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The Migration and Cytotoxic Effects on Macrophages of Different Forms of Asbestos Fibres in Rats and Mice

J.S. H a r i n g t o n

Cancer Research Unit of the National Cancer Association of South Africa, Johannesburg

K. K a n a z a w a ; M.S.C. B i r b e c k , R.L. C a r t e r and F.J.C. R o e

Chester Beatty Research Institute London.

This paper consists of two parts. The first concerns the migration of asbestos fibres from the site of their subcutaneous injection in mice. The second relates to the cytotoxic and haemolytic activities of various types of asbestos.

We recently reported the induction of injectionsite sarcomas, and of mesotheliomas, in mice injected subcutaneously with crocidolite, amosite or chrysotile (Roe et al, 1967). Mice

received 6 injections of 10 mg asbestos fibre into the subcutaneous tissues of the flanks. Of 71 mice examined post mortem, 7 developed sarcomas at the injection site. A more surprising finding was that over 50 % showed macroscopically-visible deposits of asbestos on the serosal surfaces of either the abdomen, or the thorax, or both. Proliferative changes were commonly seen in association with these deposits and 10 mice developed mesotheliomas- 4 peritoneal, 4 pleural, and 2, peritoneal plus pleural. All 3 types of asbestos gave rise to mesotheliomas, though chrysotile appeared less active than crocidolite or amosite. Removal of oils from crocidolite and amosite by exhaustive extraction with a series of solvents appeared to reduce, but did not abolish, the carcinogenic activity of the asbestos.

A problem posed by this experiment concerns the mechanism by which asbestos injected into the subcutaneous tissues of the flank reached the serosal and subserosal tissues, particularly those of the thorax.

In an attempt to elucidate the mechanism by which asbestos fibres reached the serosal and subserosal tissues of the flank from the sites of subcutaneous injection, we introduced 10 mg of crocidolite, suspended in 0.4 ml saline, subcutaneously into the right flank of mice and looked for evidence of migration in animals killed after various intervals up to 167 days. Conventional microscopy, Perl's staining, phase-contrast microscopy after micro-incineration, tissue maceration in potassium hydroxide, and electron microscopy were used in the search for asbestos fibres in tissues remote from the site of injection. To date we have seen evidence only of dissemination on a small scale via the lymphatic system, but the experiment is still in progress.

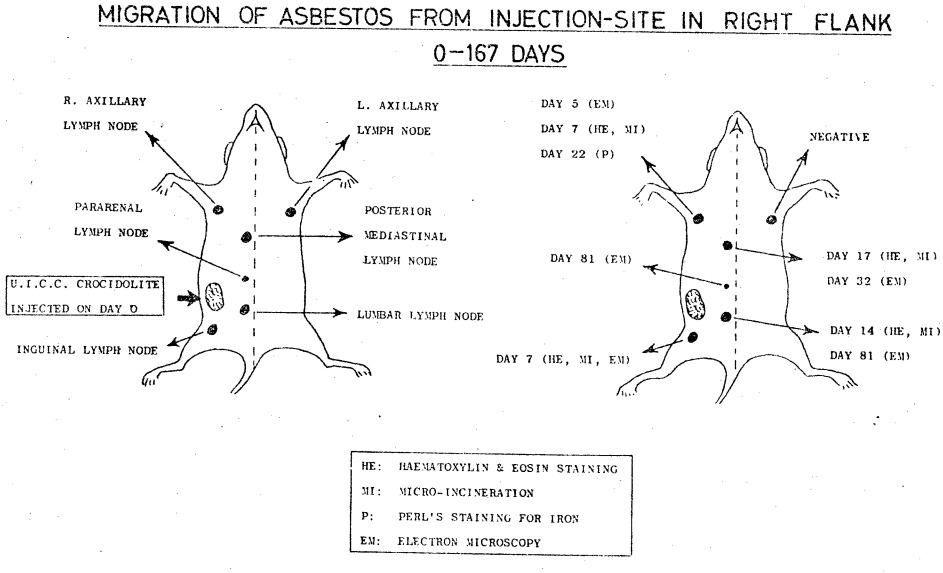

Figure 1: Migration of crocidolite fibres to various lymph nodes following a single injection of 10 mg into the right flank.

The diagram on the left of Figure 1 depicts the location of various lymph nodes in the mouse. The diagram on the right shows the dates on which crocidolite fibres were positively identified for the first time in these various nodes, and also the method of preparation by which their presence was revealed.

Within a few days of injection, phagocytes containing very small fibres were regularly found in the axillary lymph nodes. Later, larger fibres, some of them lying free in the peripheral sinuses, were found in the same node; and nodes in the mediastinal and lumbar regions began to show similar changes (Figures 2 and 3).

