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ASBESTOS FIBRES IN THE LUNGS OF CHRYSOTILE MINERS AND MILLERS—A PRELIMINARY REPORT

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Abstract—Transmission electron microscope examination of 47 autopsy lung samples from Quebec miners and millers showed that tremolite was present in approximately similar quantities to chrysotile (with crocidolite and amosite in much smaller amounts), although the quantity of tremolite compared with chrysotile in the ore worked was extremely small. These findings suggest that chrysotile was probably removed from the lungs while tremolite was retained. In spite of this removal, however, it seems that the chrysotile content of lung tissue can serve as an indicator of past exposure. The effect of the interval between exposure and death will be examined later. The design of the study does not permit evaluation of the contribution of tremolite to pulmonary fibrosis in Quebec miners and millers.

INTRODUCTION

THE USE OF the electron microscope coupled with an energy-dispersive analyser to identify and count mineral fibres in lung tissue holds great potential for extending our understanding of diseases attributable to mineral fibres. The entry of these fibres into the lung parenchyma depends on their respirability, whether they are deposited in the larger airways and excreted or whether they penetrate the small airways and alveoli and are deposited. Thereafter they may be removed by macrophages or by other means. The mineral fibre content of lung parenchyma thus depends on a sequence of processes. The behaviour of chrysotile fibres, when inhaled, appears to differ from that of the amphiboles, probably because they are curved and for this reason more easily intercepted (TIMBRELL, 1973) and subsequently excreted. Not only do chrysotile fibres apparently penetrate less well than amphibole fibres, but it is also thought that they are removed from lung tissue faster (e.g. FONDIMARE *et al.*, 1976).

Much of our present knowledge about what happens to mineral fibres after inhalation depends on whether the findings in animals under experimental conditions are also true for man when he is exposed to airborne mineral fibres in the course of his work or daily life. Few epidemiological surveys have been undertaken which incorporate both analysis of mineral fibres in lung tissue and occupational histories. Two case-control studies of malignant mesothelioma have recently been reported, one in Britain (JONES *et al.*, 1979) and one in North America (MCDONALD *et al.*, 1980). In both, the lung tissue analyses were conducted in Dr Pooley's laboratory in Cardiff by methods described by POOLEY and CLARK (1979). In both studies it was found that lungs from cases of mesothelioma contained similar quantities of chrysotile but larger quantities of crocidolite and amosite fibres than the respective control groups. The critical level of amosite or crocidolite of apparent etiological significance was one

million fibres per gram dried lung tissue; smaller quantities were found equally in cases and controls. Although the similarity in lung content of chrysotile in cases and controls suggested that chrysotile was not causally related to mesothelioma, the findings in these and similar studies cannot be interpreted confidently without knowledge of the normal pattern of retention and clearance of the various types of asbestos fibre by the lung.

An opportunity to investigate this question, particularly for chrysotile, was afforded by the continuing mortality study of a large cohort of Quebec miners and millers (MCDONALD *et al.*, 1980). Some preliminary findings of this investigation are reported here.

SUBJECTS STUDIED

A cohort comprising 11 379 persons born 1891–1920 who had worked for one calendar month or more in the chrysotile mines and mills at Thetford Mines and Asbestos, Quebec, has been studied since 1966. The mining and milling employment history of each person was known and his total cumulated dust exposure calculated in mpcf years. By the end of 1975, 4547 persons had died and of these 803 (17.6%) had had an autopsy. The autopsy rate was generally higher in recent than in the earlier deaths and in long-term than in short-term employees. Because lung tissue was required for study, the subjects were chosen of necessity from those who had had an autopsy examination. A selection of subjects was made to give a wide range of intervals between last employment and death. To avoid possible environmental exposure to chrysotile during the interval between last employment and death, only those persons who had lived more than 20 miles from the mining area after they ceased employment were chosen, unless the interval was less than 2 yr. The distribution of causes of death was similar to that of the whole cohort except that there were no accidental deaths. There were no deaths from asbestosis or mesothelioma in the series.

Samples of lung tissue from some 140 autopsies were requested from pathologists, and were obtained for 78. Failure to obtain samples was due either to material not having been kept (especially in coroner's autopsies for sudden or accidental death) or to its loss, usually in removals, fires or floods. It was not possible to standardize the part of the lung from which the study samples were taken because sampling and recording practices varied between hospitals. It has been observed that the asbestos fibre content of lung may differ by as much as a factor of two (SEBASTIEN *et al.*, 1977; Pooley, personal communication). This must be borne in mind when making inferences from the findings of this study.

