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Presence of Asbestos Bodies in Organs Other than the Lung*

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This study was designed to test the hypothesis that subjects with many asbestos bodies in their lungs at autopsy would also have asbestos bodies in various other organs. The subjects included 19 cases with diagnosis of asbestosis at death (two of these had mesothelioma, five had lung cancer) and 18 with pleural plaques but not asbestosis. Occupational histories were obtained from relatives. In subjects occupationally exposed to asbestos, large numbers of

asbestos bodies were found in the lungs, and in most of these, asbestos bodies were found in many of the other organs examined. In the 18 cases with only pleural plaques found at autopsy, considerably fewer asbestos bodies were found in the lungs. The number of other organs with one or more asbestos bodies ranged from 32 percent to 62 percent of the sites examined. The findings seem to confirm the hypothesis of the study.

When asbestos fibers are inhaled, some of those which are entrapped in the lungs become coated, by biological processes, with a material containing iron. These coated fibers have a unique ap-

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pearance under the microscope. Unlike some organic materials with which they might be confused, they are resistant to ashing, as well as to digestion by mild alkaline hydrolysis. These coated fibers are usually called asbestos bodies; the more general term, "ferruginous bodies," is sometimes preferred because objects of similar appearance can be formed by the biological coating of certain other types of mineral fibers.^{1,2}

Until recently, little attention has been given to the possibility that asbestos bodies might be present in locations other than the lungs. We became alerted to this possibility when we found one embedded in a tumor of the brain; at scientific meetings, several investigators have reported verbally that they have found asbestos bodies in other organs. Godwin and Jagatic³ reported that they found asbestos bodies in mediastinal lymph nodes, spleen, and small intestines.

The purpose of this study was to ascertain whether persons with a great many asbestos bodies in their lungs are likely to have asbestos bodies in various other organs. As it turned out, some of our subjects did not have many asbestos bodies in the specimens of pulmonary tissue which we examined.

MATERIALS AND METHODS

Our plan was to confine the study to autopsies of persons whose lungs were likely to contain a large number of asbestos bodies. With this in mind, we reviewed the records of all autopsies performed by one of us (A.S.C.) at Somerset Medical Center, Somerville, NJ, during 1970 and selected all of the cases in which asbestosis or mesothelioma had been recorded in the final diagnosis. Since many people in the area worked in an asbestos factory (and asbestos workers often develop pleural plaques), we also selected all cases in which pleural plaques had been recorded. A total of 37 such cases were found (34 men and three women). Asbestosis was recorded in 19 of the subjects, and all of these also had pleural plaques; two of these subjects died of mesothelioma, and five of them died of cancer of the lung. Pleural plaques but neither asbestosis nor mesothelioma were recorded in the

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remaining 18 subjects; one of them died of lung cancer, and one died of pulmonary fibrosis of unknown etiology.

Specimens of pulmonary tissue and renal tissue taken at autopsy (fixed in a solution of formaldehyde [formalin] and embedded in paraffin), were available from all of the 37 subjects. Similarly, preserved specimens from the heart (36 subjects), liver (35), spleen (34), adrenals (33), pancreas (30), brain (22), prostate (14), and thyroid (6) were also available, as well as a few from other sites (mediastinal lymph nodes, stomach, duodenum, colon, etc).

Relatives of the 37 subjects were interviewed to ascertain whether the subjects had ever been employed in an asbestos factory or plant in which asbestos was extensively used or had ever been employed as an insulation worker or were likely to have been exposed to asbestos in some other way, *eg* exposure to asbestos dust in making home repairs or living with an asbestos worker. In some cases, the interview indicated that exposure to asbestos dust in any of these ways was unlikely; however, since a major asbestos factory is located not far from the Somerset Medical Center, some of the subjects may have been exposed occasionally without being aware of the fact. Moreover, electron-microscopic examination of samples of air from some modern cities and industrial areas has shown that at least a few asbestos fibers are often present.⁴

Preparation of Specimens

As described in a previous study, a measured volume (20 cu mm) of pulmonary tissue was cut from a paraffin block with a sliding microtome and was placed on a glass slide on which an 18 × 18-mm grid (design by E.C.H.) had been printed by the chrome sputter process.⁵ The grid contains letters and numbers by which the location of microscopic objects can be specified from their coordinates. The lines of the grid make it easier to focus on the surface of the slide and also make it far easier to ensure the examination of all of an area of known size. Paraffin was removed from the specimen with xylene, after which the slide was placed in a low-temperature asher. In this way, most of the organic material was burned off, leaving the residue including any asbestos fibers and asbestos bodies. A drop of mounting media was applied, and a cover slip was set in place.

