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Microfiltration Method for Quantitative Study of Fibrous Particles in Biological Specimens

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Counting coated and uncoated inorganic fibers in sputum has been used to investigate the level of environmental or occupational asbestos exposure and the concentration of fibrous dusts in human lung. Inorganic fibers in sputum were counted by light microscopy after chemical digestion and microfiltration processing. The same method was used for processing gastric juice and lung tissue. There were no ferruginous bodies (FB) in sputum from 49 patients without any asbestos exposure.

The study of sputum from 125 patients with various asbestos exposure pointed out a high correlation between the number of FB in sputum and the level of asbestos exposure. These 125 patients were classified into three groups according to the type of their asbestos occupational hazard: group I, raw asbestos workers; group II, workers manufacturing asbestos products; group III, workers with mixed industrial dust exposure. For these three groups, the mean number of FB in sputum was 100, 10, and 1, respectively.

The comparison of the FB content of sputum and lung parenchyma showed the absence of FB in sputum when the concentration of FB in lung parenchyma was under 1000/cm^3 of lung parenchyma; above this concentration the number of FB in sputum was in good correlation with fiber concentration in lung parenchyma. A preliminary study with the use of gastric juice showed that gastric juice is a less sensitive sample for evaluating fiber concentration in lung.

The microfiltration method for the counting of uncoated fibers gave results as accurate as those in the centrifugation method.

Among the numerous methods now available for study of microparticles in biological specimens and air samples *(1–8)*, the quantitative microfiltration method seems to be a very good means for screening asbestos bodies or asbestos fibers in many biological specimens.

The Microfiltration Method

The processing of microfiltration after chemical digestion *(9)* is a very simple one that can be easily carried out on all biological specimens.

The ferruginous bodies (FB) and uncoated fibers can be identified and counted by mean of optical microscopy by use of phase contrast and a 40× objective. When there are many fibers, the counting is carried out on 100 fields at random; otherwise, the whole filter is scanned over by the microscopist.

The number of FB or fibers is expressed in different ways, depending on the nature of the specimens: for sputum, as total number per sputum; for gastric secretion, as number per 20 ml; for dense tissue (tumor, stomach, bowel, liver) as number per gram of dried tissue; for lung parenchyma as number per cubic centimeter.

This way of expressing results seems to be more accurate for lung parenchyma than using dry weight; indeed as lungs are fixed inflated by formalin infusion of airways, volume is a more physiological related parameter. Elsewhere, dry

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weight can be modified by a pathological increase of density of the lung tissue, whether due to fibrosis or to pneumonia, which leads to a decrease of fibers per unit of dry weight. This is probably one of the reasons why Ashcroft and Heppleston *(10)* found a lower fiber concentration in solid fibrotic areas than in almost normal areas in asbestos lungs. Nevertheless, both ways of expressing results have been compared. In 15 cases, the distribution of the ratio Z of the weight of dry lung tissue to the volume of the same tissue has been obtained from the study of 60 blocks of lung parenchyma (Fig. 1). Thus, we can see that when the count is expressed per cubic centimeter of lung tissue, it has to be multiplied by a factor of 10 to 50 to be compared with a count expressed per gram of dried tissue.

