

Liposomes in Silicosis Investigations

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The effects of quartz and sodium metasilicate on liposomes were studied in order to understand the mechanism of silicosis. 8-Hydroxyquinoline-5-sulfonic acid was tested for its *in situ* silicosis-prevention capacity. Two types of liposomes—(A) those incorporating cholesterol and (B) those without cholesterol—were used. The tests consisted of measuring permeability changes caused by the above-mentioned chemicals. Permeabilities were found to depend on membrane composition. Tests on quartz action led us to the conclusion that liposomes of this composition did not simulate the erythrocytes very well. It was also observed that absence or presence of cholesterol and the mode of contact altered the effect of quartz. Silicate destabilized type A liposomes, but this was less than that caused by quartz. This was explained by the concentration of monosilicic acid that dissolves out from quartz and silicate. When quartz was pretreated with the preventive, the type A liposomes were stabilized, but a slight destabilizing effect was observed on type B. 8-Hydroxyquinoline-5-sulfonic acid augmented the destabilizing effect of silicate, whereas it decreased the hemolytic activity of uncoated quartz, indicating a preventive potential in *in vivo*.

Introduction

It has long been recognized that silica dust, when inhaled, is fibrogenic in man and experimental animals and that it can cause silicosis.

It is not known how fibrosis is initiated. Theories on the mechanism of silicosis formation include: adsorption of foreign ions or molecules onto the surfaces of particles (1, 2a, 3), damage caused by hard and sharp surfaces (1), progressive injury by the charge of the disrupted surface silica molecules (Piezoelectric Theory) (4), and a solubility theory which states that the damage is caused by monosilicic acid liberated by the dissolution of silica (5).

Although the *in vivo* evidence is scanty, *in vitro* and *in situ* experiments produced results implying that silicic acid might be the toxic compound. Even if silicic acid is accepted as the toxic species, its state that causes the damage is not certain. There are reports about colloidal silica (2b) polymeric silicic acid (6) or monosilicic acid (7) being the cytotoxic species.

Another aspect which needs clarification is the site of action of dust damage. There are reports (7) on lysosomal membranes being the actual target for silica.

As a conclusion, it is now believed that (8) inhaled silica particles are phagocytosed by alveolar macro-

phages and soon become incorporated into secondary lysosomes via the phagosomes. The toxic properties of the dusts are related to their capacity to damage lysosomal membranes through hydrogen bonding interaction. And, upon lysis of the lysosomal membrane, lysosomal enzymes cause lysis of the cell leading after a number of steps to silicosis.

It has been found that a number of substances reduce the toxic effects of quartz. Coating quartz with aluminum oxide reduces the lytic effect (7). Other efficient protective compounds are poly(2-vinylpyridine 1-oxide), PVNO (9-11), poly(1-vinylpyrrolidone), PVP (12), and various *N*-oxide-carrying polymers (13).

The mechanism of protection by PVNO is not clear. It is known that this chemical possesses $N \rightarrow O$ groups and readily forms hydrogen bonds with silicic acid (14,15).

In this study, we aim to investigate the mechanism of silicosis initiation and the finding of a new silicosis preventative. Liposomes were chosen to simulate biomembranes. (Fig. 1).

They are composed of phospholipids, and various long-chain anions or cations can be incorporated into the liposomes to create negative or positive charges on the membrane. The diffusion of solutes from the liposomes can be measured in the same way as diffusion across biological membranes.

Since it was suggested that a kind of chelating activity is required for silicosis prevention (15), a non-toxic chelating agent, 8-hydroxyquinoline-5-sulfonic acid, was chosen as the potential preventative.

