# THE INJURIOUS EFFECT OF QUARTZ ON CELL MEMBRANES AND THE PREVENTIVE EFFECT OF ALUMINIUM CITRATE AGAINST QUARTZ

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## ABSTRACT

The injurious effect of quartz on the membranes of macrophages as well as erythrocytes and the anti-injurious effect of aluminium citrate (Al citrate) were examined. The comparative study with titanium dioxide was carried out simutaneously. The results from the present study show that quartz can cause the increases of fluidity and permeability of macrophage membranes and reduce membrane-bound water of erythrocytes, resulting in the membrane dehydration. Furthermore, quartz can change electrophoretic behavour of macrophages by increasing the negative charge density and electrokinetic potential on these cells surface. The effect of titanium dioxide on cell membranes however is very different from quartz in intensity and kinetics, and is not affected by Al citrate. The relationship between these effects was discussed and a possible mechanism was proposed for the interaction of quartz with membrane lipids resulting in membrane damage.

The preventive effect of Al citrate against membrane damage by quartz was also demonstrated. In general, the addition of Al citrate can recover all alternations caused by quartz, so that the stability and order structure of cell membranes were maintained. A hypothesis about the action of Al citrate on the surface of quartz particles to exert its anti-injurious effect was postulated in this paper.

## INTRODUCTION

It is generally accepted that the cytotoxic effect of quartz on alveolar macrophages is a key step in the pathogenesis of silicosis.[1-2] The cytotoxic mechanism postulated by Allison depends on mainly the release of hydrolytic enzymes from lysosomes after the phagocytosis of quartz by macrophages followed by cell damage.[3] However, a question that remains unanswered is whether the toxic particles directly damage the plasmic membranes of macrophages. It is well known that the contact of quartz with the cell membranes is the first event during the process of phagocytosis, no matter how the particles are uptaken into the interior of these cells. For this reason, it is desirable to elucidate the molecular interactions between quartz and cell membranes from the viewpoint of membrane toxicology.

The therapeutic effects of Al citrate on the experimental animal and patients with silicosis have been demonstrated in our previous experimental studies and clinical observations. It was also found that Al citrate is able to prevent effectively macrophages from the cytotoxicity of quartz instead of the inhibition of fibrosis.[4-6] It is, therefore, necessary to clarify its pharmacology with the goal being to provide the experimental and theoretical evidence for screening the preventive measurments and therapeutic drugs for silicosis.

On the other hand, titanium dioxide, a less toxic and usually classified as "inert dust,"[7] was also studied in this work for comparsion.

## MATERIALS AND METHODS

Macrophages were harvested from lung of guinea pig through lavage. The erythrocyte membranes of rabbit were prepared as described elsewhere.[8]

Quartz (99% pure) was supplied by Hygiene Institute of Chinese Prophylatic Medical Center. Particles diameter is less than 5 $\mu$m, among which 89.3% is less than 2 $\mu$m. Titanium dioxide with the same purity and size was selected as a control. Al citrate with Al of 9.26% was supplied by Pharmaceutical Factory of Beijing Medical University. Fluorescence probe, 1,6-diphenyl-1,3,5-hextriene (DPH) was purchased from Sigma. Adenosine 5'-triphosphate disodium salt (ATP) was produced by Shanghai Biochemical Institute of Academia Sinica.

Fluorescence polarization was determined by spectrophotofluometer Model MPF-4. Potassium (K+) content of cells was detected by Fire Atomic Absorption Spectrophotometer Model Y-3.[9] $Na^+$-$K^+$-ATPase activity was determined using the method described by Pan H.Z.[10] Viscosity of medium and surface charge of cells were measured by viscosimeter Model E and Cell Electrophoresis Autotimer Model SX-2, respectively.[11] Membrane-bound water was determined employing the method of sorption isotherms and Nicolet Fourier Transform Infrared Spectrometer Model 5DX.[8-12]

There were on the average five samples in each group. Data

were presented as mean + standard error and significance was estimated by analysis of variance. Pairing data about fluidity and permeability were treated by linear correlation and regression.