TECHNICAL METHODS

The methods used for preparation and analysis of the samples of lung tissue were similar to those described by POOLEY and CLARK (1979) and differed only in the method of digestion of the organic material. Most samples were received embedded in paraffin blocks. After three sections were cut and mounted, the lung tissue was removed from the block by melting the paraffin and washing with xylene. Acetone was used to remove fats. The sample of lung tissue was dried, diced, placed in a 25 ml test tube and 15 ml of 30% v/v hydrogen peroxide solution added. After the initial reaction, the test tube was placed in a warm oven (60°C) until tissue digestion ceased, taking care to prevent a too vigorous reaction. Most samples were completely digested in 24 to 48 h, but in some,

especially those which contained embalming fluid, the organic material was not completely digested. These were given an additional treatment with 5N potassium hydroxide and were filtered through a 0.45 μm pore size cellulose acetate filter and ashed at low temperature for 8 h. Liquid was then added to make up to 25 ml and, after ultrasonication, to suspend the solid matter, it was filtered through a 0.1 μm pore size 47 mm polycarbonate filter backed by a 5 μm pore size cellulose acetate membrane. After drying, a filter was coated with carbon in a vacuum evaporation unit, and portions of it were placed on four 200 mesh gold and copper electron microscope grids. The filter was dissolved by a modified Jaffe Wick method and the material was then ready for electron microscopy. All solvents used were checked to ensure that they contained no mineral fibres.

Fibres were counted on a Philips Electron Microscope 201 at a magnification of $\times$ 20 000. Particles were classified as fibrous if they possessed an aspect ratio of 3 : 1 or greater. A minimum of 10 grid squares (maximum 50) or 100 fibres were counted and grid squares were selected at random from the four grids prepared for each sample. Microchemical analysis was performed on fibres in the samples using a JEOL 100CX–ASID4D scanning transmission electron microscope equipped with an EDAX707B energy dispersive analyser. Where possible 100 fibres were analysed and classified as chrysotile, crocidolite, amosite, tremolite, anthophyllite or not asbestos.

FINDINGS

The mineral fibre content of 47 lung samples has been analysed so far. The findings are shown in Table 1. Most remarkable is the fact that tremolite was found in approximately similar quantities to chrysotile. In contrast, few persons had more than one million fibres of crocidolite or amosite. Table 2 shows that the higher counts of chrysotile and tremolite were found in those persons who had the higher dust exposure at work. Spearman rank correlation coefficients of cumulated dust exposure with lung fibre counts were for chrysotile, 0.40 ($p=0.005$), and for tremolite, 0.49 ($p=0.0005$).

DISCUSSION

In the reaction zones around the chrysotile-containing ore of the eastern townships of Quebec, some tremolite occurs (GIBBS and LACHANCE, 1972), but the quantity is extremely small compared with chrysotile. The approximately equal quantities of

TABLE 1. MINERAL FIBRES BY TYPE AND QUANTITY IN THE LUNG TISSUE OF 47 PERSONS (45 MALE AND 2 FEMALE) EMPLOYED IN THE QUEBEC CHRYSOTILE MINING INDUSTRY

Fibres ($\times 10^6$) per g dried lung	Chrysotile	Tremolite	Amosite	Crocidolite	Non-asbestos
<1	12	18	44	43	8
$1<10$	15	6	1	–	18
$10<100$	18	18	2	3	21
100 and over	2	5	–	1	–

TABLE 2. CHRYSOTILE AND TREMOLITE FIBRES IN LUNG TISSUE ACCORDING TO TOTAL ESTIMATED DUST EXPOSURE

Total dust (mpcf yrs)	Chrysotile Fibres ($\times 10^6$) per g lung				Tremolite Fibres ($\times 10^6$) per g lung				Total
	<1	1<10	10<100	100+	<1	1<0	10<100	100+	
<10	4	4	2	–	6	2	2	–	10
10<80	6	5	4	1	8	2	6	–	16
80 and over	2	6	12	1	4	2	10	5	21
All	12	15	18	2	18	6	18	5	47

chrysotile and tremolite found in lung tissue therefore require explanation. Either chrysotile penetrates very poorly into the lungs compared with tremolite or, more probably, it disappears or is removed. The effect of the interval between last exposure and death has not yet been analysed; this has been left until all tests are completed and the occupational histories of exposure to asbestos both in mining and milling and elsewhere have been carefully checked. In addition, the estimated dust exposure will be replaced by estimated fibre exposure.

Large quantities of tremolite were also found in a series of 20 lung samples previously examined by Pooley from the same cohort (POOLEY, 1976). Of the 20, 6 were from cases in which death was certified as due to asbestosis and 14 of other causes, although slight to moderate pulmonary fibrosis was reported at autopsy. In this series there was a little less tremolite than chrysotile. In the light of the somewhat larger quantities of tremolite found in the present series which was unselected as far as asbestosis was concerned, it seems unlikely that asbestosis in the miners and millers was attributable to tremolite rather than to chrysotile.