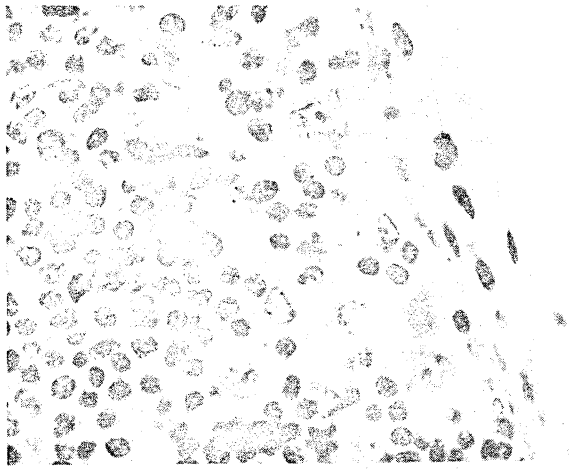

Figure 2:
Right axillary lymph node 42 days after subcutaneous injection of 10 mg crocidolite into the right flank. The subcapsular marginal sinus is packed with macrophages which are laden with asbestos fibres and hemosiderin granules.

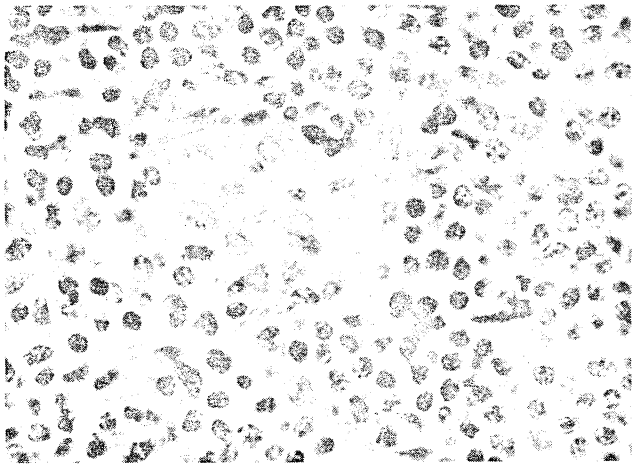

Figure 3:
The same lymph node as shown in Figure 2: an interfollicular space occupied by macrophages which have intracellular asbestos fibres. A few long asbestos fibres are seen also lying extracellularly between macrophages.

Dr. J. Davis (see p. 82 of the present Proceedings) reported the occurrence of fibres lying free in the cytoplasm of macrophages. We did not see this in our experiments: in all cases fibres were contained in phagosomes and associated with haemosiderin formation (see Figure 4).

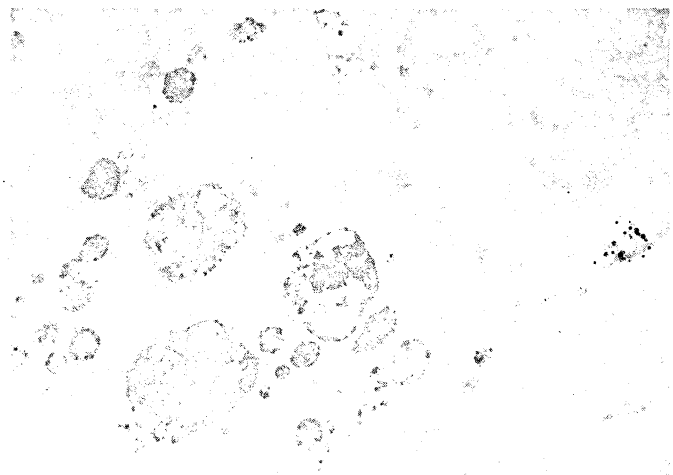

Figure 4:
Crocidolite fibres in a phagosome of a macrophage in a mediastinal lymph node of a mouse 32 days after the subcutaneous injection of asbestos.

Surgical interruption of the lymphatics between the injection site and the axilla effectively prevented the appearance of fibres in the axillary node, though migration to the lumbar

node still occurred.

In another experiment on rats, segments of large intestine were surgically isolated to form pouches, and the continuity of the remaining gut re-established. Asbestos was then introduced into the lumina of the pouches. Up to the 70th day there was no evidence of the passage of fibres through the gut wall to the serosal surface.

These more recent experiments, therefore, have not yet revealed evidence of massive migration of fibres from a subcutaneous site to serosal surfaces, and the mechanism by which this occurred in our published experiment remains to be elucidated.

The second part of this paper is possibly more relevant to the pathogenesis of asbestosis than to carcinogenesis. Earlier, we showed that silica disrupted the membranes of phagosomes of macrophages after phagocytosis and suggested that this was a primary event in the pathogenesis of silicosis (Harington and Allison, 1965). This lytic action of silica could be prevented by polyvinylpyridine N-oxide, probably because of hydrogen bond formation between the polymer and hydroxyl groups on the silica surface.

Extension of this work to asbestos - by the use of histochemical and enzymatic techniques - suggested that chrysotile (and silica) are more toxic to macrophages than crocidolite and amosite. This finding is in accord with the haemolytic activities of each of these materials.

It remains possible, however, that such results apply to certain specific experimental systems only. For example, the entire technique of macrophage culture seems to need detailed re-appraisal so that apparently "inactive" forms of asbestos, such as amosite and crocidolite, can be allowed longer access to macrophages than present techniques permit.

It is our belief that most, if not all, of the forms of asbestos we have studied are both cytotoxic and fibrogenic, and that it may be possible to inter-relate these features once longer periods of exposure of macrophages to asbestos are possible.