## RESULTS

### Cell Membrane Lipid Fluidity

We began with the examination of membrane fluidity of macrophages by measuring fluorescence polarization P and microviscosity of membrane-bound DPH. As shown in Figure 2, P values of quartz I and II groups dropped down continuously with the cultural time, resulting in more fluid membranes. It is important that the effect of quartz on membrane fluidity is not only time-dependent, but also dose-dependent (Figure 1). However, the change of membrane fluidity by titanium dioxide is much lower than that of quartz group and tends to be recovered rapidly (Figure 1).

Compared with quartz control, fluidity was decreased (e.g. P value raised) when quartz plus Al citrate was added into the cells simutaneously, although Al citrate did not affect membrane fluidity alone. Similarly, the effect of Al citrate against quartz is dose-dependent (Figure 1).

### Permeability of Cell Membrane to $K^+$

Table I presents the differences between the groups treated by several ways in membrane fluidity and permeability to $K^+$. It is interesting that the increased pemeability of macrophage membranes to $K^+$, that is, $K^+$ content of the cells was reduced, by quartz was accompanied with increasing membrane fluidity. Statistic analysis indicates the effects of quartz on both these properties of macrophage membranes exhibit very siginificant correlation (for instance, using $\eta$ and $K^+$ as X and Y, respectively, $r=0.917$, $P< 0.001$, $Y=9.059X-0.011$) (Figure 3).

Like that on fluidity, Al citrate did not influence permeability of macrophage membranes to $K^+$ by itself, but it prevented acting efficiently against the effect of quartz, except that macrophages were pretreated with Al citrate (Table I).

It is seen from Table I that membrane permeability of titanium dioxide group was lowered only slightly and it seems that no exact relationship exists between the changes of fluidity and permeability. Another important difference from quartz is that the effects of titanium dioxide are unable to be affected by Al citrate.

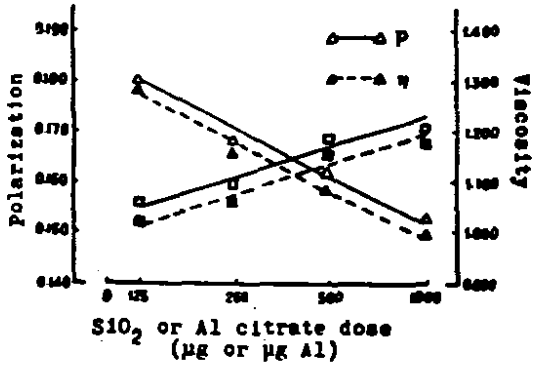


Figure 1. Dose-effect relationships of the effect of $SiO_2$ on fluorescence polarization (P) of macrophage membrane-bound DPH and lipid viscosity ($\eta$) and the antagonistic effect of Al citrate against $SiO_2$.

Δ——Δ $SiO_2$: □——□ $SiO_2$ (1 mg) +Al citrate
▲——▲ ■——■

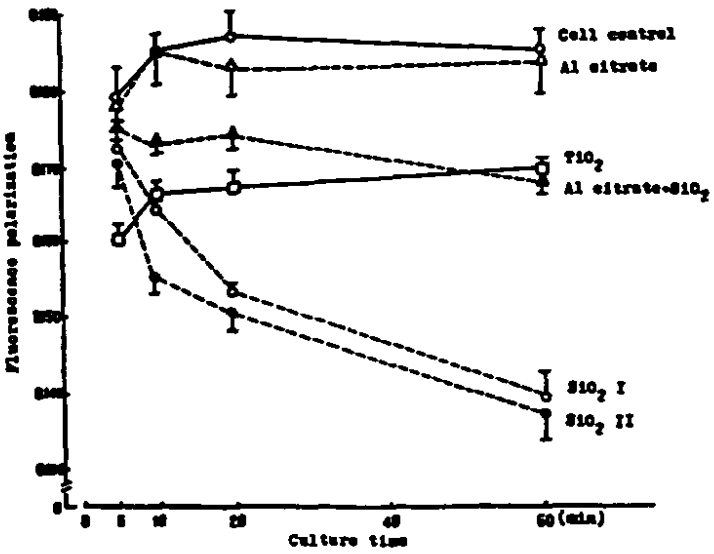


Figure 2. Kinetic curve of DPH fluorescence polarization labelled in macrophage membrane

