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# The use of liposomes in the investigation of mechanisms of asbestos damage

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**Abstract.** Asbestos-induced cell damage is initiated by a reaction at the plasma membrane. The effect of chrysotile (which is more hemolytic and releases more silicic acid than other types of asbestos) on permeability changes of liposomes has been investigated. The destabilizing effect rises when the amount of chrysotile is increased. Silicon dioxide is one major constituent which could be a hemolytic agent, and a cause of damage. It also caused an increase of permeability of liposomes.
**Key words.** Asbestosis; chrysotile; liposomes; silicic acid.

Asbestosis is a slowly progressive and persistent interstitial fibrosis of the lung, associated with the inhalation of asbestos dust and characterised by asbestos bodies and fibres appearing in large numbers in the tissue[1]. Asbestos describes a group of hydrated silicates that have a 'fibrous' morphology (defined as a 3:1 length to width ratio). Two main subgroups are recognised: serpentine and amphibole. Chrysotile ($3\,MgO{\cdot}2\,SiO_2{\cdot}2\,H_2O$), a serpentine asbestos which accounts for approximately 95% of the asbestos used worldwide, is a pliable, curly fiber made up of bundles of smaller fibrils[2]. These subunits are comprised of either multi- or single layers of silica and brucite (MgO) that form concentric, scroll-like tubes. In neutral aqueous media, the brucite becomes ionized and gives the fiber a positive charge. The chemistry of asbestos is complex. A number of trace metals, including Ni, Fe, Sb, Cr and Co, are associated with native chrysotile[3].

Chrysotile is acid-labile and can undergo chemical alteration in the lung. The process could account for the faster dissolution and disappearance of chrysotile from the lung in comparison to amphiboles, which remain for a longer time period. The occurence of mesothelioma (a tumor of the serosal cells lining the pleural and peritoneal cavities, which is an extremely rare cancer in the general population, but can account for as many as 1 in 30 of the malignangies found in asbestos workers) has generally been associated with exposure to crocidolite. However, experiments by Wagner[4] and Stanton[5] have shown that a variety of fine fibers including chrysotile produce mesothelioma when injected into the pleural cavities of rats. Red blood cells lyse after exposure to asbestos, and the release of hemoglobin can be quantified spectrophotometrically. Thus, hemolysis has been used as an index of membrane damage by asbestos[3]. Earlier studies correlated fiber charge with the capacity of the fibers to cause membrane damage[6]. It was shown that chrysotile adsorbs both proteins and phospholipids from red blood cell membranes[7]. These events appear to modify the hemolytic capacity of the fibers, and suggest that asbestos interacts with specific sites on the plasma membrane to induce hemolysis.

Experimental approaches were developed mainly in the past decade to try to elucidate the mechanism of asbestosis[8]. In the present study, to investigate the mechanism of asbestosis initiation, liposomes were chosen to simulate biomembranes. Liposomes are composed of various phospholipids, and long chain anions or cations can be incorporated into them to produce charges on the membrane. The diffusion of solutes from the liposomes can be measured in the same way as diffusion across a biological membrane. We have used this model to elucidate the effect of chrysotile asbestos on membranes. The technique provides a simple and rapid way of studying the effects of dusts and fibers on membranes.

## Materials and methods

**Preparation of liposomes.** Liposome suspensions were prepared as described previously[9,10]. Lecithin, dicetylphosphate, and cholesterol, in the molar ratios of 7:2:1, respectively, were dissolved in chloroform (40 ml) to yield a lipid concentration of 6.750 mM. Thin homogeneous films of lipid were obtained by drying at 30 °C for 3 hours. The films were swollen in potassium chromate solution (0.145 M), and the final suspension thus obtained was transferred to a dialysis tube (Sigma Stock No: 250–7 U, 3.3 × 31.5 cm), and dialysis was carried out for 30 min in phosphate buffer (75 ml, 0.1 M, pH 7.3). This process was repeated three times, and the total amount of chromate that leaked from the dialysis tube was determined spectrophotometrically at 371.5 nm by using a Zeiss PMQ II spectrophotometer (fig. 1).

