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"Ferruginous Bodies" in Guinea Pigs

Fine Structure Produced Experimentally From Minerals Other Than Asbestos

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Electronmicroscope studies of "ferruginous" bodies, produced in guinea pigs in response to fine glass fiber and ceramic aluminum silicate, have shown that the fine structure of these bodies is identical to that of asbestos bodies. All these bodies are produced intracellularly, mainly in giant cells. Their coating consists of small dense granules, approximately 60 Angstroms in diameter, that probably represent ferritin or hemosiderin. This coating may be laid down as a single dense layer or as a series of variable layers. Usually all bodies are separated from the giant cell cytoplasm by a distinct membrane, but in some of the older bodies this membrane is no longer present.

ASBESTOS bodies were probably first observed by Fahr and Feigel in 1914,[1] but they were not immediately associated with asbestos exposure. Fahr and Feigel called them strange crystals, and later in 1924, Cooke[2] suggested that because of their beaded nature they might be fungi. Stewart and Haddow[3] realized that they were an integral part of the disease. They therefore coined the term "asbestosis body," but it was later understood that the number of bodies present was not directly related to the degree of lung fibrosis. For this reason the bodies became looked upon solely as an indication of asbestos exposure. It was felt that they were produced in response to this mineral alone, and the term asbestosis body was changed to asbestos body. Gloyne, in 1932,[4] demonstrated that each asbestos body contained a central core of asbestos dust surrounded by a coating which he thought contained iron and some protein material. The iron protein nature of the capsule was confirmed by Beger[5] and Sundius and Bygden.[6]

After the 1920's the industrial hazards of asbestos exposure were well known and resulted in most countries in strict hygienic regulations for factories and mines. It was believed, however, that the use of products containing asbestos was not dangerous to the general public and did not result in the liberation of harmful asbestos dust.

This idea was repudiated by the findings of Thomson et al in 1963,[7] who reported that structures very similar to asbestos bodies were present in the lungs of 30% of unselected autopsy cases from Cape Town. It seemed, therefore, that asbestos might be becoming a general urban hazard and studies were undertaken in other cities. These confirmed Thomson's results and bodies were found in 30% of autopsies from Miami,[8] 43% from Pittsburgh,[9] and 48% from Montreal.[10] A more recent study in Pittsburgh[11] has found bodies in nearly 100% of autopsy cases. In all these reports, however, only a few bodies were found in each case and they appeared to result in no pathological changes in the lungs.

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Thomson had assumed that all the bodies found in his cases were caused by asbestos, but anthracosis bodies have been known for a long time from the lungs of coal miners. Gloyne, in 1949,[12] reported bodies similar to asbestos bodies in the lungs of workers exposed to graphite. This indicated that the process of body formation was not limited to asbestos, although, since the coal and graphite bodies had dark central cores, it was thought that they could not be confused with genuine asbestos bodies. In 1968, however, Gross et al[13] reported that structures indistinguishable from asbestos bodies could be formed in experimental animals by the injection of such materials as aluminum silicate needles, silicon carbide whiskers, and fine glass fiber. Because all the bodies contained iron, it was suggested that they and asbestos bodies should be given the general name of "ferruginous bodies." Since minerals of similar size range to those used in these experiments are ubiquitous, it now seems very likely that many of the bodies seen in the lungs of the normal urban population result from minerals other than asbestos.

The deposition of a coating containing iron around a variety of different mineral fibers indicates that a very general process is involved, and it is of great importance, therefore, to elucidate the biochemical conditions required for body formation. As a first step, an electron microscope study of a variety of experimentally produced ferruginous bodies was undertaken to see if all these bodies have the same ultrastructure, and whether this is exactly the same as asbestos bodies. Asbestos bodies from guinea pigs and one human had previously been examined by Davis in 1965,[14,15] and it was reported that these structures were always formed intracellularly within macrophages or giant cells. The body coating consisted of small granules approximately 60 A in diameter and it was suggested that these might be ferritin. The body coating was sometimes deposited as a single layer but could consist of a number of layers of variable thickness and density. Sometimes the outermost layer consisted of radially arranged fine filaments about 60 A in diameter, and it was suggested that these represented some form of calcium deposit. It has now been shown that the fine structure of apatite crystals is very similar to these asbestos body filaments, and it now seems likely that the outer layer of some asbestos bodies is made up of this material.