The findings in the Quebec miners and millers may also usefully be compared with the series of controls in the North American mesothelioma study already referred to MCDONALD, 1979). The controls were patients with pulmonary metastases from a primary tumour other than lung cancer from the same hospitals as the cases of mesothelioma in the U.S.A. and Canada in 1972. They thus give some indication of the mineral fibre content of the general population in the U.S.A. and Canada. There was much less tremolite in their lungs and the chrysotile content was somewhat lower than in the miners and millers. A very high tremolite content thus appears to be confined to the Quebec mining industry. Study of the lung tissue of factory workers exposed to Quebec chrysotile would clearly be informative.

In conclusion, these preliminary findings suggest to us that chrysotile was probably removed from the lungs of Quebec miners and millers while tremolite was retained. In spite of this removal, however, it seems that the chrysotile content of lung tissue can serve as an indicator of past exposure. Neither this investigation nor the earlier study by Pooley was designed to evaluate the extent to which tremolite contributed to the pulmonary fibrosis of chrysotile miners and millers (or, indeed, to other asbestos-related diseases). This clearly deserves to be done but will require comparison of fibrotic and other changes in chrysotile workers exposed to varied quantities of tremolite.

REFERENCES

FONDIMARE, A., SEBASTIEN, R., MONCHAUX, G., BIGNON, J., DESBORDES, J. and BONNARD, G. (1976) Variations topographiques des concentrations pulmonaires et pleurales en fibres exposes. *Ann. d'Anatomie pathol., Paris* **21**, 277–284.

GIBBS, G. W. and LACHANCE, M. (1972) Dust exposure in the chrysotile mines and mills of Quebec. *Arch. Environ. Health* **24**, 189–197.

JONES, J. S. P., POOLEY, F. D., CLARK, N. J., OWEN, W. G., POLLOCK, D. J., ROBERTS, G. H., SMITH, P. G., WAGNER, J. C. and BERRY, G. (1979) The pathology and mineral content of lungs in cases of mesothelioma in the United Kingdom in 1976. *Symposium on Biological Effects of Mineral Fibres*, IARC, Lyon, 23–27 September 1979.

MCDONALD, J. C., LIDDELL, F. D. K., GIBBS, G. W., EYSSEN, G. E. and MCDONALD, A. D. (1980) Dust exposure and mortality in chrysotile mining. *Br. J. Ind. Medicine* **37**, 11–24.

POOLEY, F. D. (1976) An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. *Environ. Res.* **12**, 281–298.

POOLEY, F. D. and CLARK, N. J. (1979) A comparison in fibre dimensions between chrysotile, crocidolite and amosite particles from samples of airborne dust and post-mortem lung tissue specimens. *Symposium on the Biological Effects of Mineral Fibres*, Lyon, 23–27 September 1979.

SEBASTIEN, P., FONDIMARE, A., BIGNON, J., MONCHAUX, G., DESBORDES, J. and BONNAUD, G. (1977) Topographical distribution of asbestos fibres in human lung in relation to occupational and non-occupational exposure. *Inhaled Particles IV* (Edited by WALTON, W. H.) part 2, pp. 435–446. Pergamon Press, Oxford.

TIMBRELL, V. (1973) Physical factors as etiological mechanisms. *Biological Effects of Ashestos*, IARC Scientific Publications, No 8, pp. 295–303. WHO, Lyon.

DISCUSSION

G. BERRY: You have shown a correlation, albeit not very strong, between the chrysotile content of the lung and exposure during life. You also state that subjects were selected to give a wide range of intervals between last employment and death, but you have not yet analysed this aspect. I think this will be an important analysis in view of the scarcity of data with documented life-exposures; you are fortunate to be working with one of the few such sets of data.

J. M. DEMENT: (1) How did lifetime fibre exposures among the cases you selected for study compare with those of the rest of the cohort? (2) Was electron diffraction used to aid in fibre identification?

Dr ALISON MCDONALD: The subject group was not selected in any way as representative of the whole cohort. It was chosen to give a large range of intervals between last employment and death and of durations and intensities of exposure. As a result, there were relatively more short and low exposures than in the main study.

Dr ROWLANDS: We found that, quite often, selected area electron diffraction was useful in the identification of fibres. However, when dealing with very small single fibrils it is often difficult to form a diffraction pattern. We are working on micro-diffraction techniques with the STEM, but at present switching back and forth from the standard mode into the micro-diffraction mode creates some difficulties. I hope to modify the instrument in the near future and think this technique will be very valuable in analysing small particles by electron diffraction.

J. BIGNON: You showed that the tremolite:chrysotile ratio in the lungs was much higher than would be expected from the relative occurrence of the two minerals; you suggested that one explanation could be a more efficient removal of chrysotile. What is known about the penetration of the curly chrysotile fibre into the alveolar spaces; would alveolar penetration be different for tremolite and chrysotile?

Dr ROWLANDS: From this study it would be rather difficult to say, because the tissue was received in the form of paraffin blocks. We do not know exactly where these blocks came from in the lung, so we cannot make any comparative evaluation. We do have some wet tissue in which we hope to eventually look at the distribution within the lung.