

PLAINTIFF'S EXHIBIT

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Cytotoxicity of Heated Chrysotile

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Cytotoxicity and hemolysis were studied in chrysotile and quartz. The biological activity of the surface seemed to be different between chrysotile and quartz. Quartz lost its cytotoxicity on heating over about 500°C. However chrysotile showed remarkable toxicity and induced hemolysis on heating between 550 and 800°C, compared with the original unheated specimens. The mice injected intraperitoneally with minerals heated in this temperature range generally died within 48 hr after injection, while those injected with untreated chrysotile or chrysotile heated in the other heat ranges did not. The products in this range were highly disordered materials. It was assumed that the change of biological effects resulting from heat treatment may be related to the disordered state of chrysotile in the process of transformation into forsterite. The relationship between chemical character and cytotoxicity of the heated chrysotile specimens was also studied.

We have studied the cytotoxicity of mineral dusts to clarify the pathogenesis of pneumoconiosis. We found that cytotoxicity and the ability to induce hemolysis depend on surface properties of the minerals' dusts. As shown in Figure 1, alkali-treated quartz showed increased acid phosphatase activity. This quartz has a strong cytotoxicity, but it did not, however, induce striking hemolysis. On the other hand, although ground quartz was weakly cytotoxic, it easily induced hemolysis.

The chrysotile has strong cytotoxicity as well as a strong ability to induce hemolysis. Both chrysotile and amphibole can induce hemolysis, and they have a strong cytotoxic effect on the macrophage. Therefore, there is a difference between the quartz and asbestos, with respect to hemolysis and cytotoxicity.

Chrysotile was heated at various temperatures for 1 hr, and then we examined biological effects of heated chrysotile determining the degree of lactic acid production and hemolysis. Hemolysis induced by chrysotile was

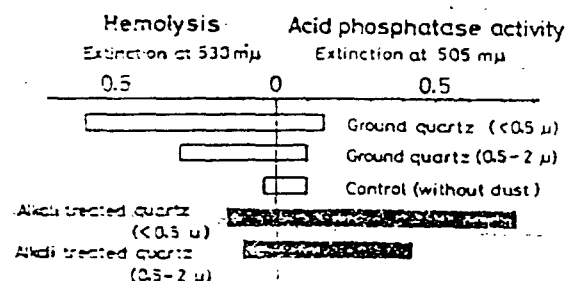


FIGURE 1. Hemolysis and acid phosphatase activation associated with quartz particles.

striking after heating in the temperature range 500-800°C (Fig. 2). On the other hand, lactic acid production increased until 500°C, decreased abruptly until 650°C, and then continued to increase with increasing temperature of heating (Fig. 2).

On DTA, chrysotile has an endothermic peak followed by an exothermic peak. X-ray and infrared analysis proved that chrysotile was converted into forsterite on heating to 500-800°C. Since chrysotile and forsterite coexist in this range, the rapid changes in the biological activity of chrysotile may be caused by the highly disordered condition during the transformation.

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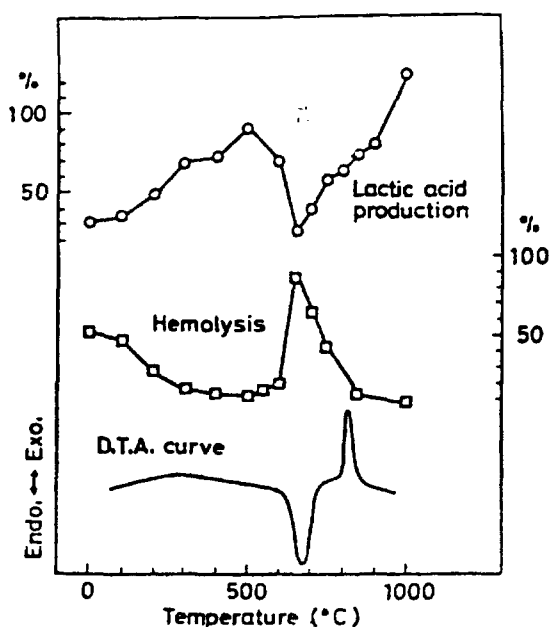


FIGURE 2. Cytotoxicity of chrysotile (UICC) treated at various temperatures.

Both the unheated sample and the sample heated at 650°C had a very high suppressing effect on the lactic acid production. However, when 0.2 ml of 10% bovine albumin was added to the cell-dust system, the two samples showed a

different behavior. The inhibitory effect of the unheated sample was remarkably reduced by presence of albumin, but the effect of the sample heated at 650°C was not reduced (Table 1).

A similar phenomenon was also observed in hemolysis. Hemolysis induced by the unheated sample was reduced by 50% by the addition of 11 μ g of albumin, but in the case of chrysotile heated at 750°C, 4000 μ g of albumin was necessary to obtain the same reducing effect. This means the unheated sample can easily absorb protein and become inert. However, absorption of protein was difficult in the heated samples. We found chrysotile heated between 650°C and 750°C to be very toxic.

This fact was confirmed by animal experiments with intraperitoneal injection of this chrysotile (Table 1). The mean gain in body weight 8 days after injection reflects this. It was very significant that all mice which were injected with chrysotile heated at 650 or 750°C died within 2 days after injection.

Further experimental studies were made to clarify the relationship between chemical character and cytotoxicity. Chrysotile was heated at various temperatures for 1 hr, then 10 mg of each sample put in water and agitated for 4 hr/day at 25°C. A supernatant was obtained by centrifugal separation, and the pH and dissolved amounts of Si and Mg in the supernatant

Table 1. Effects of heat treatment of chrysotile (UICC) on effects on macrophages, erythrocytes, and weight gain in mice.