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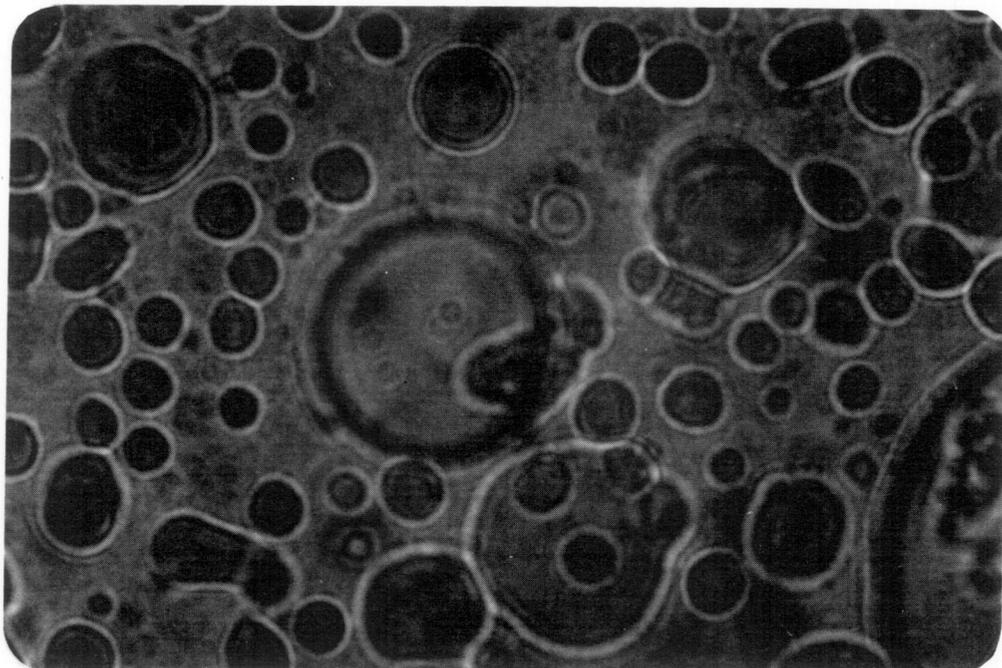


FIGURE 1. Light microphotograph of liposomes swollen in acetone and stained with Sudan III. (2000 \times).

Materials and Methods

Preparation of Liposomes

Liposomes of varying lipid composition were prepared according to the method of Weissmann and Rita (16). Their composition was (type A) lecithin, dicetyl phosphate and cholesterol in molar ratios of 7:2:1; and type B, lecithin and dicetyl phosphate in molar ratio of 7:2.

The above-mentioned chemicals were dissolved in chloroform (40 mL) to yield a lipid concentration of 6.750 mM and dried at 30°C for 3 hr. Thin homogeneous films of lipid were obtained. The films were swollen in potassium chromate solution (0.145 M), and the final suspension contained 15 μ mole of lipid/mL. The suspension was transferred to a dialysis tube (Sigma Stock No: 250-7 U, 3.3 \times 31.5 cm), and dialysis was carried out for 30 min in phosphate buffer (75 mL, disodium and monopotassium type, 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.

Release of Chromate from Liposomes

Release with Solid Material in Direct Contact with Liposomes. Quartz powder (99% pure) was dis-

persed in phosphate buffer (3.0 mL, pH 7.3) by a Sonic Membrator for 5 min at a setting of 40. The suspension was added to a dialysis tube containing the liposome suspension (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 were measured at 371.5 nm at 20-min intervals. Determinations of the blanks were performed similarly, but without quartz.

Release with Solid Material Not in Direct Contact with Liposomes. The experiment was carried out by using the apparatus and technique detailed above, with the exception that in the present case quartz suspension was added to the dialysate and not to the dialysis tube. An extra blank for the change observed in the absorbance due to the presence of colloidal silica was also used.

Release in the Presence of Silicate. Sodium metasilicate was dissolved in phosphate buffer (75 mL, pH 7.3) and the experiment was carried out with the apparatus and technique detailed above.

Treatment of Quartz with 8-HQ-5-SA

For every milligram of quartz, 0.2 mL of 0.017 M 8-HQ-5-SA solution was added to an Erlenmeyer flask containing quartz. The mixture was shaken at the rate of 90 strokes/min for 6 hr at room temperature. The solution was removed, and quartz was dried overnight at 50°C.