$SiO_2$I: the simultaneous addition of DPH and $SiO_2$ to cell medium

$SiO_2$II: the addition of $SiO_2$ to cell medium followed by the addition of DPH

The dose of $SiO_2$ or $TiO_2$ was 1 mg; the dose of Al citrate was 0.5 mg Al.

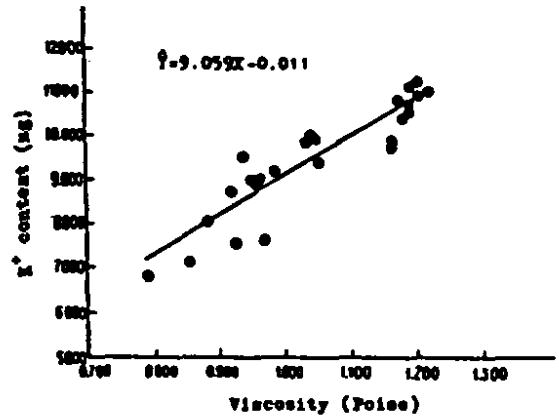


Figure 3. Scatter diagram of membrane lipid viscosity with $K^+$ concentration of macrophages administrated with $SiO_2$.

It may be involved in $K^+$ permeability, however, no change in the activity of $Na^+$-$K^+$-ATPase could be found after the treatment of three cell preparations with quartz (Table II), indicating that the increased permeability is related closely to the change in lipid fluidity.

## Membrane-bound Water

Membrane hydration of quartz group at the defferent ralative humidities (RH), particularly at higher RH, was reduced markedly from sorption isotherms curve (Figure 4) and data listed in Table III. In IR spectra, VOH shifts largely to lower frequency (Figure 5) and the results represented in Figures 5 and 6 are identical, for instance, at 76% RH, the hydration and VOH peak position in both of control and quartz groups are 18.8% and 3535 $cm^{-1}$, and 10.1% and 3447 $cm^{-1}$, respectively. It is clear that the dehydration of cell membranes was caused by quartz and has a significant does effect relationship (Table IV).

Whereas membrane hydration in either quartz plus Al citrate group or the pretreated quartz group with Al citrate is higher than quartz control (Table III) and their VOH peak position shifts towards the higher frequency (Figures 5, 6). The effect of Al citrate against quartz exists also a dose-effect relationship (Table IV). Membrane-bound water under the treatment by the different ways is presented in Figure 7. The similar results are found from two quartz groups pretreated with Al citrate and $AlCl_3$. However, the effect of titanium dioxide on membrane-bound water is not only lower than quartz, but also was not recovered by the pretreatment of Al citrate (Figure 7).

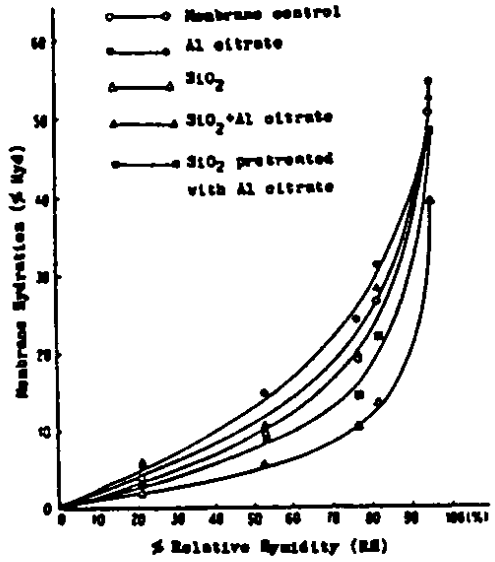


Figure 4. Sorption isotherm of water on red blood cell membranes treated by different ways at 20°C.

