deposition in the cells. However, the possibility cannot be ruled out that other types of modified LDL (for example, oxidized) may also potentiate the effect of glycosylated and desialated LDL on intracellular lipid accumulation. In our view, data on the synergic effect of the two types of modified LDL, like data on the increase in atherogenic potential of LDL modified by the two methods, may be one explanation of the high ability of sera from patients with coronary heart disease accompanied by diabetes to stimulate intracellular lipid accumulation.

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EFFECT OF CYTOTOXIC DUSTS ON FORMATION OF ACTIVE FORMS OF OXYGEN BY RAT PERITONEAL MACROPHAGES

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There is at present a steady increase in attention paid to the role of free oxygen radicals in the mechanism of injury to cell structures by dust particles and also in the study of free-radical processes during the development of dust diseases of the lungs [1, 2, 4, 8, 11]. Dust particles induce activation of the "respiratory burst" of phagocytic cells (neutrophils, monocytes, alveolar and peritoneal macrophages) [7, 11], during which the uptake of oxygen and glucose by the cells is increased and active forms of oxygen (AFO) are produced, especially the superoxide anion-radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) [6, 14]. Investigations have shown that the cytotoxic activity of different dusts correlates with the quantity and rate of generation of AFO by the cells [4, 7, 11]. Dust particles can induce AFO formation also by other extracellular pathways:

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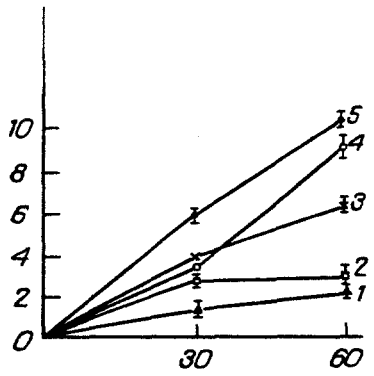

Fig. 1

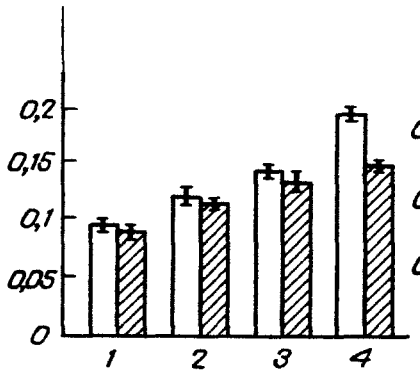

Fig. 2

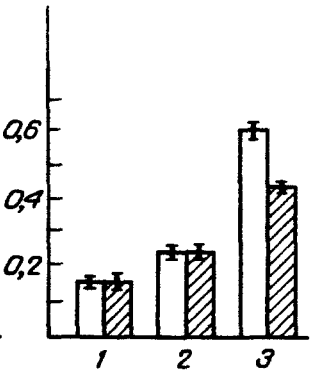

Fig. 3

Fig. 1. Superoxide radical formation in rat peritoneal macrophages activated by various dusts and by opsonized zymosan. Abscissa, incubation time (in min); ordinate, superoxide radical formation (in nmoles $O_2^-/10^6$ cells). 1) Control; 2) crocidolite; 3) quartz; 4) opsonized zymosan; 5) opsonized zymosan + 0.3 mM NaCN.

Fig. 2. Effect of quartz and crocidolite on destruction of DOR in rat macrophages. Ordinate, change in optical density per hour (in relative units). 1) DOR; 2) DOR + cells; 3) DOR + cells + quartz; 4) DOR + cells + crocidolite. Unshaded columns — without ethanol, shaded columns — with ethanol.

Fig. 3. Action of dusts on LPO in phagocytes. Ordinate, MDA formation (in μM). 1) Cells; 2) cells + quartz; 3) cells + crocidolite. Unshaded columns — without ethanol, shaded columns — with ethanol.

for example, as a result of catalytic conversions of O_2^- and H_2O_2 on the surface of minerals some extremely reactive hydroxyl radicals (HO·) are formed [12]. In this case the catalytic centers are ions of metals of variable valency, incorporated into the surface structures. These results were obtained for asbestos and quartz particles in cell-free systems by methods of electron paramagnetic resonance and chemiluminescence.

During contact of isolated phagocytic cells with dust particles two processes may perhaps take place: enzymic formation of AFO and their subsequent conversion by interaction with the surface of the mineral. Thus the content of different AFO in the dust—phagocyte system will depend on the composition and structure of the mineral particle. Second, various AFO, which constitute the molecular basis for injury to biostructures, may determine the specific nature of the pathogenic activity of the dust samples tested. On the basis of this hypothesis we set out to identify and determine the quantity of radical products formed in a suspension of rat peritoneal macrophages under the influence of asbestos fibers and quartz particles.

EXPERIMENTAL METHOD

Peritoneal macrophages were isolated from noninbred albino rats without preliminary stimulation of the outflow of cells into the peritoneal cavity [5]. Viable macrophages were identified by the trypan blue test and suspensions containing not less than 80% of viable cells were used in the experiments. A differential cell count was carried out on films stained with hematoxylin and eosin. The suspension of peritoneal exudate cells contained up to 50% of neutrophils and 20-30% of macrophages, while the rest consisted of lymphocytes.

O_2^- formation was assessed spectrophotometrically by measuring superoxide dismutase-(SOD)-sensitive reduction of cytochrome c [10]. The incubation mixture contained (in a volume of 1 ml) $2 \cdot 10^6$ cells in Hanks' solution (pH 7.4), 50 μM cytochrome c, 1 mg of the sample of dust or opsonized zymosan with or without 20 μg SOD. The reaction was initiated by adding a suspension of particles to the cells. After incubation for 30 and 60 min at 37°C with constant mixing the samples were centrifuged and absorbance at 550 nm was recorded in the supernatant. The rate of O_2^- generation was calculated with the aid of the coefficient of extinction of cytochrome c, which is $2.1 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$.

