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STUDIES ON THE EFFECT OF QUARTZ, BENTONITE AND COAL DUST MIXTURES ON MACROPHAGES *IN VITRO*

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Summary.—The effect of quartz, bentonite and coal dusts as well as the effect of the artificial mixture of these dusts on TTC reduction and extra- and intra-cellular lactate dehydrogenase activity in peritoneal rat macrophages was determined *in vitro*. The cell-membrane-damaging effect of quartz caused a significant extracellular release of lactate dehydrogenase. Bentonite caused no extracellular enzyme release, which leads us to believe that the biological effect of this dust is shown by decrease in intracellular lactate dehydrogenase activity. TTC reduction was inhibited equally by quartz and qentonite. In mixtures of quartz (60%)–bentonite (40%) dust the specific effect of quartz was inhibited by bentonite *in vitro* and also *in vivo*. We obtained the same results with coal–quartz–bentonite dust mixtures *in vitro*. Our experiments show that comparison of the biological effects of artificial dust mixtures and airborne dust samples is justified, and prove that performing various examinations simultaneously give fuller particulars on the probable biological effect of mineral dusts.

DAMAGE to macrophages by mineral dusts has a role in the pathogenesis of the pneumoconioses. This is one more reason for giving attention to the investigation of macrophages even under *in vitro* conditions (Marks and Nagelschmidt, 1959; Allison, Harington and Birbeck, 1966). According to Münch, Beck and Manojlovik (1971) and Beck, Holt and Manojlovik (1972) regarding estimation of the cytotoxic effect, those methods are suitable which indicate the changes in permeability of the cell membrane, such as changes in the number of cells stained with erythrosin B, or lactate dehydrogenase (LDH) activity measured in the supernatant.

It has already been established in experiments on the biological effect of dusts occurring in coal-mining and mineral-mining that dust samples of quartz, bentonite, illite and other kaolins are all cytotoxic to macrophages *in vitro* (Adamis and Timár, 1976; Timár *et al.*, 1977). Whereas, on the other hand coal dust from mines where silicosis is a hazard proved to be inert both in animal and in *in vitro* experiments (Timár *et al.*, 1962).

In our present work we examined the effect of quartz, bentomite, and coal dusts and the effect of the artificial mixture of these dusts on the LDH activity and TTC reduction of rat peritoneal macrophages *in vitro*.

MATERIALS AND METHODS

Peritoneal macrophages were obtained, under sterile conditions, from male Sprague–Dawley rats (CFY strain, body wt 200–250 g), two days following the i.p. administration of Tyrode solution containing 0·01% glycogen. For determination of extracellular and intracellular LDH activity, each test tube contained $4{\cdot}4 \times 10^6$ cells which were incubated in Tyrode solution. The cells stuck to the bottom of the test tubes in 1 h, the medium was poured off and 2·2 ml fresh Tyrode was measured into the test tubes, the cells were incubated with and without dust for 3 h at 37°. The determination of the LDH released from the cells was carried out on the supernatant. The intracellular LDH activity was determined by the method of Welscher and Cruchaud (1976) following extraction with Triton X-100 (1 ml 0·2% Triton X-100 was added to the monolayer). The determination of extracellular and intracellular LDH (EC 1.1.1.27) was carried out by Boehringer's UV test (Boehringer GmbH, Mannheim) using a Spekord UV-VIS

spectrophotometer at 340 nm. The activity was expressed as mU/culture.

TTC reduction activity (TTC-RA, TTC= 2,3,5-triphenyltetrazolium chloride) was determined by two methods (Robock, 1974; Marks and James, 1959). Following the method of Robock (1974) the macrophages were incubated in Tyrode (0% serum) for 3 h, and following that of Marks and James (1959) the macrophages were incubated in Tyrode containing 30% homologous serum for 24 h, after which the TTC-RA was determined.