20mg $SiO_2$: 8.333 mg Al(Al citrate)

Table I

Polarization (P) of Membrane-bound DPH and Its Lipid Microviscosity ($\eta$) with $K^+$ Content of Macrophages

| Groups | time (min) | P $\bar{X}\pm SE$ | $\eta$ $\bar{X}\pm SE$ | $K^+$ $\bar{X}\pm SE$ |
|---|---|---|---|---|
| Control | 20 | 0.185±0.003 | 1.352±0.042 | 11.557±0.149 |
| | 60 | 0.184±0.002 | 1.329±0.018 | 11.533±0.099 |
| $SiO_2$ | 20 | 0.152±0.002 | 0.984±0.019 | 9.239±0.192 |
| | 60 | 0.141±0.003 | 0.885±0.031 | 7.431±0.205 |
| $SiO_2$+Al citrate | 20 | 0.171±0.001 | 1.183±0.007 | 10.612±0.227 |
| $SiO_2$ pretreated with Al citrate | 20 | 0.172±0.001 | 1.195±0.010 | 10.694±0.254 |
| Cell pretreated with Al citrate | 20 | 0.185±0.004 | 1.350±0.043 | 11.608±0.181 |
| Cell pretreated with Al citrate+$SiO_2$ | 20 | 0.154±0.003 | 1.000±0.029 | 9.498±0.205 |
| $TiO_2$ | 20 | 0.164±0.002 | 1.104±0.019 | 10.616±0.264 |
| | 60 | 0.163±0.002 | 1.096±0.025 | 9.384±0.231 |
| $TiO_2$+Al citrate | 20 | 0.164±0.002 | 1.111±0.022 | 10.549±0.258 |
| $TiO_2$ pretreated with Al citrate | 20 | 0.163±0.001 | 1.100±0.015 | 10.647±0.279 |

$K^+$($\mu g/2x10^6$ cell); 1mg $SiO_2$ or $TiO_2$; 0.5mg Al

Table II

$Na^+$-$K^+$-ATPase Activities ($\mu$M Pi/mg protein) of Three Cell Preparations

| Groups | adhesion cell | suspension cell | cell homogenate |
|---|---|---|---|
| | $\bar{X}\pm SE$ | $\bar{X}\pm SE$ | $\bar{X}\pm SE$ |
| Control | 0.672±0.099 | 0.806±0.144 | 1.156±0.190 |
| $SiO_2$ | 0.646±0.113 | 0.794±0.142 | 1.099±0.232 |
| Al citrate | 0.616±0.099 | 0.799±0.137 | 1.136±0.057 |
| $SiO_2$+Al citrate | 0.666±0.119 | 0.794±0.128 | 1.162±0.168 |
| $TiO_2$ | 0.627±0.095 | 0.862±0.192 | 1.098±0.187 |

The doses of $SiO_2$ and Al citrate were 300μg and 125μg Al respectively; the enzymatic activities were determined at 1 hr. of culture.