In the experimental studies of ferruginous bodies, the following minerals were used: ceramic aluminum silicate, silicon carbide whiskers, and fine glass fiber. In addition to this, a commercial preparation of elastin was also tested to check on the structural similarity between elastosis bodies[16] and other ferruginous bodies.

Materials and Methods

In the first experiments, mineral samples were injected intratracheally into hamsters as reported by Gross et al[13] in 1968. It was found, however, that although the resulting ferruginous bodies were common enough to be easily found with the light microscope, they were too dispersed to be found with the electron microscope except on rare occasions. It was known, however (Davis, unpublished data), that the intrapleural injections of asbestos dust into guinea pigs resulted in the production of large granulomas which contained many asbestos bodies after as little as two weeks. For this reason it was decided to use the intrapleural technique with other minerals. For these experiments, 25 mg of test material was suspended in 1 ml of physiological saline and injected into the right lower thoracic cavity of guinea pigs. The animals were killed between two and six weeks after injection and the resulting granulomas were divided into two segments. One was fixed in formol saline for light microscope examination and the other was fixed in buffered osmium tetroxide for electron microscope study.

The light microscope sections were stained by Perl's method for iron. This stains the coating material of the ferruginous bodies a dense blue, making them easily visible against the background of a pale, neutral red counterstain. The material for electron microscopy was embedded in epoxy resin and stained with lead citrate.

The injected materials were as follows:

1. Glass fiber, a commercial sample with an average fiber diameter of 0.05μ to 0.99μ. Initially

supplied as a felt, this was finely ground before injection.

2. Aluminum silicate, an uncoated ceramic fiber with a median diameter of 2μ. Fifty percent of the fibers were under 75μ in length and many filaments were shorter than 15μ. No free silica was detected in the fibers.

3. Silicon carbide whiskers, 99.5% silicon carbide. Fiber diameter ranged from 0.5μ to 3μ, and fiber length ranged from 100μ to 750μ.

4. Elastin, a commercial sample consisting of relatively short thick fibers with diameters of 5μ to 15μ and lengths from 50μ to 200μ.

Observations

When the animals were examined six weeks after injection, it was found that all test materials had caused the formation of large granulomas. These were fairly evenly distributed over both visceral and parietal pleura and the diaphragm. The histological patterns of these granulomas varied, however, with the different materials. Granulomas produced in response to glass or aluminum silicate were very cellular and consisted mainly of giant cells mixed with free macrophages and some fibroblasts. There was some deposition of reticulin fibers in these lesions from about two weeks after injection, but collagen was limited to a capsule around each granuloma. In the case of elastin, however, although the foreign material was quickly encased in a thin collagenous capsule, the cellular response around the elastin fibers was poor. In some areas, macrophages and fibroblasts infiltrated between the individual elastin fibers, but other areas remained quite acellular.

Perl's stain revealed that with both glass fiber and aluminum silicate, some of the fibers were coated with iron-containing material as little as two weeks after injection. By six weeks many ferruginous bodies, some of them beaded, were present in the granuloma, and they appeared in all respects similar to asbestos bodies produced in guinea pigs by the intrapleural injection of chrysotile asbestos (Fig 1 to 3). In the lesions produced by elastin, Perl's staining appeared negative after two weeks, but by six weeks some of the fibers were beginning to stain blue. In this case, however, the elastin fibers were much larger than asbestos bodies and could not be confused with them.

Electron miscroscope examination revealed that the cellular response to fine glass fiber and aluminum silicate was exactly similar to that induced by asbestos dust. Although the glass fiber used had an average diameter of 0.05μ to 0.99μ, much of the dust was thinner than this and some of it was as small as 250 A in diameter. These particles were, therefore, almost as small as crystals of chrysotile asbestos. The aluminum silicate dust was much coarser and very little of it was below 0.5μ in diameter.

All sizes of dust were taken up into macrophage or giant cell phagosomes, and these quickly contracted either to surround the dust with a closely apposed membrane or to form dust containing secondary lysosomes, as previously reported for asbestos dust by one of us (J.M.G.D., 1967). As with asbestos dust, it was found that the coating of dust to form ferruginous bodies was the exception rather than the rule, and it would appear that well below 1% of dust was ever involved in this process. Ferruginous bodies were always intracellular, usually in giant cells, and the deposition of their coating appeared in all respects similar to the formation of asbestos bodies.