The quartz used in our experiments was supplied by Mr M. T. R. Reisner (Essen, Federal Republic of Germany). The quartz DQ 12 is used as standard dust in experimental silicosis research. The bentonite sample (Istenmezeje, Hungary) was supplied by Mr Z. Juhász and the further processing into particle sizes smaller than 2 μm was done in the Dust Laboratory of this Institute. The coal dust samples were airborne samples collected by means of a cyclotron in the mines of Mecsek (Hungary). The particle size of the airborne fraction was less than 5 μm. The dust mixtures—quartz–bentonite, quartz–coal and coal–quartz–bentonite—were prepared from the above mentioned samples, just before the distribution of the cells.

Analytical grade chemicals were used in our experiments. The values shown in the figures mean an average$\pm$s.d. of 6 cultures. Statistical significance was calculated by Student's t test.

RESULTS

After 24 h incubation the TTC-RA in the medium containing 30% serum was reduced by half compared to the control, by 150 μg quartz DQ 12, 30 μg bentonite and 750 μg coal.

After 3 h incubation in Tyrode (0% serum) 50% decrease in activity was provoked by an amount of about 480–500 μg quartz DQ 12 and bentonite, and in the case of coal dust by above 1000 μg (Fig. 1).

In the case of quartz DQ 12 extracellular LDH activity increased roughly in parallel with the dose of dust; no difference was observed between doses of 200 and 600 μg of bentonite, but a decrease in effect was found at a dosage of 1000 μg. With coal there was no notable LDH release, the level of which was significantly lower than that of quartz DQ 12 (Fig. 2). In the case of quartz DQ 12 and bentonite, intracellular LDH activity decreased with dose; the coal sample showed no significant change compared to the control. Total LDH activity (extracellular+intracellular) was similar to control in the case

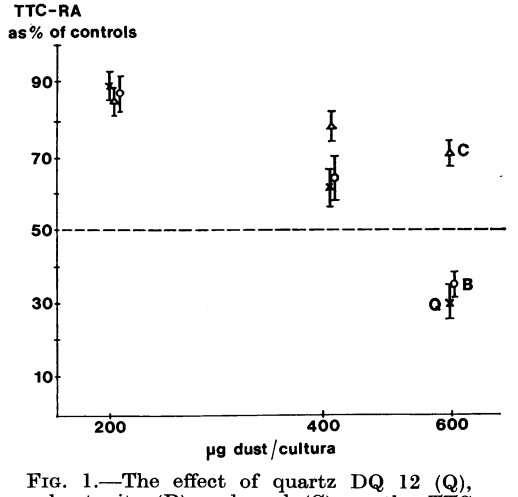

FIG. 1.—The effect of quartz DQ 12 (Q), bentonite (B) and coal (C) on the TTC reduction of macrophages in Tyrode.

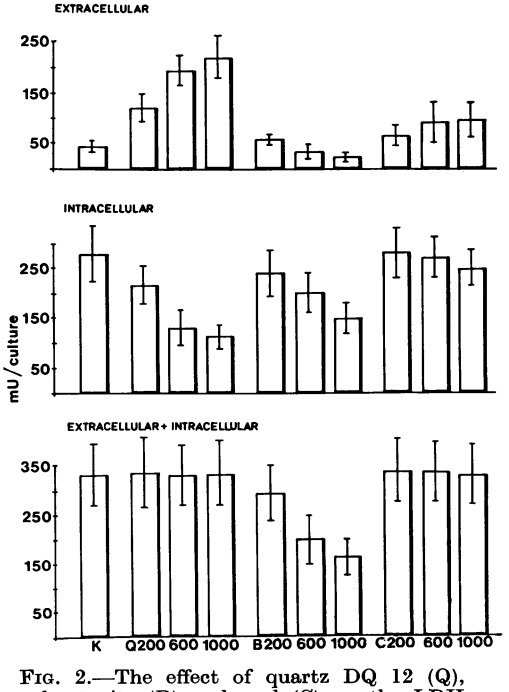

FIG. 2.—The effect of quartz DQ 12 (Q), bentonite (B) and coal (C) on the LDH activity of macrophages (K = control; the numbers show the amount of dust in μg/culture).

of quartz DQ 12 and coal, while the decrease observed in the case of bentonite was proportional to dose (Fig. 2).