Table III

Hydration of Erythrocyte Membranes of Several Groups at the Different Relative Humidity (RH)

| Groups | Hydration(%) | | | | |
|---|---|---|---|---|---|
| | 95%RH | 81%RH | 76%RH | 52%RH | 20%RH |
| Control | 50.2 | 26.1 | 18.8 | 9.2 | 4.0 |
| $SiO_2$ | 39.1 | 13.3 | 10.1 | 5.8 | 2.3 |
| Al citrate | 54.1 | 30.9 | 23.8 | 14.7 | 5.7 |
| $SiO_2$+Al citrate | 52.0 | 28.1 | 19.4 | 10.6 | 5.8 |
| $SiO_2$ pretreated with Al citrate | 47.5 | 21.6 | 14.3 | 9.1 | 3.2 |

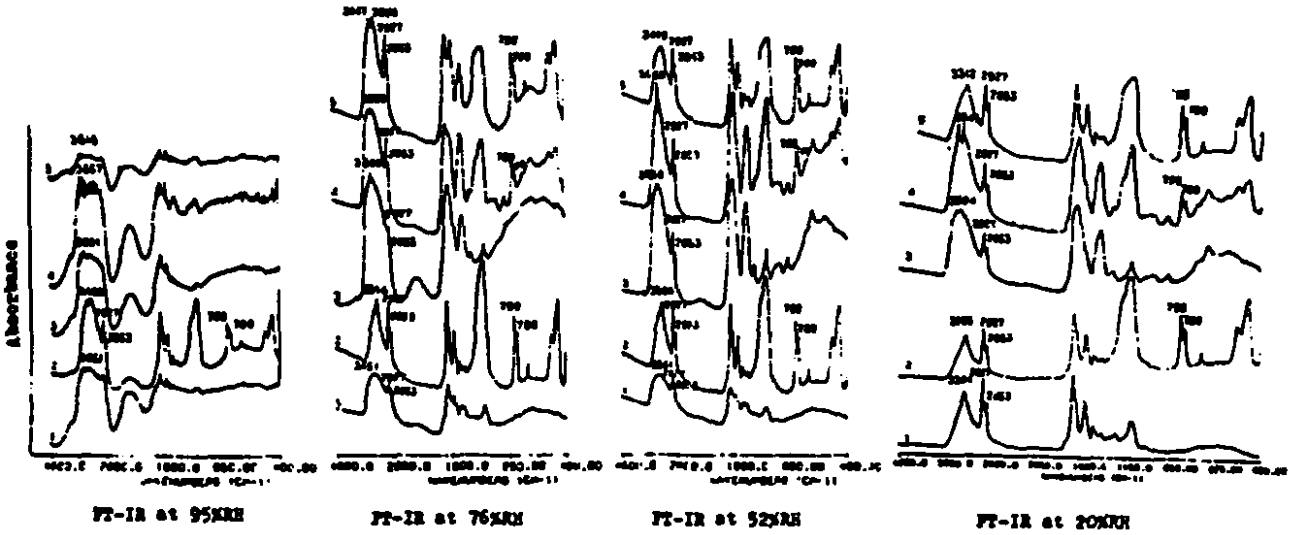


Figure 5.

1. Membrane control
2. $SiO_2$
3. Al citrate
4. Al citrate + $SiO_2$
5. $SiO_2$ pretreated with Al citrate
2. Omg $SiO_2$: 0.833 mg Al