The body coating consisted of dense granules approximately 60 A in diameter which probably represent ferritin (Fig 4 to 6). Most frequently the granules were deposited in a single dense layer up to 5μ thick (Fig 7), but occasionally they were arranged in layers of uneven thickness and density. In many cases there was a distinct membrane between the ferruginous body and the surrounding cell cytoplasm (Fig 6), but this was not always present (Fig 4). It can be seen in Fig 4 that the good preservation of many cytoplasmic membrane structures in close proximity to the ferruginous body precludes the possibility that a surrounding

membrane has been destroyed during fixation.

In the case of the elastin lesions the ferruginous material still consisted of small ferritin-like granules approximately 60 A in diameter, but in this case the granules were not deposited as a coat around the elastin fiber but penetrated into the matrix of the fiber itself. This impregnation was found most frequently in areas where the masses of elastin fibers were well infiltrated with macrophages and fibroblasts, but giant cells were not found, and only rarely were the elastin fibers small enough to be phagocytosed by a single macrophage. The formation of elastosis bodies would appear, therefore, to be an extracellular process in contrast to other types of ferruginous body. The earliest sites of granule deposition were usually found in areas where the elastin fibers were closely opposed to one another, and the areas of impregnation appeared to extend inwards from adjacent points on the surfaces of both fibers to form hemispherical masses. The greatest density of granular deposition was usually

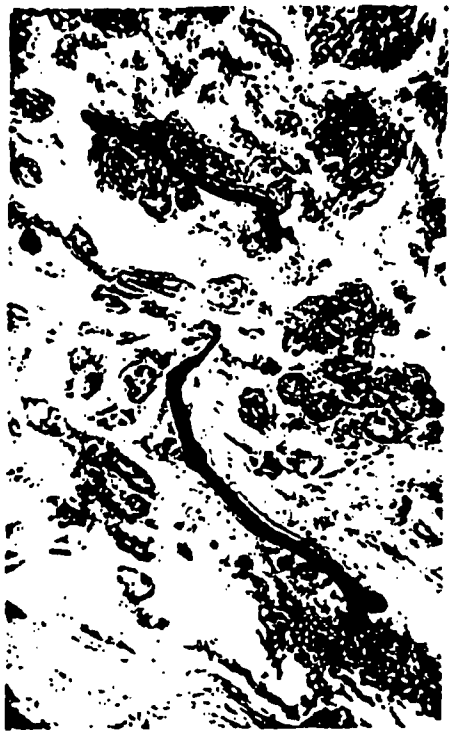


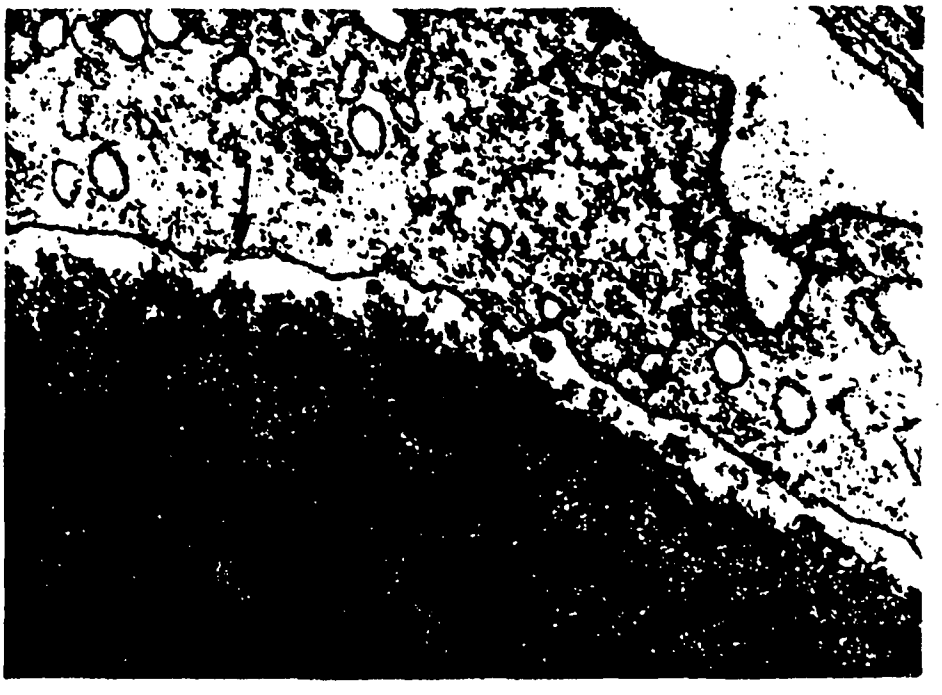
Fig 1.—Asbestos bodies produced in guinea pig pleural cavity six weeks after injection of chrysotile asbestos dust. This illustration is presented for comparison with Fig 2 and 3 (Perl's stain, reduced from × 800).