The effect of quartz–bentonite and quartz–coal dust mixtures on macrophage LDH activity is shown in Fig. 3. In the case of quartz DQ 12 and quartz–coal dust mixtures, the amount of cellular enzyme release was significantly higher than with the control. Whereas with quartz–bentonite dust mixtures extracellular LDH activity was significantly lower than in those mentioned above, no significant change being observed compared to the controls with quartz (600 μg)–bentonite (400 μg) mixtures. Intracellular enzyme activity decreased significantly in all cases; total LDH activity was similar to the control in the case of quartz DQ 12 and quartz–coal mixtures, but in the case of quartz–bentonite mixtures a significant decrease was seen.

The effect of coal–quartz, coal–quartz–bentonite dust mixtures on the LDH activity of macrophages is summarized in Fig. 4. Extracellular LDH activity was significantly increased with coal–quartz mixtures containing 10% and 20% quartz. If the dust mixture contained 10% quartz and also 10% bentonite (or 20% quartz and 20% bentonite) the LDH activity was the same as that of coal. The amount of intracellular LDH differed significantly

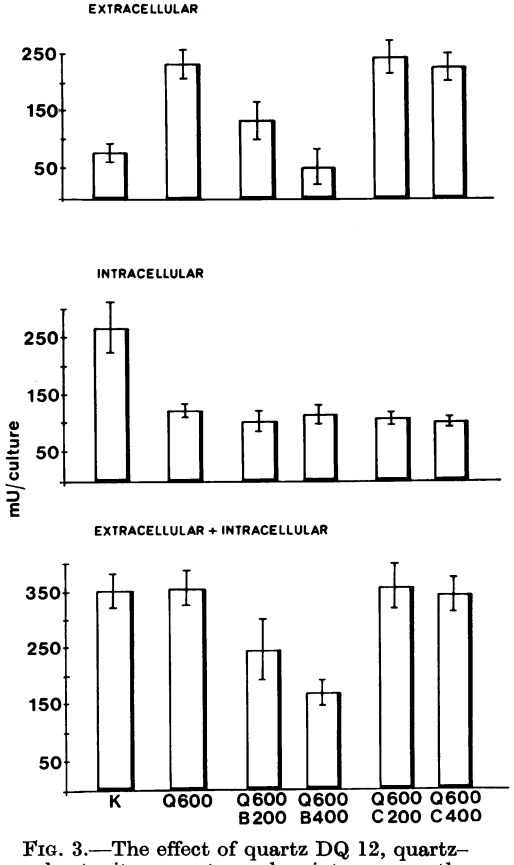

FIG. 3.—The effect of quartz DQ 12, quartz–bentonite, quartz–coal mixtures on the LDH activity of macrophages (K = control, Q = quartz DQ 12, B = bentonite, C = coal; the numbers show the amount of dust in μg/culture).

