Adjuvant Effect of Silica (Tridymite) on Antibody Production.* (27527)

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Although previous investigations suggest that immunologic reactions may occur during development of the silicotic nodule(1), a search for specific antibodies either to silica or lung tissue has yielded inconsistent results (2). A non-specific stimulation of the immune response by silica appears more likely and is supported by preliminary data indicating that treatment of animals with silica may increase production of antibodies against protein antigens(3,4). The present work was undertaken to confirm and extend this observation and to provide some clues as to its mechanism.

Material and methods. Crystalline *silica* particles, 1-3 μ in diameter, were prepared from tridymite† according to the procedure of Occella and Maddalon(5). The dust was suspended in saline (50 mg/ml) and sterilized by autoclaving; the suspension was well shaken and injected in the marginal ear vein of rabbits or in the femoral vein of rats. *Kaolin* and *coal* (anthracite) dusts of 1-5 μ in diameter were prepared similarly.

As *antigens* twice crystallized ovalbumin‡ was dissolved in saline, in a concentration of 2 mg/ml before use. Sterile horse serum§ was used undiluted.

Twenty-one chinchilla rabbits of either sex, about 3 kg in weight, were injected with 210 mg silica divided in 3 doses of 70 mg given on alternate days. Three rabbits received a single injection of 200 mg of kaolin and 3 one of 200 mg of coal dust. At various intervals (Table I) after the injections the animals were immunized with 6 mg of ovalbumin given as 3 intravenous injections of 2 mg every third day. Two additional control groups of 4 animals each, not treated with dust, were subjected to the same immunization, one group at the beginning and the other at the end of the experiment. Blood was drawn from the central artery of the ear 7 days after the last antigen injection.

Ten Sprague-Dawley rats of either sex, weighing about 250 g, were injected with 50 mg silica in one injection. They were immunized with horse serum 42 days later with an intravenous injection of 1 ml, followed 10 days later by a subcutaneous injection of 0.1 ml, and finally, 2 days later, by a second intravenous injection of 1 ml. A control group of 7 animals was similarly treated with horse serum without exposure to silica, and another control group of 7 animals was treated with silica and then received saline instead of horse serum. Four days after the last injection, blood was collected from the heart under ether anesthesia and the animals were then sacrificed. Precipitating antibodies in rabbit sera were determined by the quantitative method of Heidelberger and Kendall(6) and antihorse serum antibodies of rat sera by a semi-micro precipitin technic(7).

Blocks from spleen, liver, lung, lymph nodes, heart, kidney and gonads were quickly frozen in isopenthane at –70°C and stored at –20°C until processed for immunofluorescence. Sections were cut, stained and observed as previously described(8). For detection of antibody the direct and indirect technics were used employing fluorescein-labeled horse serum or horse serum followed by fluorescein-labeled anti-horse antiserum prepared in rabbits.

Results. Pre-treatment with silica dust considerably enhanced the production of antibodies in rabbits immunized with ovalbumin (Table I). This was more conspicuous in animals which had received the silica before the antigen than in those treated simultaneously with silica and ovalbumin. The anti-

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† Tridymite, a pseudohexagonal orthorombic form of crystalline silica, was obtained from pure quartz, treated as described(5).

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TABLE I. Antibody Response to Ovalbumin in Rabbits Intravenously Treated with Silica Dust.

No. rabbits	Mineral dusts injected	Days elapsed between injection of dust and ovalbumin	Antibody nitrogen* Single values	Mean
3	Silica	0	16, 16, 60	31.0
3	"	15	56, 36, 81	57.6
3	"	30	47, 72, 43	54.0
4	"	60	115, 112, 126, 117	120.0
8	"	90	276, 180, 236, 450, 315, 428, 814, 537	404.5
3	Kaolin	30	12, 32, 14	19.3
3	Coal	30	23, 20, 18	20.3
4	None	—	23, 8, 4, 11	11.5
4	None	—	17, 15, 7, 22	15.0

* Expressed in μg/ml of serum.

body response was highest when immunization was performed with the longest delay after silica treatment. Rabbits pre-treated with kaolin or coal gave an antibody response similar to that of controls or only slightly higher. Only rats previously injected with silica produced measurable amounts of antibodies (Table II).

Immunofluorescence studies indicated numerous antibody containing cells in the spleen and lymph nodes of silica-treated rats (Fig. 1A) while in the controls treated with horse serum alone only few scattered antibody containing cells were present. In addition in silica-treated animals both antigen and antibodies were detected in the perivascular regions of several organs (lungs, heart, kidneys, spleen) (Fig. 1B) whereas the controls that received antigen only showed none.