Table 1. Relation between the type of liposome and the amount of chromate ion trapped and released.

Liposome	Total chromate ions trapped $\times 10^5$ mole/2 ml	Chromate ions released after 140 min, % of total trapped	Chromate ions released after 140 min $\times 10^5$ mole
Type A	6.54 ± 1.68	28.99 ± 3.38	1.86 ± 0.34
Type B	4.10 ± 0.81	48.39 ± 9.98	1.98 ± 0.58

Hemolysis Test

In this experiment, quartz was added to phosphate buffer (4 mL, pH 7.3), and the mixture was sonicated for 1 min at a setting of 40. Sheep erythrocyte solution (4 mL, 3%) was added and incubated for 2 hr at 37°C. The sample was centrifuged (10 min, 2000 rpm) and the amount of released hemoglobin in the supernatant was determined spectrophotometrically at 541.5 nm. A blank was prepared under the same conditions but without quartz.

Results and Discussion

In Table 1 the amount of chromate ions trapped in and released from type A and type B liposomes is shown. Type A liposomes entrapped about 60% more chromate than type B.

When the amounts of chromate ions released from liposomes are compared, it is observed that a lower percent of ions trapped is released from type A than from type B liposomes. It seems that the retention and release of ions by the liposomes depends on the composition of lipid mixture used in liposome preparation; thus the presence of cholesterol facilitates intake but retards release of chromate. When quartz was not in direct contact with liposomes (type A), it was observed that the permeability of the liposomes increased (Fig. 2), whereas when quartz was in direct contact, permeability was decreased (Fig. 3), so that it was lower than that of the control. Quartz cannot pass through the dialysis

bag; therefore, the differences between direct and indirect contact of quartz could be explained by the effect of a chemical that dissolves from quartz. Monosilicic acid is the only solute obtained from the dissolution of quartz, and the indirect effect caused by quartz must therefore be due to monosilicic acid.

Production of decreased permeability by quartz (in direct contact) indicates that increase in the permeability of the liposomes (which might be considered damage) is not caused by direct contact of quartz. This invalidates the "mechanical damage" and "damage due to adsorption on the solid" theories and supports the solubility theory.

When silicate was allowed to interact with liposomes, it was observed that permeability of the liposomes increased (Fig. 4). Increased permeability indicates a destabilizing effect on liposomes. Thus, this finding provides further support for the interaction between liposome and a chemical that dissolves from quartz. Figure 4 also shows that the destabilizing effect of silicate decreased as its concentration increased. High concentrations of silicate might cause an increase in the extent and degree of polymerization of monosilicic acid or silicates (2c), leading to a reduction in the amount of silicate passing through the dialysis membrane.

Quartz did not have any hemolytic activity when its amount was low, but increasing the amount caused increased hemolytic activity (Fig. 5). This was not in agreement with our quartz-liposome results (Fig. 3) and this might be due to the differ-

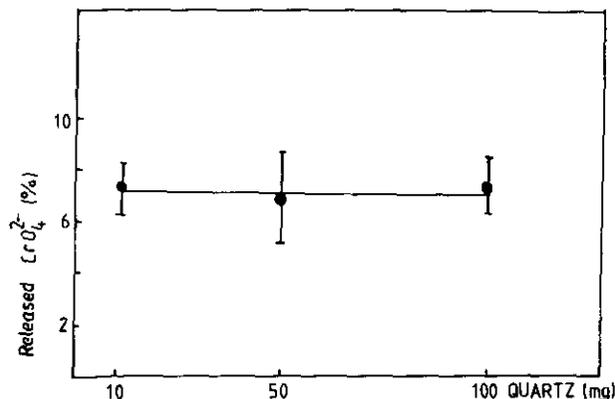


FIGURE 2. Release of chromate from liposomes by the action of quartz when there is no direct contact.