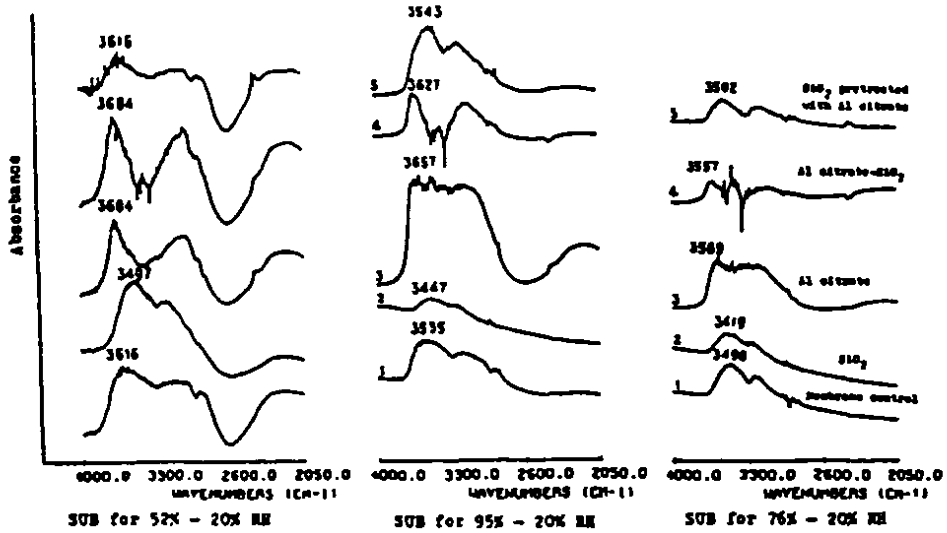

Figure 6.

Table IV

The Effects of Different Doses of $SiO_2$ and Al Citrate on Membrane-bound Water

| $SiO_2$(mg) | $\nu_{OH}(cm^{-1})$ | Al(mg) | $\nu_{OH}(cm^{-1})$ | |
|---|---|---|---|---|
| 0.5 | 3395 | 0.417 | 3419 | |
| 1.0 | 3364 | 0.833 | 3558 | 3460 |
| 2.0 | 3314 | *1.668 | 3607 | 3493 |
| 3.0 | 3288 | | | |

* $SiO_2$(2.0mg)+Al citrate

## Cell Membrane Charge

As shown in Figure 8, electrophoretic mobility of macrophages sped up rapidly following the addition of quartz. The result indicates that the interaction of quartz with macrophage surface causes increasing negative electrophokinetic potential (ξ-potential) and charge density on the membrane surface. Similar to that on membrane fluidity and permeability, the effect of quartz on membrane charge of macrophages has also significant time-dependent and dose-dependent relationships (Figures 8, 9).

Al citrate can decrease electrophoretic mobility of macrophages by itself like its effect on membrane-bound water. The effect of quartz is almost abolished by the addition of a high dose of Al citrate (Figure 9). Of particular interest, the effect of quartz on membrane charge can be decreased by the pretreatment with Al citrate (Figure 8).

As illustrated in Figure 8, the increment by titanium dioxide is lower and its kinetics are very different from that of quartz, although it increased also electrophoretic mobility of macrophages.

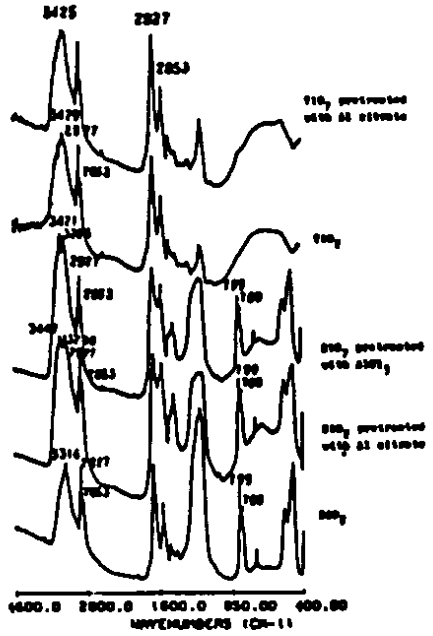

Figure 7. PT-IR or erythrocyte membranes treated by the different ways at 76% RH.

2.0 mg $SiO_2$ or $TiO_2$

## DISCUSSION

In previous studies, the cytotoxicity of quartz on macrophages was evaluated usually by measuring the activities of LDH and ACP and cell death rate.[3,6,13-15] The enzymatic activities may reflect indirectly the palsmic and lysosomal membranes damage caused by quartz, but their changes did not occur until after one hour of the incubation of cells with quartz. It is obvious that the indirect interaction between quartz and macrophage membranes, particularly its early effect need to be observed in order to establish the injurious effect of quartz on the membranes. It is for this purpose that the present studies was carried out.