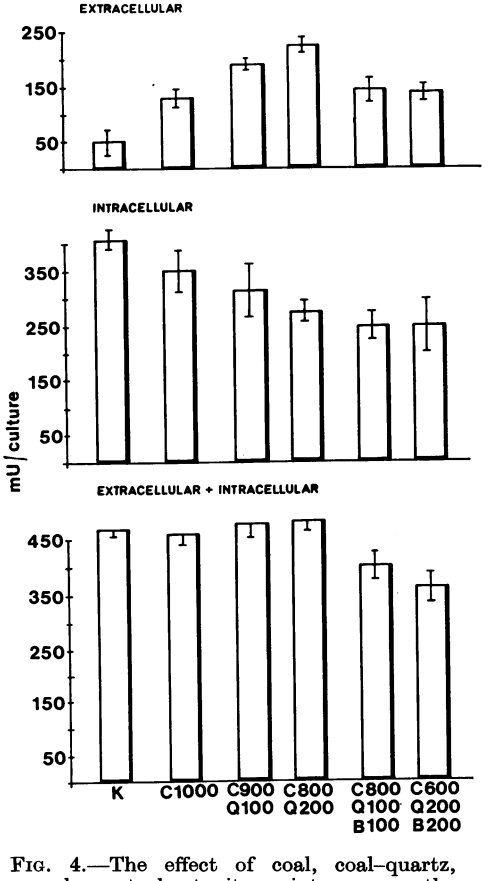

FIG. 4.—The effect of coal, coal–quartz, coal–quartz–bentonite mixtures on the LDH activity of macrophages. (K = control, Q = quartz DQ 12, B = bentonite, C = coal; the numbers show the amount of dust in μg/culture).

from that of coal in the coal–quartz–bentonite mixtures and in coal (800 μg)–quartz (200 μg) mixtures. Total activity showed a difference in the coal–quartz–bentonite mixtures only when compared to the control.

DISCUSSION

Quartz and bentonite inhibited TTC reduction in media without serum as well as in media containing 30% serum; coal showed no inhibitory effect on the macrophage cell.

The damaging effect on macrophages can be estimated accurately in the case of quartz and coal based on the amount of LDH released into the medium; however, this estimation cannot be done in case of bentonite. We obtained similar results in earlier studies on membrane permeability by aluminium silicates (Adamis, 1976).

Further conclusions concerning the effect mechanism of dusts can be drawn on the basis of the LDH activity values. We have established that the damaging effect of quartz on the macrophage membrane causes a significant release of enzyme extracellularly, but has no influence on total enzyme activity; total LDH activity corresponds with that of the control. The biological effect of bentonite can be understood on the basis of the decrease in intracellular LDH activity. It follows that the cause of the decreased total LDH activity is due to the intracellular effect of bentonite. The mechanism of the intracellular effect requires further study.

Timár *et al.* (1966) showed in their *in vivo* experiments that, in dust mixtures prepared arbitrarily—40% bentonite (montmorillonite)–60% quartz mixture—the bentonite inhibited the productive fibrosis caused by quartz dust and brought about a storage-type reaction. In dust mixtures of bentonite (20%)–quartz (80%), the effect of quartz in the production of fibrosis predominated. A similar phenomenon was observed in our *in vitro* experiments, in that the quartz–bentonite mixture containing 40% bentonite was able to inhibit the extracellular release of LDH that is an effect of quartz. In the presence of 25% bentonite the amount of the enzyme released into the medium was significantly smaller than occurs with quartz alone, but higher than the control figure.

Schlipköter, Seemayer and Manojlovic (1976) showed that anthracite inhibited the toxic effect of quartz in their *in vitro* experiments. This fact has not been confirmed in our experiments with coal–quartz mixtures. We suppose that the effect described by Schlipköter *et al.* (1976) may have depended upon the origin of the coal used, its geological evolution, ash content, *etc.*

Concerning the effect of mineral dusts on macrophages, our knowledge has increased significantly in recent years, but the potential or probable effect *in vivo* of the airborne dusts ("mixed dusts") collected from the atmosphere of mines and workshops, on the basis of *in vitro* experiments, remained unsolved. That was the reason for this investigation, namely to study the effects of the kinds of coal–quartz–bentonite dust mixtures which we encountered in practice (Fig. 4). In these mixtures 10–20% bentonite inhibited the characteristic *in vitro* effect of quartz (enzyme release into the medium), but the intracellular effect remained unchanged. Our studies show that the comparative examination of the biological effects of artificial dust mixtures of known composition and airborne dust samples is justified. In ongoing experiments we are studying the effect of quartz, bentonite, coal and their artificial mixtures respectively on the LDH activity of macrophages in the presence of serum.

On the basis of our experimental results we believe that the biological estimation of the effect of dust by means of a single *in vitro* model-examination is impractical at present. Our experiences show that examining various parameters simultaneously gives more useful information on the probable biological effects of mineral dusts.

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