was then placed in saturated Na_2SO_4 solution, centrifuged and the floating debris removed. This procedure was repeated once with Na_2SO_4 solution and thrice with water. The material was kept in trypsin solution at $\text{pH} 7.0$ and 15° for 24 hr., then separated centrifugally. The solid was washed thoroughly with water, then hydrolysed with 20% (w/w) HCl for 24 hr. at 100° in a sealed tube.

Method of analysis. The hydrolysate, which was bright yellow and contained some humin and insoluble mineral material, was filtered and diluted, and samples were dried in a vacuum desiccator over P_2O_5 and NaOH . The samples contained large quantities of FeCl_3 . Amino acids other than hydroxyproline were determined chromatographically by the method of Moore & Stein (1951) as modified by Eastoe (1955). Hydroxyproline was determined by the method of Neuman & Logan (1950) as modified by Leach (1960). As a direct determination of hydroxyproline on the hydrolysate gave a high value, due to the iron salts, the analysis was carried out on the appropriate fractions from the early (usually discarded) part of the chromatographic separation of basic amino acids. The iron was separated by 2–3 fractions from the hydroxyproline peak. Insufficient material was available for the determination of cystine and tryptophan. The final analytical values were calculated on the basis of the total weight of amino acid residues recovered.

Adsorption isotherms. Into each of several tubes was placed a known weight (0.1–0.2 g.) of milled Rhodesian chrysotile asbestos together with the selected quantity of albumin (crystallized bovine plasma albumin; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex) or $\text{FeNH}_4(\text{SO}_4)_2$. Each sample was made up to 10 ml. with water. The tubes were sealed and slowly rotated in an incubator at $37 \pm 1^\circ$ for 24 hr. The asbestos was then filtered off and the iron in the filtrate was estimated by the thiocyanate method or the protein by Folin-Ciocalteu reagent.

The effects of iron on the adsorption of albumin was studied by consecutive adsorption experiments in which chrysotile was first treated with the quantity of albumin that experiment had shown would be completely adsorbed. Ferric iron was then added in a quantity that was completely adsorbed. Further albumin was added to the solution. A series of such experiments was carried out in which various quantities of albumin were used in the final adsorption with the object of constructing an adsorption isotherm.

Davis (1964) observed deposits of ferritin near to asbestos in electron micrographs of lung sections and suggested that the coating of the asbestos body might be ferritin. Adsorption studies were therefore made with ferritin by using chrysotile on which had been adsorbed a layer of albumin, i.e. 0.048 g. of albumin/g. of chrysotile.

RESULTS

Amino acid residues. The results of the analysis, carried out on 6.4 mg. of sample, are given in Table 1. The outstanding features are that, though collagen contains about 90 hydroxyproline residues/1000 total residues, the value for the protein of the asbestos body was only 6; for glycine residues the corresponding values are 324 and 88, for leucine 26 and 122, and for phenylalanine 14 and 51. Clearly, the principal protein present in the asbestos body cannot be collagen; from the hydroxyproline content, the collagen could represent no more than 7% of the protein.

The composition of the protein resembles that of the general lung protein and is also similar to that of serum γ -globulin, globins and haemoglobins. The composition of apoferritin, the protein part of ferritin, shows similarities to that of the asbestos coating. There are, however, differences; thus the values for leucine and glutamic acid suggest that, if the coating were mainly ferritin, then our material was contaminated with 30% of other protein.

Iron. The hydrolysate contained iron equivalent to 5.7% of Fe calculated on the sample of asbestos bodies.

Adsorption of albumin. The amount of albumin adsorbed from solution by chrysotile is related to the initial concentration of albumin in Fig. 1 (curve A). This isotherm shows that adsorption is complete until a maximum value is approached at

Table 1. *Amino acid composition of asbestos bodies and the published values for collagen, lung tissue and apoferritin*

The composition of human lung was calculated from the values of Mütting & Wortmann (1954), assuming the hydroxyproline content of rat lung protein to be similar to the human materials. The values for the other amino acids of the two materials were very similar. The value of 3.7 g. of cystine/16 g. of N was not used in the calculation. The composition of human collagen is that given for human tendon collagen by Eastoe (1955). The values for asbestos bodies (corrected) represent those for asbestos bodies corrected for lung tissue content assuming hydroxyproline to represent the degree of contamination. The composition of apoferritin was calculated from the values of Harrison, Hofmann & Mainwaring (1962). N.D., Not determined.