**Release due to solid material in direct contact with liposomes.** Chrysotile was dispersed in phosphate buffer (3.0 ml, pH 7.3) by a Sonic Membrator for 5 min at the setting 40, and the suspension was transferred to a dialysis tube containing the liposome suspension

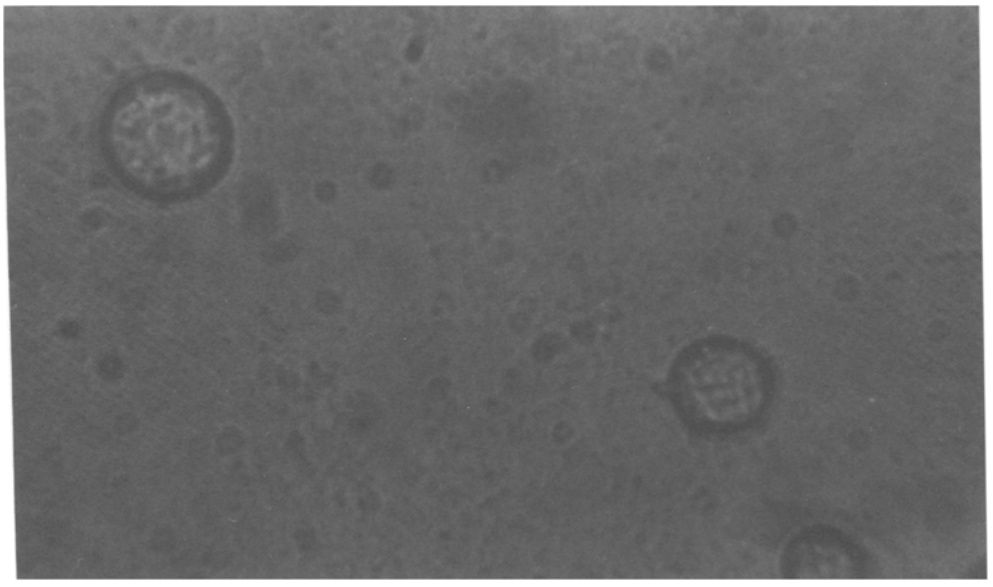

Figure 1. Polarized light microphotograph of liposomes swollen in acetone and stained with Sudan III (2000×).

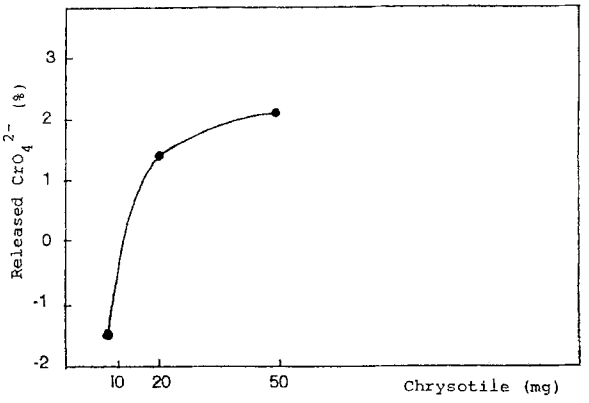


Figure 2. Effect of the amount of chrysotile on release from liposomes.

(2.0 ml). The mixture was dialysed in phosphate buffer (75 ml, pH 7.3) for 140 min at room temperature with constant stirring. Release of ions from liposomes was measured at 371.5 nm every 20 min (fig. 2). Determinations of the blanks were performed similarly, but without chrysotile.

**Determination of the amount of chromate trapped in liposomes.** The amount of chromate used for swelling was known (4.5 ml, 0.145 M). The absorbance of the swelling solution after swelling was measured at 371.5 nm. With the help of a calibration curve the amount of chromate in the solution was calculated. The difference between the amounts of chromate entrapped in and/or adhering to the liposomes was determined. After dialysis the three dialysate solutions were combined and the amount of chromate was also determined spectrophotometrically. The difference between two values gave the amount of chromate that was entrapped in liposomes. Since the amount of liposomes used for swelling was known, the amount of chromate in a unit amount of liposomes was easily calculated. This value and the release of chromate from liposomes were then used as a reference in order to calculate % chromate released.

**Release of $CrO_4^{2-}$ (%) caused by silicate solution.** Amounts of sodium metasilicate pentahydrate which were equivalents of 10, 50 and 100 mg $SiO_2$ were dissolved in phosphate buffer to yield silicate concentrations of 0.167 mM, 0.830 mM and 1.670 mM. The experiment was carried out using the apparatus and technique described above. An extra blank for the change observed in the absorbance due to the presence of silicate was also taken into account (fig. 3).