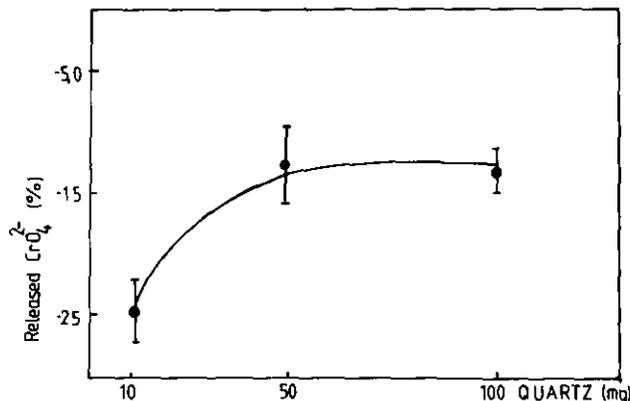


FIGURE 3. Effect of quartz on release from liposomes when there is direct contact.

ences in membrane compositions of erythrocytes and liposomes.

Results obtained with 8-hydroxyquinoline-5-sulfonic acid indicates that when it acts alone (17) or with silicate on type A liposomes (Fig. 6) it is toxic. However, when it is used for coating quartz, the function is dependent on the type of liposome used. When type A liposome is the target, it has a stabilizing action and when type B liposome is used it is destabilizing (Fig. 7). In both cases quartz is not in direct contact with liposome. One can then conclude that in both cases the release of silicic acid is prevented by the coat. The only other way of influencing membrane permeability is by osmotic pressure. It seems that the presence of a compound with ionic groups (and unable to pass the barrier) causes the organized membrane to be further stabilized, whereas it has the reverse effect on the type B (and more fluid) membrane.

The effect of 8-hydroxyquinoline-5-sulfonic acid on erythrocytes when used simultaneously with un-

coated quartz was to stabilize it (Fig. 5). It is found that quartz and 8-hydroxyquinoline-5-sulfonic acid do not interact (Fig. 8).

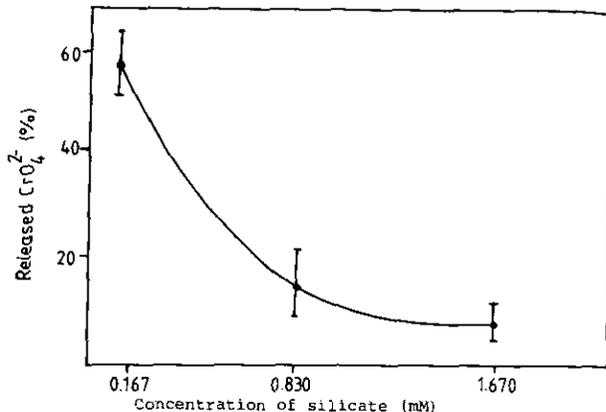


FIGURE 6. Relation between the release of CrO_4^{2-} (%) and the amount of silicate in the presence of 8-HQ-5-SA (0.08 μmole of 8-HQ-5-SA for every mg of SiO_2 in silicate).

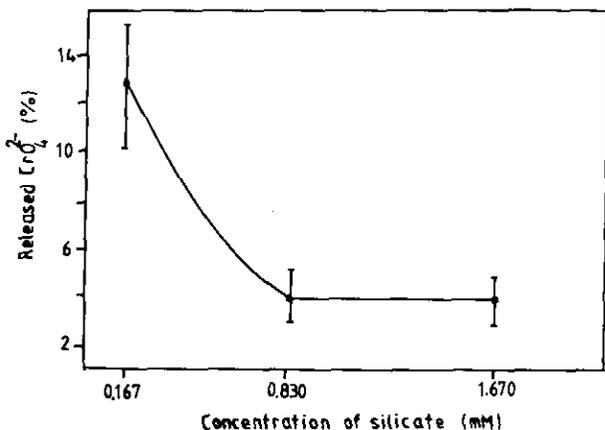


FIGURE 4. Relation between the release of CrO_4^{2-} (%) and the amount of silicate.