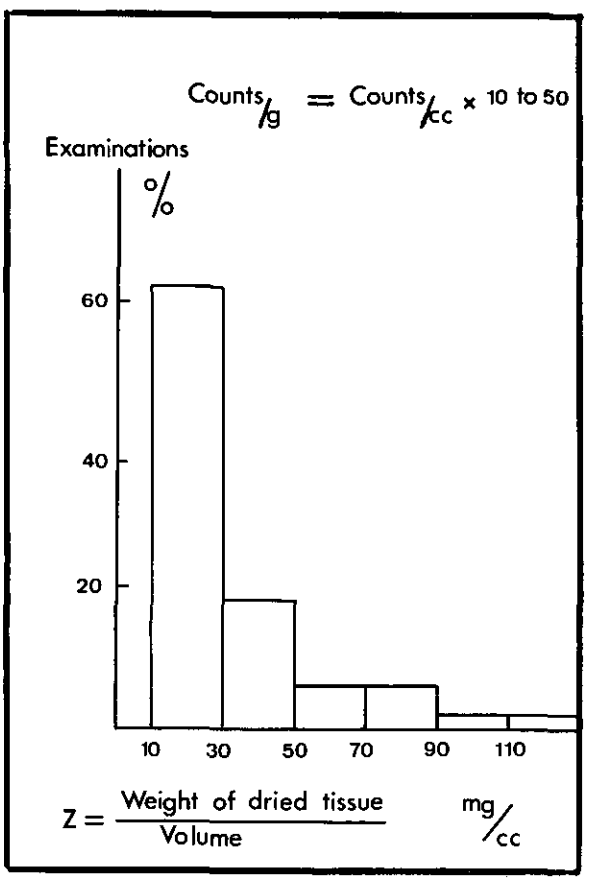

FIGURE 1. Frequency distribution of the ratio Z of the weight of dried lung parenchyma to the volume of the same formalin fixed samples.

Fibers in Sputum

Recently, we had the opportunity to use the microfiltration method for the study of 200 sputums from 125 patients, all suspected of having been exposed to asbestos. These patients were divided into three groups according to the type of occupational asbestos exposure (Table 1): group I, raw asbestos workers, i.e., patients having worked in milling, spinning, and projecting raw asbestos (16 cases); group II, workers having manufactured or used products containing asbestos as for insulation, brake-lining installation, or repair (42 cases); group III, workers exposed to industrial mixed dust, including asbestos (64 cases).

Ferruginous Bodies (FB) or Coated Fibers

In 31 cases, three sputums have been examined in order to assess the validity of studying one, two, or three sputums (Fig. 2). In group I with the greatest occupational exposure, numerous coated fibers (FB) were found in all cases since the first examination; at the opposite side; in group III, with low asbestos exposure, the results were positive only in 5% of the cases at the first examination, while they became positive in almost 50% of the cases after the study of three sputums. This stresses upon the need of studying at least two or three sputums, although, so far, this method is time-consuming.

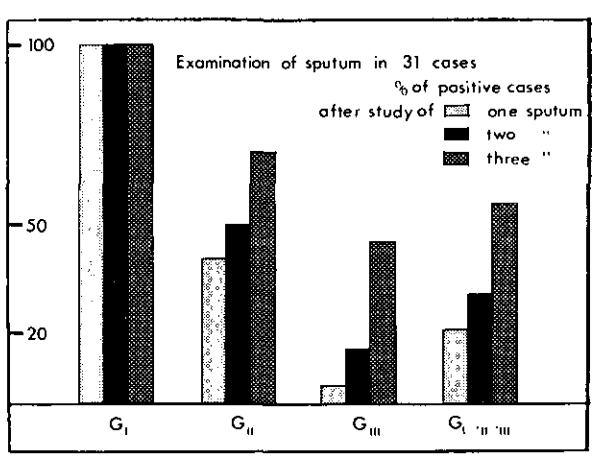

FIGURE 2. Percentage of positive cases observed in each group after study of one, two or three sputum (G denotes group).

Table 1. Distribution of the patients according to occupation type.

	200 Sputums of 125 patients (122 M, 3 F), (mean age 54 years)		
	Group I	Group II	Group III
Occupational group	Raw asbestos workers	Workers manufacturing products containing asbestos	Workers with industrial mixed dust exposure
No. of cases	16	42	64
Mean age	52 years	53 years	56 years

For this series of 31 cases, with several sputums studied, the highest value of ferruginous bodies was used for the survey.

The distribution for the different kinds of diseases, as indicated in histograms for the three occupational groups (Fig. 3), showed that ferruginous bodies were found in the sputum of 7/9 cases of mesothelioma, 21/23 cases of lung fibrosis, and 16/23 cases of lung carcinoma.