Fig 2.—Ferruginous bodies produced in guinea pig pleural cavity six weeks after injection of glass fiber (Perl's stain, reduced from × 800).

Fig 3.—Ferruginous body produced in guinea pig pleural cavity six weeks after injection of ceramic aluminum silicate fibers (Perl's stain, reduced from × 800).

Fig 4.—Surface of ferruginous body formed around glass fiber. Body is lying in cytoplasm of giant cell at point where two macrophages have not completely fused and where interdigited phagocytic processes are still visible. Although surface membranes of these processes are excellently preserved, there is no clear membrane between body and rest of cytoplasm. It can be seen that body coating consists largely of small dense granules approximately 60 A in diameter (arrows) (× 110,000).

Fig 5.—Ferruginous body formed in response to glass fiber in cytoplasm of giant cell. This body is surrounded by very distinct membrane (arrows) (× 78,000).



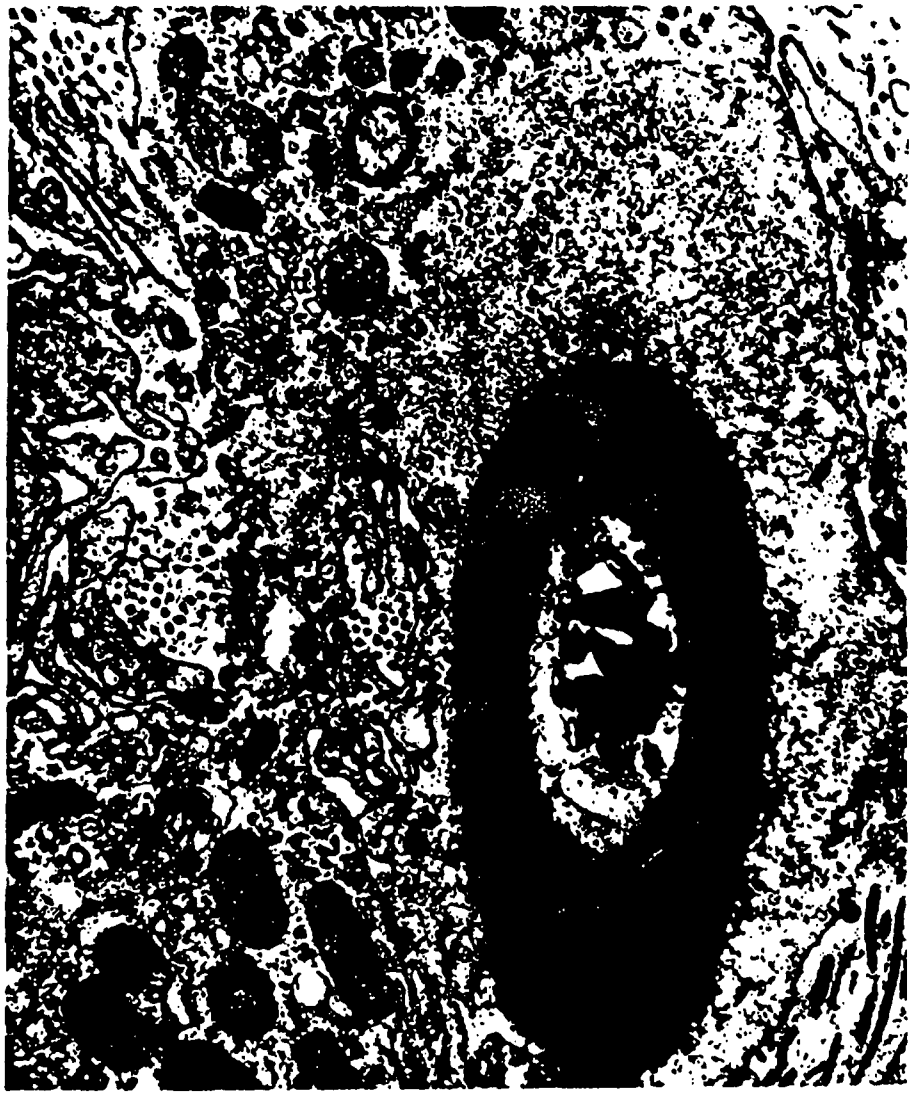
found near the surface of the elastin fiber and became progressively less further in. In many cases the innermost layers of the area of deposition consisted not of granules, but a radially arranged array of fine filaments about 60 A in diameter (Fig 6). The size of impregnated areas found in elastin fibers varied considerably, and in some cases the whole fiber was involved (Fig 8). It therefore seems logical to suggest that the impregnation is a gradual process which proceeds until the whole elastin fiber is saturated with the ferruginous materials. Where the impregnation has involved a complete fiber, the 60-A granules extend throughout and no layer of fine filaments is present. It must be emphasized that by no means all elastin fibers in any area are impregnated within the duration of this experiment, and completely unchanged fibers can often be found adjacent to others in which impregnation is complete (Fig 8). After it was discovered that the formation of elastosis bodies was by a process of impregnation, a control experiment was undertaken to