Discussion. Silica particles have a marked adjuvant effect on antibody production. This effect is lasting and is even more evident when the animals are immunized some time after the treatment with silica. This was particularly clear in rats, because the control animals did not produce measurable amounts of antibody. Rats are known to be poor producers of precipitating antibodies(9). Other dusts such as kaolin and coal have at best only a slight adjuvant effect not comparable in magnitude to that of silica. The increase of the adjuvant effect of silica with time elapsing between injection of silica and antigen is in contrast to Freund's adjuvant(10) or endotoxins(11) which are most effective if injected with the antigen. Likewise silica differs from the finely divided inorganic dusts (*e.g.*, $AlPO_4$) that act mainly by adsorption of antigen(10). The presented observations may, instead, be explained by the exuberant and progressive proliferation of reticuloendothelial cells in the spleen and lymph nodes induced by silica treatment. Rabbits injected with crystalline silica particles exhibit a progressive enlargement of spleen and lymph nodes up to 10 times the normal size (12). The immunohistochemical observations indicate that after an antigenic stimulation many more antibody-containing cells are found in the reticuloendothelial system of silica-treated animals. Therefore the adjuvant effect of silica may be related to proliferation of cells in the R.E.S., especially of those cells involved in antibody production. This is in accord with the possibility that a quantitative alteration of immunological reactivity plays a role in the pathogenesis of silicosis.

TABLE II. Effect of Treatment with Silica Dust on Antibody Production in Rats Injected with Horse Serum.

No. of rats	Injection of Silica	Horse serum	Antibody titer*
10	+	+	256, 32, 64, 128, 256, 128, 256, 64, 128, 64
7	0	+	0 0 0 0 0 0 0 0
7	+	0†	0 0 0 0 0 0 0 0

* Each value is the reciprocal of the serum dilution still giving a visible precipitation at interface in the capillary ring test.

† These animals were injected with physiological saline.

Summary. Crystalline silica particles (1-3

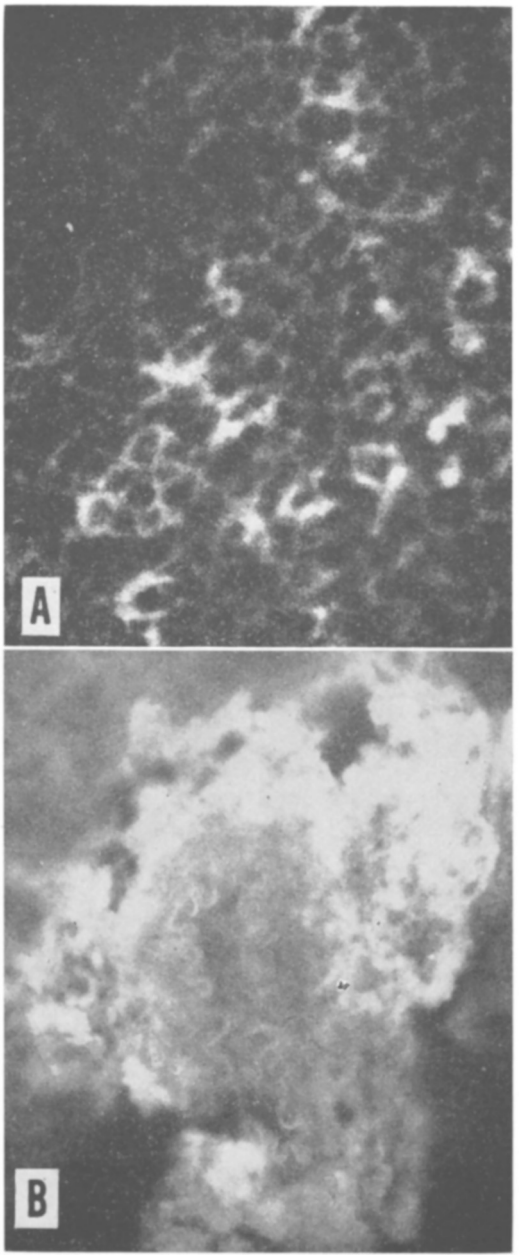

FIG. 1. Rat pre-treated with silica and immunized with horse serum. Tissue sections were treated with horse serum followed by fluorescein-labeled anti-horse serum

A) section of spleen showing many antibody containing cells (x 250)
B) section of kidney with antibody present mainly in adventitia of a middle size artery (x 250).

μ in diameter) were injected intravenously into rabbits and rats. At various intervals from 0 to 90 days the animals were immunized with ovalbumin or horse serum. Antibody production was greatly enhanced in silica-treated animals. The antibody titer was higher when the interval between silica and antigen injection was longer. Many more antibody-containing cells were observed in silica-treated animals. It is suggested that the adjuvant effect of silica is related to the proliferation of immunologically competent cells in the R.E.S. This might play a role in the pathogenesis of silicosis.

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