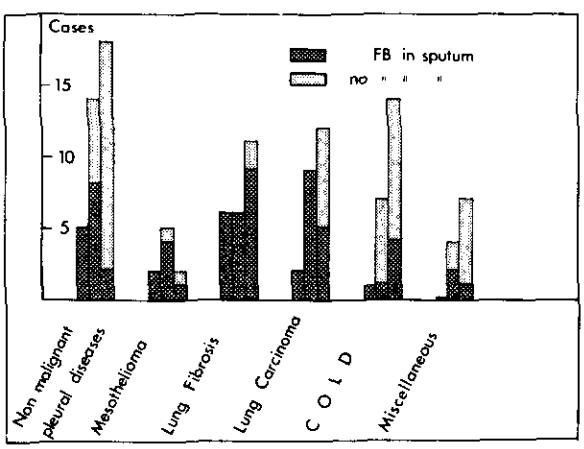

FIGURE 3. Distribution of the 125 patients according to the kinds of diseases. (COLD denotes chronic obstructive lung diseases). The occupational groups are indicated on the histograms (G_I on the left; G_{II} in the middle; G_{III} on the right).

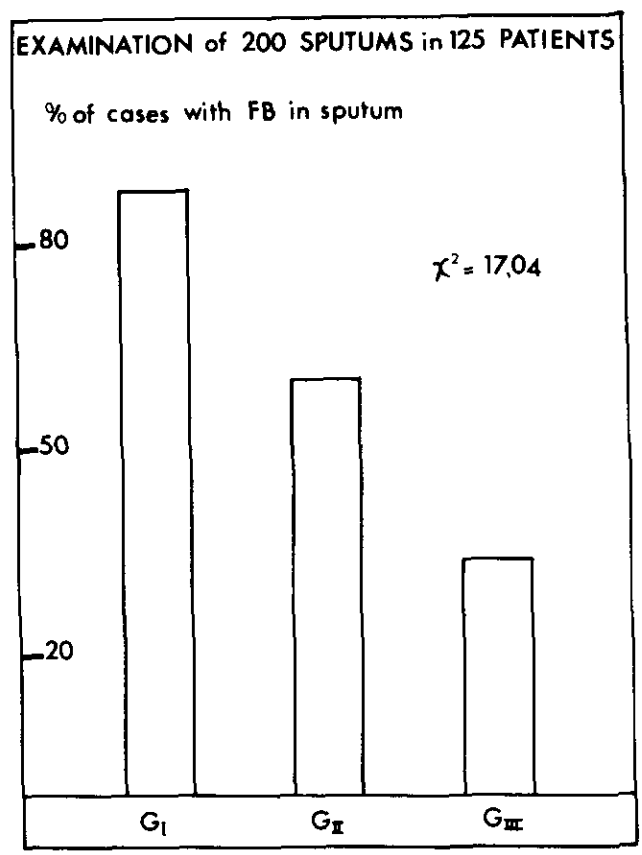

FIGURE 4. Percentage of cases with ferruginous bodies (FB) in sputum observed in each occupational group.

In the 125 patients studied, evidence was clearly demonstrated for a significant association between the presence of ferruginous bodies in sputum and the degree of asbestos occupational exposure (Fig. 4): the more severe the exposure, as in group I, the higher was the percentage (85%) of positive cases. Conversely, the less severe or only probable the exposure, the lower was the percentage of positive cases. However, in group III, at least 25 percent of the cases had ferruginous bodies in their sputum, indicating a dusty lung, as will be discussed

further. Otherwise, in a previous publication *(9)*, we showed that people without asbestos exposure had no FB at all in their sputum. Correlation between ferruginous bodies in sputum and occupational exposure to asbestos is better shown when quantitative data are used (Fig. 5). The number of ferruginous bodies in the sputum as indicated in a log-scale ordinate was more than 10 in almost all the patients with severe occupational exposure. In the two cases with less than 10 FB in the sputum, only one sample was examined.