	Amino acid composition (residues/1000 total residues)				
	Asbestos bodies	Human lung	Human collagen	Asbestos bodies (corrected)	Apoferritin
Hyp	6	34	92	0	0
Hyl	1	N.D.	9	0	0
His	19	25	6	17	32
Lys	46	65	22	41	51
Arg	31	29	49	31	55
Met	9	19	6	7	19
Asp	103	49	48	117	113
Thr	59	86	18	53	36
Ser	68	84	37	65	51
Glu	110	117	72	109	150
Pro	68	73	126	67	17
Gly	88	45	324	98	65
Ala	122	101	111	128	90
Val	82	74	25	84	45
Ile	21	29	11	19	22
Leu	112	102	26	113	155
Tyr	4	18	4	1	35
Phe	51	52	14	51	46
Trp	N.D.	N.D.	0	N.D.	5
CyS	N.D.	N.D.	0	N.D.	13

Fig. 1. Adsorption isotherms of albumin (A), serum proteins (B) and ferritin (C) on chrysotile asbestos. The continuous straight line from the origin indicates complete adsorption.

related by measuring the surface area because the chrysotile cannot be separated mechanically into single fibres. When a sample of chrysotile was used that had received more mechanical treatment the maximum adsorption was higher but the type of curve was not altered. The maximum adsorption was affected by the presence of other solutes. Sodium chloride or phosphate decreased the maximum value but again the type of curve was unchanged. When normal horse serum was equilibrated with chrysotile in the same way, the adsorption isotherm relevant to the serum proteins resembled that given by albumin alone (Fig. 1, curve B).

The results indicate that the non-specific adsorption of protein alone is unlikely to produce the thick layer found in the asbestos body. They also suggest that all fibres reaching the alveoli are, within a short time, likely to be covered with a thin layer of protein that has been adsorbed from the lymph.

Adsorption of ferritin. Ferritin was completely removed from solution until 0.13 g. of ferritin was adsorbed/g. of asbestos (Fig. 1, curve C).

about 45 mg. of albumin/g. of chrysotile. This maximum value probably corresponds to a monolayer on the albumin, but it cannot be directly

Fig. 2. Adsorption isotherm of ferric iron on chrysotile asbestos. The continuous straight line from the origin indicates complete adsorption.

Adsorption of ferric iron. Chrysotile was equilibrated with solutions of ferric ammonium sulphate. The adsorption curve, shown in Fig. 2, shows a break at about 0.08 g. of Fe^{3+} /g. of asbestos. This value was unchanged when the chrysotile was previously coated with albumin.

Consecutive adsorption of iron and albumin. Though the maximum amount of albumin that would adsorb on untreated chrysotile was 35 mg./g. of chrysotile, asbestos that had been previously treated with albumin and then iron adsorbed 123 mg./g. This was not a maximum value, all the albumin being removed from solution; there are practical difficulties if even higher concentrations of albumin are used.

DISCUSSION

From this evidence it is certain that the asbestos body is not an asbestos fibre coated with collagen deposited by fibroblasts. The analytical values for proline, hydroxyproline, leucine and glycine are all too low for a high proportion of collagen to be acceptable as the main part of the protein envelope. Yet the analytical values do allow about 7% of collagen, and this may account for the few fibres Davis (1964) demonstrated on the surface of some asbestos bodies by electron microscopy. This structure of the surface would explain why Beattie (1961), who used a very sensitive method that may well have given information only about the surface, concluded that the capsule of an asbestos body is made up of collagen or 'collagen-like substance'.

The adsorption isotherms might be interpreted as meaning that the capsule of an asbestos body is built up either by adsorption of ferritin or other preformed iron-protein complex, or that iron and protein may be separately adsorbed and built up to a thick layer on the fibre. In either case it would seem that the metal determines the continued increase in the thickness of the organic deposit; a

metal-free protein layer did not, in our experiments, increase beyond a monolayer.

Histological evidence has been presented (Holt & Young, 1967) that suggests that the coating of an asbestos body is formed from the cytoplasm of an alveolar macrophage. This chemical evidence supports the suggestion. The iron of the coating is possibly derived from disintegrating erythrocytes ingested by the phagocytes. This would explain why in the early stages the very small asbestos fibres in some cells give a Prussian blue reaction for iron whereas those in other cells do not. In the electron micrographs of transverse sections of asbestos bodies published by Davis (1964) there are patches of the coating where iron is absent.

The iron content of the asbestos bodies was much lower than the values of 16.4% (Cooke, 1935) and 29.2% and 65.7% (Sundius & Bygden, 1937) quoted earlier, and lower than values of up to 23% of Fe (i.e. 0.5–1.0 iron atom/peptide link) given for liver and spleen ferritin (Granick & Michaelis, 1942). A lower value would be expected, however, if, as the analytical values suggest, the coating of the asbestos body also contains proteins other than ferritin.

This work was supported by the Asbestosis Research Council.

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