Fig 6.—Area of impregnation in elastin fiber. Center of impregnated area consists of small dense granules approximately 60 A in diameter, while outer layer consists of fine filaments of similar diameter that probably represent apatite crystals (× 74,000).

make sure the initial sample of elastin was not impregnated in any way. For this purpose, elastin fibers from the commercial samples were embedded directly in epoxy resin and examined in the electron microscope. No areas of impregnation were found.

In a few cases the elastin fibers used in this experiment were small enough to be phagocytosed, and when this occurred it was sometimes found that the fiber was separated from the majority of the cytoplasmic organelles by a pale area showing a fine granulation (Fig 9). It is possible

Fig 7.—Ferruginous body produced in response to glass fiber lying in cytoplasm of giant cell. The cytoplasm around dense "ferritin" of body is devoid of cytoplasmic organelles and probably corresponds to pseudopodial ectoplasm. There are hints of limiting membrane between body coating and this pseudopodial material, but this is not very distinct (× 35,000).



that a membrane exists at the point of contact between the elastin fiber and this granular layer, but if so it must be very closely apposed to the fiber. It seems certain, however, that no membrane exists between the pale granular layer and the rest of the cell cytoplasm. Some of the ferruginous bodies from glass and aluminum silicate were also separated from the cytoplasmic organelles by a pale granular area (Fig 7). In this case a membrane was sometimes present between the body and the granular area, but never betwen this area and the rest of the cytoplasm. These pale granular areas are very similar to the structure of pseudopodia of ameboid cells and were reported in lung macrophages by Collet and Reuet-Normand in 1967.[17] To find that such areas occasionally remain for some time around large particles is perhaps not surprising.

Comment

The finding that the fine structure of ferruginous bodies formed from a number of different mineral fibers is identical to that of genuine asbestos bodies reemphasized the suggestion of Gross et al in 1967 that it is extremely difficult to distinguish

Fig 8.—Area from center of mass of elastin fibers in guinea pig pleural cavity. There is slight infiltration of fibroblasts between elastin fibers and some deposition of collagen or reticulin. The variability of impregnation of elastin fibers is shown by fact that while three fibers are almost completely impregnated and appear black in photograph, one large fiber (F) shows no signs of impregnation at all (× 9,000).

Fig 9.—Small elastin fiber that has been phagocytosed by a macrophage. Cell cytoplasm immediately surrounding fiber is quite devoid of cell organelles and probably corresponds to pseudopodial ectoplasm (× 40,000).

between any of these structures with the light microscope. Where a mineral fiber is large or pigmented, it may be possible to rule out asbestos as the core, but in many cases this cannot be done. This is especially true of the finest glass fiber which has similar dimensions to asbestos. For this reason electron microscope examination is the only reliable method of distinguishing between the various types of mineral core in a sample of ferruginous bodies, and even then differentiation is often only possible if an electron diffraction pattern can be obtained from the mineral. Because of these difficulties, it is at present impossible to critically evaluate the finding of ferruginous bodies in members of the normal urban population. It may be that many of these are genuine asbestos bodies, but on the other hand

other minerals may predominate and very few of these bodies be due to asbestos. A number of studies are at present being undertaken to recognize the mineral core of bodies found in unselected autopsies, but the examination of even one body is tedious and no definite results are yet available.