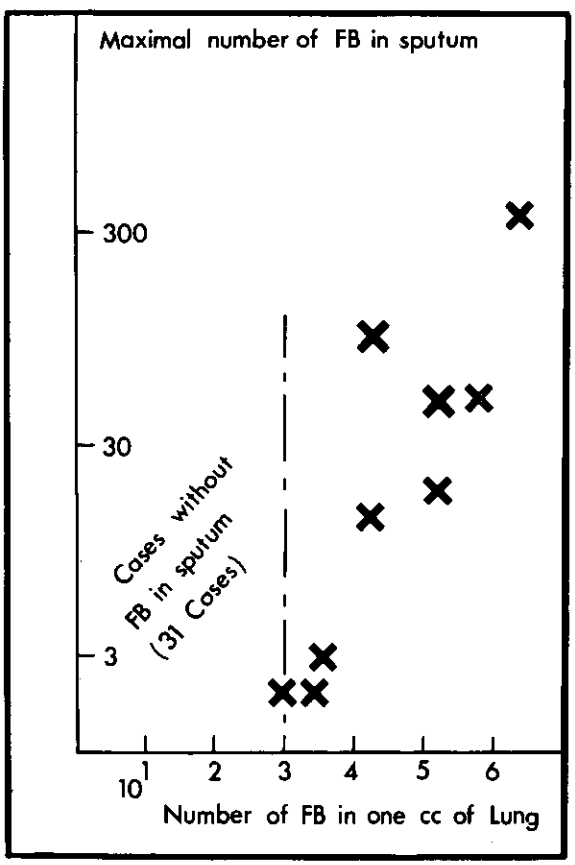

FIGURE 5. Quantitative correlation between the maximal number of ferruginous bodies (FB) in sputum and the type of occupational exposure. For every patient with FB in sputum, this number is plotted in on the logarithmic scale.

Uncoated Fibers

Uncoated fibers have been counted in only 14 cases (eight sputum examinations and six lung parenchyma examinations). In both studies (sputum and parenchyma), the ratio of uncoated fibers to all fibers, coated and uncoated, was wide, ranging from 15 to 85%. This is an apparent discrepancy with the data of Ashcroft and Heppleston *(10)*, who, using the centrifugation method, established a mean ratio of uncoated fibers to total fibers as 70%, with a low standard deviation.

In order to test the validity of the microfiltration method for counting uncoated fibers, the following study was carried out. Samples of lung were digested; following centrifugation at 1600*g* for 30 minutes, the supernatant was aspired, leaving 1–2 ml of fluid above the deposit. The sediment was resuspended by pipetting in this volume for counting in a Malassez chamber. The proportion of coated and uncoated fibers was assessed in the whole chamber. Then, remaining sediment was filtered by the usual technique (Millipore filter, pore size 3 μm) but the fluid was recovered and filtered through a Millipore filter (pore size 0.05 μm). Then the proportion of coated and uncoated fibers was determined: first on the surface of the filter 3 μm pore size, secondly in ashes of the same filter after low temperature ashing (LTA) for estimating the proportion of fibers which could have remained in the thickness of the filter, thirdly on the surface of the 0.05 μm pore size filter.

Preliminary results showed that no coated or uncoated fibers remained inside the 3 μm pore size filter membrane. Otherwise, in every case no coated fiber has been seen on the surface of the 0.05 μm pore size filter membrane, with the exception of only one case where the examination of the 0.05 μm pore size filter showed a negligible proportion (about 0.05%) of uncoated fibers lost during the filtration process through the 3 μm pore size membrane. Thus, as far as the proportion of coated and uncoated fibers is concerned, the results obtained by the microfiltration method were as accurate as those obtained by centrifugation. Otherwise, the microfiltration avoids some of the disadvantages of the centrifugation such as breaking, slitting or losing fibers *(10)*.