This procedure is satisfactory for pulmonary tissue which, because of air spaces, is not very dense; it is not satisfactory for the more dense tissue of other organs. Therefore, an improved procedure was developed by one of us (H.D.K.) for the purpose of this study.

The approximate surface area of the paraffin-embedded tissue was measured and the thickness and number of sections necessary to yield 20 cu mm of tissue calculated. The paraffin block was sectioned in increments of 25 to 40 mm as necessary, using a sliding microtome. The sections were transferred to a 25-ml Erlenmeyer flask and were extracted twice by decantation with 5-ml portions of xylene, finishing with 3 ml of acetone, and were then evaporated to dryness on a warm plate. Washings of xylene were decanted cautiously under a magnifying glass. Infrequently, when particulate matter failed to settle properly, the washings were filtered separately and examined for asbestos bodies. Five ml of a 5 percent aqueous solution of potassium hydroxide was added to the deparaffined tissue in the flask. The flask and contents were heated at 85 degrees C to 90°C under reflux conditions for one hour. After cooling, the alkaline suspension was diluted with about 5 ml of water and was filtered by suction in a Pyrex microanalysis filtering device (Millipore) using

MF paper (mixed esters of cellulose) of 25-mm diameter and a pore size of 0.45 micrometers. The filter paper (25-mm diameter) is clamped between the base of the filter and the 15-ml funnel (diameter, 15.5 mm at the base), resulting in a round filter cake approximately 16 mm in diameter. (When properly centered, no fibers or asbestos bodies occur outside the boundaries of the grid.) The flask and filter were washed down with several portions of water.

The microscopic slide with the grid was prepared with a light coating of a water-soluble adhesive; then the filter paper was removed from the filter, blotted lightly on the clean side, and centered, coated side down, on the grid. The water-soluble adhesive was a commercial glue (Elmer's glue) diluted 1:1 with water just before use and applied with a swab. (Trials using either albumin or gelatin adhesives at various dilutions showed inferior performance). The paper was blotted and dried in warm air. The slide was then transferred to the asher and ashed for two hours at 200 W and 250 ml/min of a gas mixture of 10 percent oxygen and 90 percent helium, at 1.3 mm Hg of vacuum. Under these conditions the ashless filter paper and the upper surface adhesive were oxidized away, leaving fixed on the slide any asbestos bodies, fibers, and other resistant solids present in the tissue.

Examination of Slides

In a previous study, we found that when a slide contains under 100 asbestos bodies, it is usually possible to count them accurately.⁵ When a great many are present, there are usually clumps consisting of asbestos bodies, fragments of asbestos bodies, and uncoated fibers. An individual clump may contain only a few intact asbestos bodies, or it may contain dozens of them. Under these conditions, accurate counting is impossible; therefore, the following plan was used.

The grid is divided into 18 rectangular areas. These areas were examined microscopically one at a time until over 100 definite asbestos bodies were counted. If and when over 100 were found, the remaining areas were scanned for the presence of clumps and individual microscopic fields containing many objects which resemble asbestos bodies. When these were found, they were carefully studied to determine whether the objects were actually asbestos bodies. If the examiner concluded that the slide probably contained a great many more than 100 definite asbestos bodies (probably hundreds or even thousands of them), then "TMTC," meaning "too many to count," was recorded. Otherwise the examiner recorded more than 100. If no clumps were found and the slide appeared to contain not many more than 100 definite asbestos bodies, then all of the 18 areas were examined as described subsequently, and an actual count was made.