Hydroxyl radicals were determined on the basis of destruction of 2-deoxy-D-ribose (DOR) [13]. In a total volume of 0.5 ml the samples contained $3 \cdot 10^6$ cells in Hanks' solution (pH 7.4), 5 mM DOR, 1 mg dust, with or without 80 mM ethanol. Combined incubation was carried out at 37°C with constant mixing for 30 min. Next, 0.6 ml of 1% thiobarbituric acid (TBA) and 2.8% TCA were added to all the samples, which were heated on a water bath (100°C) for 15 min, cooled, and centrifuged (400g, 4°C), after which the change in absorbance at 532 nm in the supernatant was determined. The intensity of lipid peroxidation (LPO) in the macrophages was found by determining the quantity of malonic dialdehyde (MDA) by the reaction with TBA [9]. Zymosan was opsonized with 50% serum.

Dust samples: the quartz was obtained from the Lyubertsy deposits with a particle size (over 90%) not exceeding 5 μ; asbestos was used in the form of crocidolite (from UICC, Canada), with particles not more than 10 μ long.

EXPERIMENTAL RESULTS

Dust samples in cell-free systems at physiological pH values catalyze the reaction of O_2^- formation: for instance, 1.78 ± 0.1 nmole O_2^- is formed in 60 min on a quartz surface. Meanwhile, asbestos (crocidolite) had virtually no effect on SOD-sensitive reduction of cytochrome c. In the case of activation of macrophages by crocidolite and quartz, O_2^- generation during the first 30 min was virtually identical, and was about 2.5-3 times greater than the control (Fig. 1). For comparison, we give the results of activation of the cells by opsonized zymosan particles. During incubation for 1 h radical formation by macrophages under the influence of optonized zymosan was 9.0 ± 0.3 nmoles, of quartz 6.2 ± 0.2 nmoles, and of crocidolite 2.9 ± 0.3 nmoles; in other words, under the influence of asbestos the level of O_2^- generation remained the same as after 30 min. In the control, an increase in the quantity of superoxide also was observed, although it took place more slowly.

Inhibition of endogenous SOD of the macrophages was produced by adding 0.3 mM NaCN to the reaction mixture; in this concentration, it does not inhibit cellular respiration. Treatment with NaCN led to an increase in the concentration of the superoxide during stimulation of the phagocytes by opsonized zymosan. For instance, after incubation for 30 min the O_2^- concentration during inhibition of SOD was 5.8 ± 0.3 nmoles, whereas in the absence of cyanide it was 3.5 ± 0.1 nmoles.

Thus, during the action of the test dust samples on phagocytic cells, generation of the superoxide radical takes place. This process depends on the incubation time and the type and concentration of the dust sample. After incubation for 60 min macrophages form twice as much O_2^- in response to stimulation by quartz than to stimulation by crocidolite. Opsonized zymosan had the strongest effect on O_2^- generation by the cells.

HO· generation under the influence of the test dust samples in solution at physiological pH (7.4) was studied in the presence and in the absence of ethanol, for DOR destruction can take place not only due to free HO·, but also to "site-specific" hydroxyl radicals, formed actually at the point of contact between DOR molecular and dust particle. DOR degradation inhibited by ethanol reflects the level of free HO· in solution. In a cell-free system, in medium with 100 mM potassium-phosphate buffer, during incubation for 30 min the quartz particles had no significant effect on DOR, evidence of the absence of HO·. Crocidolite, on the other hand, induces both general degradation of DOR (by 28%) and ethanol-inhibited degradation (by 8%). Consequently, HO· are formed mainly "site-specifically" in solution under the influence of crocidolite.

In the presence of peritoneal exudate cells, which carry out phagocytes of quartz, total degradation of DOR increased a little (by 18%), whereas crocidolite significantly increased both total (by 63%) and ethanol-inhibited (by 33%) DOR degradation (Fig. 2). Thus, in a suspension of cells ingesting asbestos fibers, the hydroxyl radicals found are formed mainly by the Haber-Weiss reaction in the presence of ferrous ions present in the composition of the fiber:

$$O_2^- + Fe^{2+}\text{-asbestos} \xrightarrow{+2H^+} HO\cdot + HO^- + Fe^{3+}\text{-asbestos}$$

The ability of different types of asbestos, including crocidolite, to catalyze LPO was demonstrated on phospholipid preparations and in microsomes. We studied the effect of quartz and crocidolite on lipid peroxidation in rat peritoneal macrophages (Fig. 3). Quartz increased the quantity of MDA formed by 53%, crocidolite by 300%. Ethanol, in a concentration of 80 mM, which did not affect cell viability in the course of the experiment, inhibited LPO induced by crocidolite by about 30%. Probably the leading role in the intensification of LPO by crocidolite particles is played by hydroxyl and, in particular, by "site-specific" hydroxyl radicals, whereas the superoxide anion-radicals of oxygen, formed under the influence of quartz particles, are less able to initiate LPO.

Thus, by contrast with quartz, which generates mainly O_2^- during activation of peritoneal macrophages, crocidolite leads to the formation mainly of hydroxyl and "cryptohydroxyl" radicals, which have a high ability to initiate LPO. Generation of these AFO by asbestos is one cause of its greater cytotoxicity and mutagenicity [3], and also probably of its ability to induce the development of malignant neoplasms of the pleura and lungs.

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