Number of FB in Sputum, Lung Parenchyma, and Gastric Juice

The study of gastric juice is now in progress,

and only preliminary results can be given (Table 2). It consists in sodium hypochlorite digestion of 20 ml of gastric juice and microfiltration through a millipore membrane of 3 μm pore size. The gastric juice was sucked out on ambulatory patients. Preliminary results indicate that the time from awakening of the patient to the gastric juice aspiration is a very important parameter which could explain the low correlation found till now between the number of FB in sputum and the number of FB in gastric juice. We are now improving the method by doing gastric aspiration on recumbent patients as soon as they awake up.

Elsewhere, in 38 cases, the number of ferruginous bodies per cubic centimeter of lung parenchyma has been compared to the number found in the sputum. Figure 6 shows that 1000 ferruginous bodies per cubic centimeter of lung, which should correspond to about 50000/g of dried lung tissue, represent a critical concentration value: under that concentration, in 31 cases, the sputum was always negative; on the other side, in 9 cases with a number of FB in the lung parenchyma above this threshold value, the number of ferruginous bodies in the sputum correlated well with the parenchymal concentration as shown here by these preliminary data.

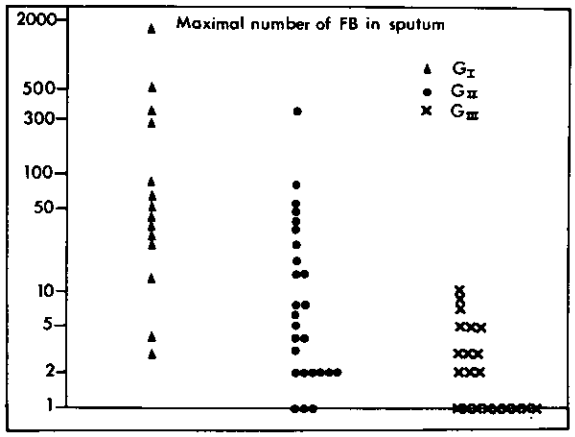

FIGURE 6. Correlation between the maximal number of ferfuginous bodies (FB) found in sputum and in lung parenchyma. Both number are plotted in a log scale. All the 31 cases without FB in sputum, not dotted on this diagram, had less than 1000 FB/cm³ of lung parenchyma.

Conclusion

The microfiltration of the sputum appears as a reliable method allowing quantitation of the level of FB and uncoated fibers actually present in the lung.

Indeed, FB are unequivocally identified on the filters and cannot be assigned to a background contamination. Otherwise, as a significant correlation exists between the number of FB and small uncoated fibers visible with the electron microscope at a ratio of about 1:1000 *(10, 11)*, the quantification of FB, much easier safer and faster, could allow an accurate enough appreciation of the number of inhaled fibers.

Table 2. Preliminary results of correlated ferruginous bodies (FB) found in sputum, gastric juice, and lung parenchyma.

Case	Sex	Age	Occupational exposure	Length of exposure, years	Cessation of exposure, years	Diagnosis	Number of FB in gastric juice, per 20 ml	Number of FB in sputum	Number of FB in the lung, per cm³
1	M	63	Brake maintenance	20	15	Pleurisy	0	4	—
2	M	48	Insulation	25	—	Pleural plaques	0	0	925
3	M	47	Insulation	2	30	Pleural plaques	4	48	135,000
4	M	72	Insulation	50	6	Pleural plaques	0	0	—
5	M	42	Asbestos spray	17	—	Lung fibrosis	0	37	—
6	M	33	Asbestos spray	2	12	Pleurisy	8	29	—
7	M	60	Asbestos spray	21	4	Lung fibrosis	2500	1850	—

Thus, the quantitative determination of FB in sputum, and perhaps also in gastric juice, could be used as an efficient laboratory screening test for the diagnosis of asbestos, particulary when occupational or environmental exposure is unknown, and as an accurate tool for the control of workers actually exposed to inhale asbestos dust.

Acknowledgement

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