## Results and discussion

The direct effect of chrysotile on liposomes was studied by the change of permeability. When chrysotile was in direct contact with liposomes (fig. 2) it was seen that permeability of the liposomes was lower than that of the control when very low amounts of chrysotile were used. This indicates a slight stabilizing effect on liposomes. The permeability increased to values higher than that of the control with an increase in the amount of chrysotile. This indicates that a destabilizing effect appears when the amount of chrysotile is increased. When silicate was allowed to interact with liposomes, it was observed that the permeability of the liposomes increased (fig. 3). This finding provides support for an interaction between liposomes and a chemical that dissolves out of chrysotile. Direct contact shows a similar trend to that of quartz, but chrysotile causes higher destabilization[10].

Theories on the mechanism of damage of asbestos are divided into physical and chemical[11]. The hypothesis of

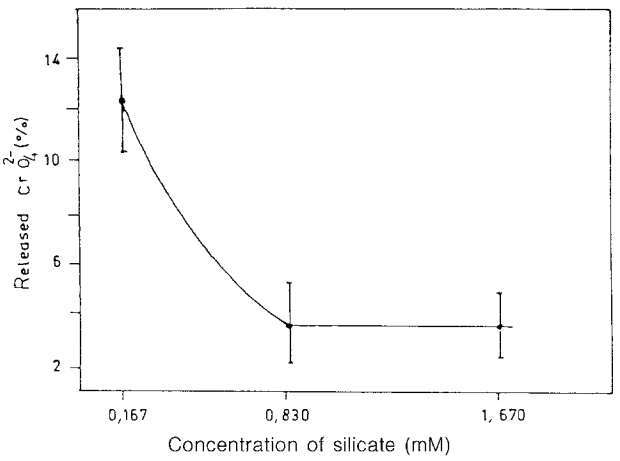


Figure 3. Relation between the release of $CrO_4^{2-}$ (%) and the amount of silicate.

physical damage suggests that mechanical irritation attributable to the embedded fibers or to the ensuing biological response may be the key factor. This idea was not viewed with favour by early investigators, but received support from the studies of Stanton and Wrench[5] who concluded that the damage was primarily related to the structural shape of fibers rather than to their chemical composition. However, there are experiments pointing to the importance of physicochemical factors in causing toxic effects[12]. For certain interactions, 3-dimensional characteristics become important in determining cellular responses such as phagocytosis; however, the degree of reactivity is also dependent on the charge on fiber, a property that could be altered after a period of time in the respiratory tract[8]. It is thought that longer, thinner fibers could be more carcinogenic in the induction of mesothelioma, and more cytotoxic to a variety of cells, but there is no clear relationship between fiber size, cytotoxicity and carcinogenicity[8]. It is evident that various cell types respond differently to asbestos. However, surface charge still appears to be an important determinant of both hemolytic and hypersecretory activity.

According to the chemical hypothesis, a principle inherent either in the silicate core of asbestos fibers or in some other factors derived from them is the ultimate damaging entity. Thus far, factors other than silicate have received the most attention. Harington suggested that metals present in asbestos (e.g. iron, aluminum, magnesium, chromium, nickel) may have played an important role in the adsorptive capacity of fibers[3]. There was a widespread belief that divalent magnesium ions are the cause of asbestosis[13–16]; the damage could be due to organized magnesium groups on the surface of the fibers. It is also thought that asbestos reacts at the tissue-air interface with epithelial cells and macrophages, and the hemolytic ability of asbestos has been used as a measure of its interaction with the membranes and its ability to cause asbestosis[17]. It is reported that chrysotile has the highest Mg/Si ratio of the different forms of asbestos[15] and is also the most potent hemolytic form[18]. However, magnesium hydroxide or chloride do not hemolyze erythrocytes[17], which seems to contradict the idea that magnesium ions are the cause of asbestosis. Since silicon dioxide is the other major constituent, it could be the hemolytic agent. The amount of silicic acid released by chrysotile is at least twice as much as is released by other asbestos samples, which gives support to the idea[19].

Our demonstration of an interaction between liposomes and the chemical that dissolves from chrysotile shows a similar trend to that of the earlier evidence that showed a relationship between hemolysis and the solubility of silicic acid from asbestos. The ability of chrysotile to release silicic acid could be of importance for understanding the cause of asbestosis.

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