Membrane fluidity plays an important role in membrane function.[16,17] Fluorescence Probe DPH used in this experiment can be inserted into the hydrocarbon region of lipid bilayers and fluorescence polarization depends on microviscosity of that region. The decrease of P under the action of quartz elucidates that the motion of lipidic molecules was increased due to the lowered membrane lipid microviscosity, resulting in disruption of membrane structure. Moreover, the fact that quartz can increase fluidity of liposomes prepared from lecithin and cholesterol also suggests that quartz interacts mainly with membrane lipids.[18]

In regard to the study of permeability, we have demonstrated that the reduction of $K^+$ content in macrophages can anticipate the enhanced activities of LDH and ACP in culture medium following the addition of quartz to these cells and is responsible for the cytotoxicity.[6] The present paper establishes further the correlation between both changes of permeability of macrophage membranes to $K^+$ and their mambrane fluidity by quartz. Likewise, the mechanism of the increased permeability is considered to be associated with the effect of quartz on membrane lipids, but not on $Na^+$-$K^+$-ATPase.

Bound water is a major component of biological membranes and is required for the structural stability of lipid bilayers and the normal function. A novel information about the effect of quartz on membrane "water structure" was obtained from the experiment of membrane-bound water of erythrocytes. The membrane IR spectra show bydration-dependent changes in the stretching vibration band of bound water, namely VOH shifted to the lower frequency with decreasing hydration. The result from subtract spectra (SUB), which can exclude absorbance of several groups besides water at 3000-3800 $cm^{-1}$ region, is consistent with the effect. A turning point of membranes hydration from sorption isotherms curve is at about 76% RH, at which hydration of normal erythrocytes membranes is 18.8% and its VOH peak position is 3535 $cm^{-1}$, whereas hydration of quartz group is only 10.1%, and its VOH peak position exhibits red shift to 3447 $cm^{-1}$. The decrease of membrane-bound water does not provide lipidic molecules with a necessary condition required for hydrophilic and hydrophobic interactions, so that the order degree of biomolecular layers was not maintained. Indeed, Clifford et al have found the changes of structure, such as phase separation of cholesterol from lipid, in membrane dehydration.[19] Thus dehydration by quartz is associated with increasing fluidity or permeability. On the other hand, charges on the membrane surface will alter relatively because the dehydration has made water molecules separate from some groups on membranes which are bound to them. This is further supported by the results from cell electrophoretic experiments.

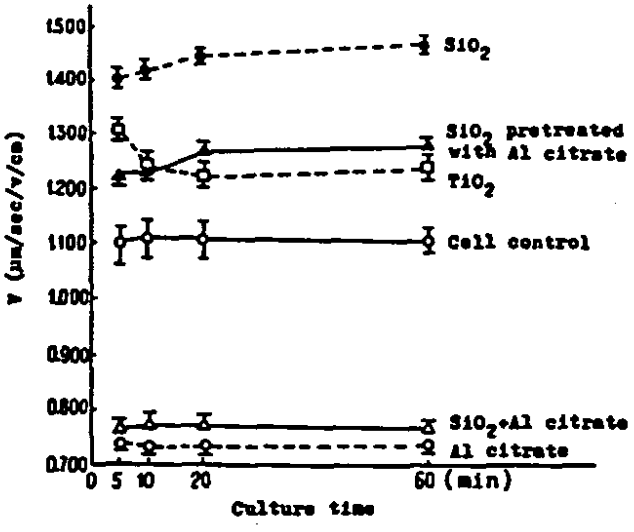


Figure 8. Kinetic curve of electrophoretic mobility (V) of macrophages treated by different ways.