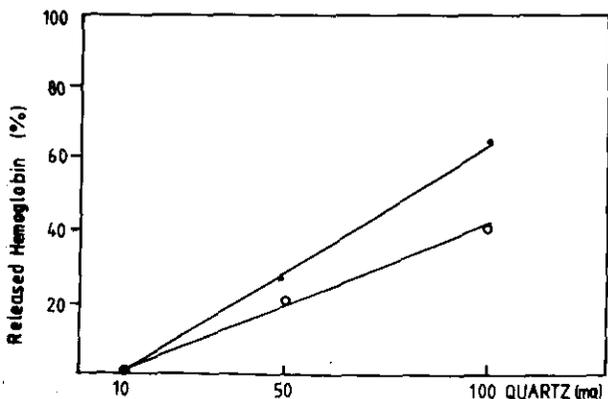


FIGURE 5. Effect of quartz and quartz 8-HQ-5-SA on hemolysis of sheep erythrocytes: (●) quartz, (○) quartz and 0.080 μmole 8-HQ-5-SA (with no pretreatment).

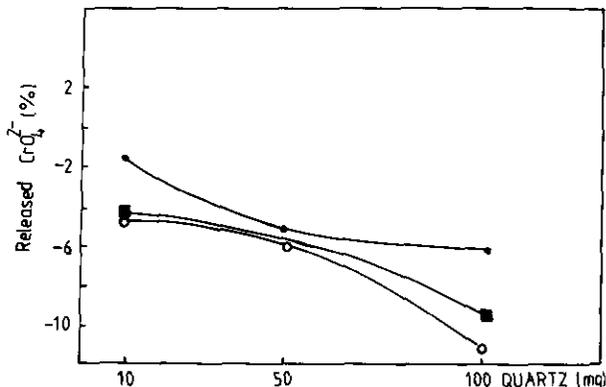


FIGURE 7. Effect of 8-HQ-5-SA on the release of chromate ions from liposomes: (●) quartz pretreated with 8-HQ-5-SA on type A liposomes; (■) quartz pretreated with 8-HQ-5-SA on type B liposomes; (○) quartz on type B liposomes.

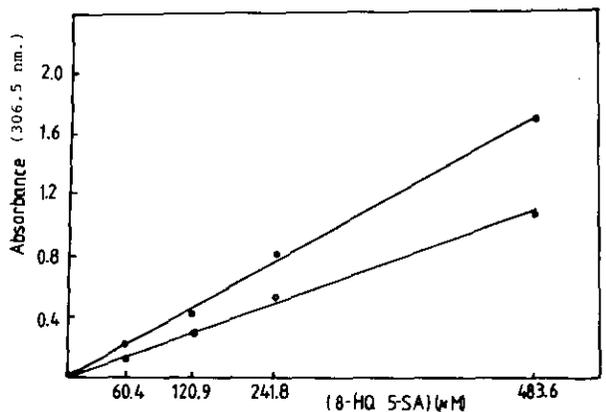


FIGURE 8. Interaction of 8-HQ-5-SA with quartz (100 mg) in phosphate buffer: (●) 8-HQ-5-SA; (○) 8-HQ-5-SA and quartz.

Although there is a decrease in the absorbance when 8-HQ-5-SA and quartz are put together, the λ_{max} did not change, and the decrease was 37% for all 8-HQ-5-SA concentrations, indicating that the decrease in absorbance is not due to the interaction.

Thus it can be deduced that 8-hydroxyquinoline-5-sulfonic acid has a stabilizing effect on erythrocyte membranes. 8-Hydroxyquinoline-5-sulfonic acid might bind to the membrane without altering permeability and cause less membrane area to be available for interaction with quartz. In this way a preventive effect can be obtained.

Conclusion

The potential preventive 8-hydroxyquinoline-5-sulfonic acid proved to be a silicosis retarder with erythrocytes, whereas a toxic effect was observed on liposomes. One can then conclude that 8-hydroxyquinoline-5-sulfonic acid might prove to have a protective effect in living systems. Further *in vitro* and *in vivo* investigations are necessary.

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