Each slide was examined microscopically by one of us (V.R.P.) under phase contrast using a 25 × objective (Neofluor) (N.A. 0.60) and 12.5 × wide-angle eyepieces. Every object found at this magnification which even slightly resembled an asbestos body was studied carefully at a higher magnification using a 40 × objective (Neofluor) (N.A. 0.75) under both phase contrast and ordinary illumination. Whenever an object was considered to be a "definite" asbestos body or "probably" an asbestos body, a sketch of it was drawn on the record sheet, and the coordinates were noted. (The coordinates together with the sketch made it easy to find the object again for review or photography.) Some of the bodies may have been formed around a nonasbestos mineral fiber. When indicated, more than 100 or TMTC was re-

corded. The time required varied considerably, with the average time being about four hours per slide.

From the recorded findings a count was made of the total number of "definite" asbestos bodies and the total number of "probable" asbestos bodies for each slide. The distinction between "definite" asbestos body and "probable" asbestos body was only a matter of certainty in the mind of the examiner. Often, lack of certainty was due to inability to see the feature of an object clearly. This can occur in any one or a combination of the following circumstances: (1) the object is partially obscured by particles, fibers, or asbestos bodies; (2) the object is not parallel to the surface of the slide; or (3) the object is so small that the critical features are barely within the resolving power of the microscope. In sections with many definite asbestos bodies, there are usually also many objects which are probably pieces of broken asbestos bodies. Such fragments are difficult to evaluate, and to count all of them would result in overestimating the number of intact asbestos bodies originally present. Finally, sometimes an object is seen which looks very much like one of the sorts of asbestos bodies typically found in the lungs of people with known heavy occupational exposure to asbestos dust; but it somehow "does not look quite right." The examiner has a choice of calling it "probably" an asbestos body or *not* an asbestos body. That difficult choice is at least better than being forced into a definite answer of "yes" or "no."

It should be noted that when and if a fairly large number of definite asbestos bodies were found on a slide, then the examiner stopped recording "probable" asbestos bodies.

RESULTS

Lung

Two different analyses were made, one confined to definite asbestos bodies and another in which no distinction was made between definite and probable asbestos bodies. Except for absolute numbers, the two analyses yielded essentially the same results. Therefore, to save space and to be on the conservative side, we have confined this report to "definite" asbestos bodies.

Many definite asbestos bodies were found in each of the specimens of pulmonary tissue from the 19 subjects with asbestosis (TMTC in 12 cases and more than 100 in seven cases). Interviews with relatives revealed that 17 of these 19 subjects had been employed in an asbestos factory, and one had worked in a factory where asbestos is used extensively. The remaining man was a welder and had worked in a plant where printing presses were made.

In 18 cases, pleural plaques were found at autopsy, but asbestosis had not been noted. Perhaps some of these subjects had asbestosis but to such a limited degree as not to come to attention in a routine autopsy. These 18 cases fell into two distinctly different groups in respect to the number of asbestos bodies found on the slides prepared from pulmonary tissue.

In eight cases, more than 100 asbestos bodies were found. The information from interviews on these subjects was as follows: four had worked in an

Table 1—Ten Subjects with Less than 100 Asbestos Bodies in Sections of Lung

Case, Sex	Age at Death	No. of Asbestos Bodies in Lung	Information from Interview
M	46	48	Laborer; no evidence of asbestos exposure
M	68	38	Plumber in factory where asbestos used extensively
F	46	25	Housekeeper and worker in bakery
F	45	22	Owned auto business; worked briefly in asbestos factory when young
M	49	17	Auto mechanic
M	53	10	Service station employee
M	54	9	Mechanical designer and draftsman
M	62	5	Inspector in factory where asbestos is used extensively
M	50	3	Laborer in lumber yard; handled asbestos siding
F	45	0	Nurse; never occupationally exposed to asbestos dust; died of pulmonary fibrosis

asbestos factory, and one had worked at a factory where asbestos is used extensively; one was a plumber who also insulated pipes; one was a furnace repairman; and one was a machinist.

In the remaining ten cases, less than 100 asbestos bodies were found in the pulmonary specimens. A description of these cases is shown in Table 1. Four of them probably had slight or brief occupational exposure to asbestos dust. The automobile mechanic may perhaps have repaired brake linings containing asbestos. Some of the others may perhaps have been exposed to asbestos dust in some way without our informants being aware of the fact.