Heat treatment temperature, °C	Lactic acid production without albumin, % of control	Lactic acid production with albumin, % of control	Hemolysis, % of control	Amount of albumin required to prevent hemolysis by 50%, μ g	Gain of body weight 8 days after injection, g
Untreated	30	112	52	11	8.0
200	48		28	36	
300	66		17	38	7.2
500	90		12	21	7.7
650	25	36	87	9000	Died
750	58		44	4000	Died
850	73		15	540	6.4
1000	125		8	10	5.1

were determined. This procedure was repeated on successive days for 15 days. The pH value of the supernatant of the samples heated at 650 and 750°C was higher than that of the other samples (Fig. 3). Amounts of dissolved Si and Mg in the supernatant of this group were also larger (Figs. 4 and 5).

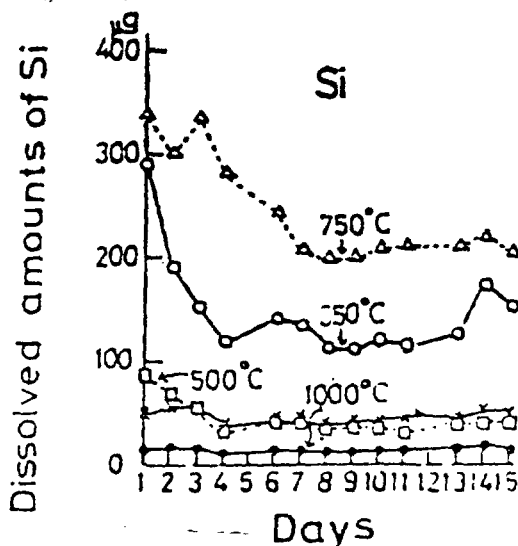


FIGURE 3. Amount of dissolved Si in supernatant as a function of temperature of heating of chrysotile: (x) unheated; (□) 500°C; (o) 650°C; (Δ) 750°C; (•) 1000°C.

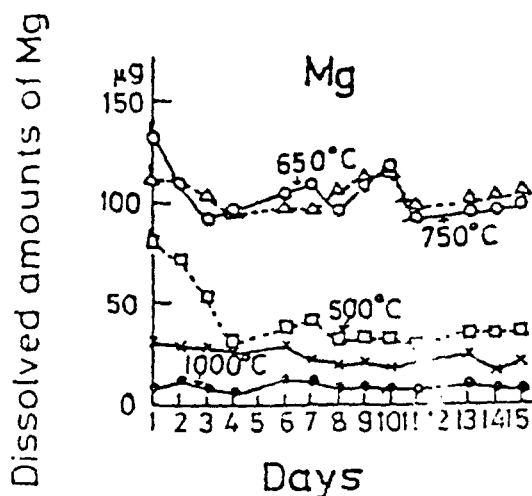


FIGURE 4. Amount of dissolved Mg in supernatant as a function of temperature of heating of chrysotile: (x) unheated; (□) 500°C; (o) 650°C; (Δ) 750°C; (•) 1000°C.

Figure 6 shows data obtained from supernatant in the experiment in the first day. The

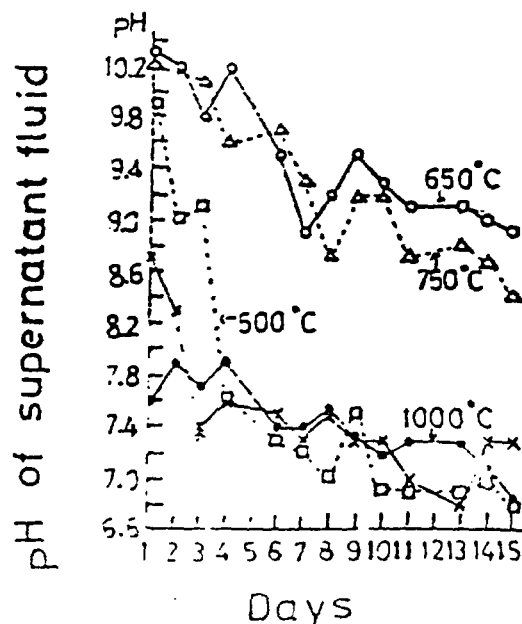


FIGURE 5. pH of supernatant as a function of temperature of heating of chrysotile: (x) unheated; (□) 500°C; (o) 650°C; (Δ) 750°C; (•) 1000°C.

Nature of supernatant fluid in the first day

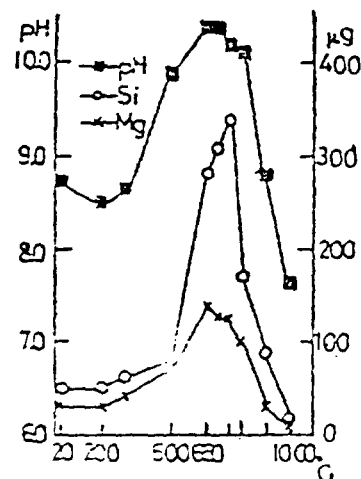


FIGURE 6. Nature of supernatant fluid as a function of temperature of heating: (□) pH; (o) Si; (x) Mg. All data obtained on the first day.

supernatant from chrysotile samples heated at 650 and 750°C show higher pH and larger amounts of dissolved Si and Mg (Fig. 6).

The effect of each sample on macrophage was estimated by incorporation of ¹⁴C-leucine into cell protein. When the pH of the suspension and/or supernatant fluid were adjusted at 7.8, the effect on macrophage were nearly same.

When we did not adjust the pH value, the data of the effect on macrophage was not constant. This means that incorporation of ¹⁴C-leucine into macrophages depends on the pH of the solution. The differences in pH may be caused by differences in surface properties of the samples. However hemolysis due to chrysotile heated at 650°C did not depend on pH of the solutions.