500μg $SiO_2$ or $TiO_2$; 250 μg Al

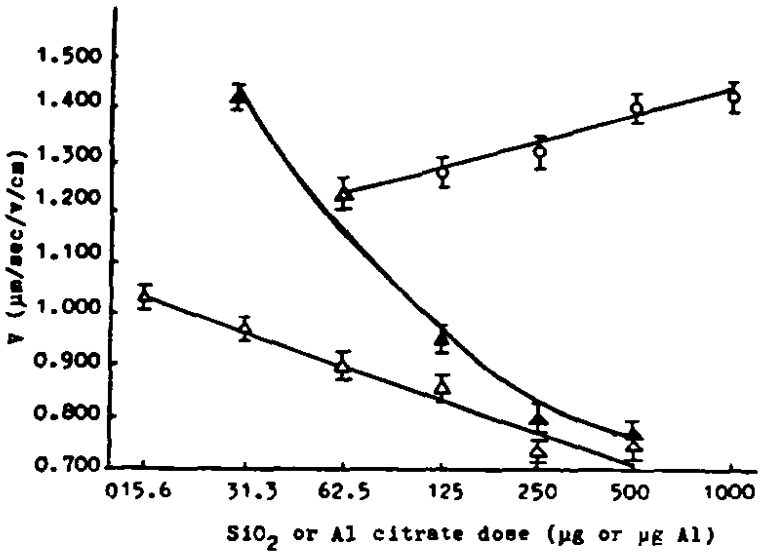


Figure 9. Dose-effect relationships of $SiO_2$, Al citrate and $SiO_2$ (500 μg) + Al citrate on electrophoretic mobility (V) of macrophages.

O—O $SiO_2$; Δ—Δ Al citrate; ▲—▲ $SiO_2$ + Al citrate

It seems more possible that quartz interacts with the positively charged groups, such as $-N^+(CH3)3$ riched in membrane phospholipids, so positive charge on the cell surface is neutralized partly and negative charge density increases relatively. Nash et al and Depasse et al presented the indirect evidence that quartz is easy to attract amide phosphate and quatenary ammonium groups and suggested that the attraction is responsible for haemolysis of quartz toward erythrocytes.[20-21]

Compared to quartz, the effects of titanium dioxide on cell membranes are not only much lower in intensity, but also very different in kinetics, for instance, the changes of fluidity, permeability and cell electrophoresis can not enhance permanently with the culture time, whereas tended to recover rapidly and remained constant. Of interest, the results from morphology is quite in accordance with biophysical and biochemical determinations.[22] Whether the membrane damage is caused will depend on physical and chemical properties of different particles. The fact that the effects of titanium dioxide on cell membranes were not affected by Al citrate may give some insight to the difference between quartz and titanium dioxide in their surface structure and affinity for ions, such as $-N^+(CH3)3$ and $A13^+$.

Another part of this paper focuses on the anti-injurious effect of Al citrate and its mechanism. In general, the increased membrane fluidity, permeability and negative charge density were declined, but the decreased membrane hydration were enhanced following the addition of Al citrate, so that the function, stability and order structure of cell membranes can be recovered and maintained. The observation by scanning electron microscope convinced us of the antagonistic effect of Al citrate once more.[22]

The mechanism is discussed through the compared antagonistic effects of several ways of the administration. It seems that Al citrate will affect membrane-bound water and charge by it self if the addition of it into cell medium without washing, but the its effect of disappeared after the cells were washed.[11] These findings suggest that Al citrate combines with certain membranes, even though the combination is not firm and matters little to its effect against quartz. No preventive effect was found in fluidity and permeability experiments of macrophages pretreated with Al citrate. Moreover, Al citrate alone did not influence these properties of macrophage membranes. From these it is considered at least that the preventive effect of Al citrate is not produced by its direct action on cell membranes.

The preventive effects of Al citrate and $AlCl_3$ were examined through the pretreatment of particles. The results show that this pretreatment way can effectively resist membrane damage by quartz. On the other hand, the fact that AlC13 exhibits a similar action indicates that the pharmalogical effective component of Al citrate is mainly Al itself, which explains why many kinds of soluble Al agents processes a similar effect of treatment for silicosis. Attention should be paid to the potential significance of the special action of Al on quartz in preventive and therapeutic silicosis.

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# II


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