Other Organs

Findings in slides prepared from nine different organs are summarized in Table 2. The top part of Table 2 includes all of the 37 subjects. The second part is confined to the 12 subjects in whom a very large number of asbestos bodies (TMTC) had been found in the slide prepared from pulmonary tissue. The third part of Table 2 is confined to the 15 subjects in whom more than 100 definite asbestos bodies (but less than TMTC) had been found in the slide prepared from pulmonary tissue. The fourth part of Table 2 is confined to the ten subjects in

Table 2—Asbestos Bodies Found in Nine Different Organs in Relation to Number Found in Small Specimen of Pulmonary Tissue

Group and Organ	Subjects with Tissue Avail- able	No. of	No. of Asbestos Bodies		
		Subjects with 1 + Asbestos Bodies (percent)	Total Defi- nite Bodies	Mean Maximum	
				No. per Subject	per Subject
All subjects (n = 37)					
Kidney	37	14 (38)	132	3.6	82
Heart	36	13 (36)	218	6.1	88
Liver	35	16 (46)	84	2.4	26
Spleen	34	21 (62)	660*	19.4	>100
Adrenals	33	16 (48)	178	5.4	29
Pancreas	30	14 (47)	272	9.1	116
Brain	22	5 (23)	30	1.4	20
Prostate	14	6 (43)	42	3.0	12
Thyroid	6	6(100)	517*	86.2	>100
TMTC in pulmonary tissue (n = 12)**					
Kidney	12	9 (75)	122	10.2	82
Heart	11	7 (64)	204	18.6	88
Liver	12	9 (75)	70	5.8	26
Spleen	9	9(100)	469*	52.1	>100
Adrenals	12	10 (83)	123	10.3	25
Pancreas	9	9(100)	246	27.3	116
Brain	4	3 (75)	24	6.0	20
Prostate	5	5(100)	41	8.2	12
Thyroid	4	4(100)	370*	92.5	>100
>100 Bodies in pulmonary tissue (n = 15)†					
Kidney	15	3 (20)	5	0.3	2
Heart	15	5 (33)	12	0.8	4
Liver	13	5 (38)	11	0.9	4
Spleen	15	10 (67)	187	12.5	47
Adrenals	13	5 (38)	26	2.0	14
Pancreas	13	5 (38)	26	2.0	14
Brain	11	1 (9)	2	0.2	4
Prostate	6	1 (17)	1	0.2	1
Thyroid	2	2(100)	147	73.5	134
<100 Bodies in pulmonary tissue (n = 10)					
Kidney	10	2 (20)	5	0.5	4
Heart	10	1 (10)	2	0.2	2
Liver	10	2 (20)	3	0.3	2
Spleen	10	2 (20)	4	0.4	2
Adrenals	8	1 (12)	29	3.6	29
Pancreas	8	0	0	0	0
Brain	7	1 (14)	4	0.6	2
Prostate	3	0	0	0	0
Thyroid	0	0	0	0	0

*In one specimen of spleen and two of thyroid, more than 100 asbestos bodies were found, but exact number could not be determined. We have arbitrarily assumed that 150 were present.

**TMTC, Too many to count (asbestos bodies).

†Does not include specimens showing TMTC.

whom less than 100 definite asbestos bodies had been found in the slide prepared from pulmonary tissue. The number of asbestos bodies refers to "definite" asbestos bodies only; objects described as "probable" asbestos bodies are not included in this table.

As shown in the first part of Table 2, the proportion of slides with one or more definite asbestos bodies varied from six out of six (thyroid) down to five out of 22 (brain). In the six organs for which 30 to 37 specimens were available, from 38 percent (14/37) to 62 percent (21/34) of the slides had one or more asbestos bodies. We were very surprised at how many asbestos bodies were found on some of the slides; for example, 88 were found on one slide prepared from the heart, and more than 100 were found on one slide prepared from the spleen.

The last three parts of Table 2 show that there was a definite relationship between the number of asbestos bodies found in pulmonary tissue and the number found in tissue from other organs. That is to say, far more asbestos bodies were found in other organs when a very large number (TMTC) were found in the pulmonary specimen than when relatively few were found.