On the other hand, it seems true to say that not all forms of asbestos are carcinogenic. We believe that, for experimental purposes at least, the fibrogenic and the carcinogenic actions of asbestos should be clearly distinguished.

In so far as our work on haemolysis is concerned, we were very pleased to hear of Dr. Schlipköter's confirmation of this (see p. 67 of present Proceedings) which encourages us to report further developments in this field.

Our earlier finding that haemolysis by chrysotile could be prevented by the chelating agent, EDTA, and by phosphate ions, but not by polyvinylpyridine N-oxide, led us to believe that magnesium in the ionic form was the principal cationic reactant on the chrysotile surface.

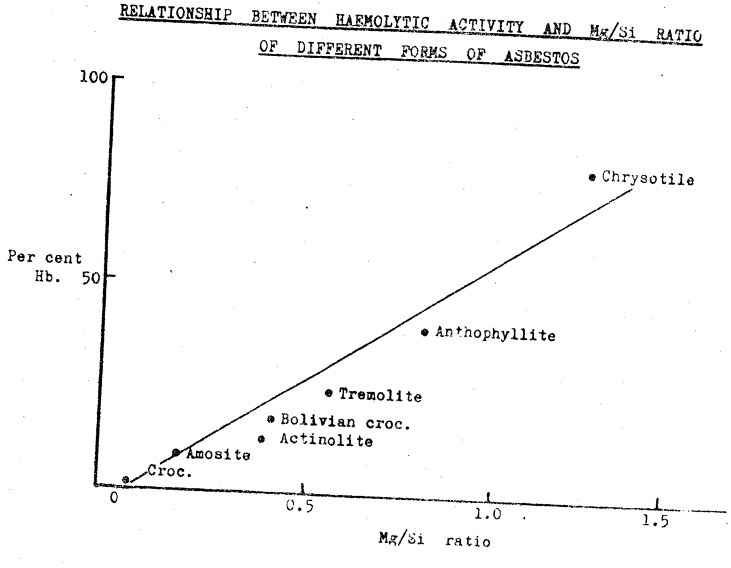

Figure 5:

Correlation between magnesium: silicon ratio and haemolytic activity.

This is supported by a correlation found to exist between the haemolytic activity on sheep cells and the magnesium:silicon ratio of each form asbestos tested (see Figure 5). The positions relative to each other of Cape crocidolite, with its 1 to 4 % magnesium and the interesting Bolivian crocidolite, with 13 % magnesium, suggest that undefined terms such as "crocidolite" may obscure differences of considerable biological importance.

The haemolytic effects of all the forms of asbestos (Figure 5) are significantly prevented by EDTA, suggesting again that metals are the main reactants. In our hands, polyvinylpyridine N-oxide had no preventive effect whatever on any of the forms of asbestos tested.

Even with the fairly clear results which have been obtained in the haemolysis work, one must be careful not to oversimplify their significance. For example, it transpires that sheep cells (conventionally used in many studies of haemolysis) are among the least susceptible to lysis by asbestos (see Figure 6.).

HAEMOLYTIC ACTIVITY OF DIFFERENT FORMS OF ASBESTOS ON DIFFERENT ERYTHROCYTES

MINERAL	Monkey	Man	Guinea Pig	Rabbit	Sheep	Rat	Frog
Chrysotile	93	87	75	73	72	65	57
Amosite	76	60	51	45	27	19	68
Crocidolite	33	23	18	43	0	19	0

Figure 6:

Haemolysis of erythrocytes from various animal species by different types of asbestos fibre. Note that amosite is highly effective against sheep red cells and those of at least four other species and that crocidolite shows marked activity both against sheep and rabbit erythrocytes.

Summary

1) We previously reported that, following their subcutaneous injection into mice, crocidolite, amosite and chrysotile induced both sarcomas at the site of injection and mesotheliomas of the pleura and peritoneum. In addition, there was evidence of selective migration of asbestos fibres to submesothelial tissues.

2) We now report that, up to 5 months after a single subcutaneous injection of crocidolite, the only evidence of migration seen was on a small scale via lymphatics.

3) It would appear at first sight from our work on the cytotoxicity of asbestos that chrysotile is more toxic to macrophages than amosite or crocidolite. However, we believe that this finding may only represent an inadequate exposure of the macrophages to the apparently "inactive" forms of asbestos.

4) The haemolytic activity of various types of asbestos against sheep erythrocytes appear to vary directly with the magnesium:silicon ratio of the asbestos.

5) The haemolytic activity of asbestos varies with the type of erythrocyte used. Sheep cells are among the least susceptible, and crocidolite and amosite - relatively inactive to the sheep cell - are markedly lytic to the erythrocyte of several other species.

References: R o e , F.J.C., Carter, R.L., Walters, M.A., and Harington, J.S. (1967) "The Pathological effects of subcutaneous injections of asbestos fibres in mice: migration of fibres to submesothelial tissues and induction of mesotheliomata." Int. J. Cancer, 2, 628 - 638. - H a r i n g t o n , J.S., and Allison, A.C. (1965). "Lysosomal enzymes in relation to the toxicity of silica". Med. Lavero, 56, 471 - 484. -