The finding that the formation of elastin bodies is a process of impregnation rather than coating raises some interesting queries regarding the chemical processes involved in the formation of all types of ferruginous body. The impregnation of basement membranes by ferritin is a well-known phenomenon,[18] and colloidal iron is used as a stain for acid mucopolysaccharides.[10,20] It may be, therefore, that some iron-containing molecules have an affinity for certain proteins, and perhaps some mucopolysaccharides, and will selectively impregnate aggregates of these materials. It has been known for a long time that asbestos body coating contained both iron and protein, but the discovery that granules very similar to ferritin constituted the bulk of the coating material suggested that this iron-containing protein might make up the coating exclusively. One of us (J.M.G.D., 1965), however, showed that some layers of asbestos body contained little ferritin, and it may be that body formation involves initially the coating of the dust with a layer of protein or mucopolysaccharide containing no iron. Later, ferritin may impregnate this layer in a number of patterns to produce either single- or multiple-layered bodies.

References

1. Fahr, and Feigel: Aerztlicher Verein in Hamburg, *Munchen Med Wschr* 61:625-626 (March) 1914.

2. Cooke, W.E.: Fibrosis of the Lungs Due to the Inhalation of Asbestos Dust, *Brit Med J* 2:147 (July) 1924.

3. Stewart, M.J., and Haddow, A.C.: Demonstration of the Peculiar Bodies of Pulmonary Asbestosis (Asbestosis Bodies) in Material Obtained by Lung Puncture and in the Sputum, *J Path Bact* 32:172 (Jan) 1929.

4. Gloyne, S.R.: The Asbestosis Body, *Lancet* 219: 1351-1355 (June) 1932.

5. Beger, P.J.: Uber die Asbestosiskorperchen, *Virchow Arch* 290:280-353 (March) 1933.

6. Sundius, N., and Bygden, A.: Der Staubinhalt einer Asbestosislunge und die Beshaffenheit der sogenannten Asbestosis korperchen, *Arch Gewerbepath Gewerbehyg* 8:26-34 (March) 1937.

7. Thomson, J.G.; Kaschula, R.O.C.; and Macdonald, R.R.: Asbestos as a Modern Urban Hazard, *S Afr Med J* 37:77-81 (Jan) 1963.

8. Thomson, J.G.; Path, F.C.; and Graves, W.M.: Asbestos as an Urban Air Contaminant, *Arch Path* 81:458-464 (May) 1966.

9. Cauna, D.; Totten, R.S.; and Gross, P.: Asbestos Bodies in Human Lungs at Autopsy, *JAMA* 192:371-373 (May 31) 1965.

10. Anjilvel, L., and Thurlbeck, W.M.: The Incidence of Asbestos Bodies in the Lungs at Random Necropsies in Montreal, *Canad Med Assoc J* 95: 1179-1182 (Dec) 1966.

11. Utidjian, M.D.; Gross, P.; and de Treville, R.T.P.: Ferruginous Bodies in Human Lungs, *Arch Environ Health* 17:327-333 (Sept) 1968.

12. Gloyne, S.R.; Marshall, G.; and Hoyle, C.: Pneumoconiosis Due to Graphite Dust, *Thorax* 4: 31-38 (March) 1949.

13. Gross, P., et al: Pulmonary Ferruginous Bodies: Development in Response to Filamentous Dusts and a Method of Isolation and Concentration, *Arch Path* 85:539-546 (May) 1968.

14. Davis, J.M.G.: The Ultrastructure of Asbestos Bodies From Guinea Pig Lungs, *Brit J Exp Path* 45:634-641 (Dec) 1964.

15. Davis, J.M.G.: The Ultrastructure of Asbestos Bodies From Human Lungs, *Brit J Exp Path* 45: 642-646 (Dec) 1964.

16. Gough, J.: Differential Diagnosis in the Pathology of Asbestosis, *Ann NY Acad Sci* 132: 368-372 (Sept) 1965.

17. Collet, A., and Reuet-Normand, C.: Aspects Infra-structuraux de la Traversee de la Paroi Alveolaire du Poumon par des Cellules Migratrices, *Sem Hop Paris* 43:1928-1937 (June) 1967.

18. Farquar, M.G., and Palade, G.E.: Glomerular Permeability: II. Ferritin Transfer Across the Glomerular Capillary Wall in Nephrotic Rats, *J Exp Med* 114:699-716 (Nov) 1961.

19. Curran, R.C.; Lovell, D.; and Clark, A.E.: Mucopolysaccharides in Peritoneal Granulomas in the Rat, *J Path Bact* 91:429-439 (April) 1966.

20. Roy, S., and Ghadially, F.N.: Synthesis of Hyaluronic Acid by Synovial Cells, *J Path Bact* 93: 555-557 (April) 1967.