The fourth part of Table 2 is confined to the ten subjects described in Table 1. They had pleural plaques but neither asbestosis nor mesothelioma; and only 48 or fewer definite asbestos bodies were found in slides prepared from pulmonary tissue. From interviews, it appears that three had had some occupational exposure to asbestos dust; and perhaps a fourth (the auto mechanic) had repaired asbestos brake linings. For the remaining six subjects, interviews provided no evidence of exposure to asbestos dust. At least for these six subjects, it seems very unlikely that the lesions described as pleural plaques (because of their gross appearance at autopsy) were caused by asbestos. Furthermore, in the absence of proof of exposure to asbestos dust (and by light-microscopic examination alone), it is impossible to say whether an object having the appearance of an asbestos body was formed about an asbestos fiber. Some of them may have been "ferruginous bodies" formed about a fiber of some other sort.

As previously mentioned, a few specimens were included from each of several sites other than the nine organs listed in Table 2. Other tissues in which one or more asbestos bodies were found include mediastinal lymph nodes, bone, duodenum, stomach, colon, omentum, and spinal cord.

The number of organs in which definite asbestos bodies are found depends in part upon how many and which organs are examined for asbestos bodies. This is taken into account in Table 3. Twenty-five

Table 3—Number of Different Organs in Which One or More Asbestos Fibers Were Found

Group and Organs Studied	No. of Subjects	No. of Organs with + 1 Body								
		0	1	2	3	4	5	6	7	8
All subjects (n=37)										
6 specific organs*	25	6	4	2	3	4	3	3	0	0
Any 7+ organs**	29	6	3	3	3	2	3	2	3	4
TMTC in pulmonary tissue (n=12)										
6 specific organs*	7	0	0	0	2	0	2	3	0	0
Any 7+ organs**	9	0	0	0	0	0	1	2	2	4
>100 Bodies in pulmonary tissue (n=15)†										
6 specific organs*	11	3	1	1	1	4	1	0	0	0
Any 7+ organs**	12	3	0	1	3	2	2	0	1	0
<100 Bodies in pulmonary tissue (n=10)										
6 specific organs*	7	3	3	1	0	0	0	0	0	0
Any 7+ organs**	8	3	3	2	0	0	0	0	0	0

*Slides from six specific organs (kidney, heart, liver, adrenal glands, spleen, and pancreas) were examined for each of these subjects.

**Slides from seven to ten different organs were examined for each of these subjects.

†Does not include specimens showing TMTC

subjects had tissue examined from all of the following six organs: kidney, heart, liver, spleen, adrenal glands, and pancreas. In six of the subjects, no definite asbestos bodies were found in any of these six organs; and in three subjects, definite asbestos bodies were found in all six of these organs. As shown on the first line of the second part of Table 3, a very large number (TMTC) of asbestos bodies were found in pulmonary tissue from seven of the same subjects. In these subjects, asbestos bodies were found in three to six of the specified organs; in three of the nine subjects, asbestos bodies were found in all six of the specified organs.

The following is presented only to give an idea of how widely asbestos bodies may be distributed. Twenty-nine subjects had specimens of tissue from seven to ten different sites examined. These sites include the nine organs listed in Table 2, plus stomach, duodenum, colon, and mediastinal lymph nodes. There were very few subjects in whom more than nine sites were examined. The findings in the subjects are shown on the second lines of each part

of Table 3. It is sufficient to say that in one subject, definite bodies were found on slides from each of the eight different sites examined.

DISCUSSION

One should not draw too-sweeping conclusions from findings in so few as 37 subjects, all of whom came to autopsy in the same hospital and many of whom had worked in an asbestos factory in which chrysotile was the principal type of asbestos used. Nevertheless, the findings strongly suggest that when people have such a degree of exposure to asbestos dust that a great many asbestos bodies are formed in the lungs, then asbestos bodies are very likely to be present in one or several other organs as well.

There are two different possibilities concerning the presence of asbestos bodies in other organs: (1) they may have been formed in the lungs and then migrated to another organ; or (2) asbestos fibers may have migrated to another organ and have been coated there, thus forming an asbestos body. We are inclined to favor the latter possibility.

Electron-microscopic studies have shown that only a tiny proportion of the asbestos fibers present in the lungs of asbestos workers become coated, with the resultant production of asbestos bodies.⁶ Assuming that this is also the case in other organs, then the finding of even one or two asbestos bodies in a fragment of tissue from an organ suggests that the entire organ contains a very large number of asbestos fibers.

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