ELECTRON-MICROSCOPIC STUDY OF THE TISSUE RESPONSE OF THE LUNGS TO QUARTZ DUST WITH DIFFERENT DEGREES OF DISPERSION

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Differences in the tissue response of the lungs to the action of quartz dust of varied degree of dispersion were studied by methods of light and transmission and scanning electron microscopy. It is suggested that the characteristic clinical manifestations of dust pathology are connected with the degree of dispersion of silica. Dust containing the largest particles of quartz mainly gives rise to the development of dust bronchitis. The most cytotoxic "middle" fractions lead to the development of nodular forms of silicosis, whereas the highly dispersed very small quartz particles lead to the formation of diffuse-sclerotic changes in the lungs.

KEY WORDS: quartz; electron microscopy; fibrogenicity of dust.

Experimental studies by Soviet and western workers have shown that the fibrogenicity of quartz dust increases with an increase in the degree of dispersion only up to a certain limit (1-2 μ), after which it falls steadily [4, 6, 7, 9]. During exposure of animals to a dust aerosol of the same chemical composition, reproducible results as regards the severity of fibrosis can thus be obtained only if dust with identical degrees of dispersion is used.

Originally the reason for the reduction in fibrogenicity of quartz dust particles under 1 μ in diameter was considered to be that these tiny dust particles are eliminated more rapidly from the lungs via the lymphatics, and also that they are more soluble in the tissue fluids. It was shown more recently that the decrease in fibrogenicity of highly dispersed fractions of quartz dust is mainly due to changes in the physicochemical properties of the surface of the dust particles [1].

The study of changes in the character of the pathological effect of silica on macrophages depending on particle size and on the character of formation of the tissue response to dust particles of different degrees of dispersion is of great scientific and practical interest. The gap in our knowledge in this respect is one of the main causes of the protracted discussion on critical particle size that determines the curve of separation of dust fractions during two-stage measurement of the dustiness of the air. This situation can be to some extent explained by technical difficulties in the study of the dependence of the mechanism of action of silica on the degree of dispersion of dust particles. The point is that even after repeated fractionation dust always contains a certain amount of dust particles of larger and smaller size than those of the "calculated" diameter, and optical light microscopy cannot be used to study the cellular response to individual dust particles. Electron microscopy provides more extensive opportunities in this respect.

To study the mechanism of action of silica disintegration aerosols of different degrees of dispersion, light, transmission, and scanning electron microscopy were used. Male albino rats weighing 150-200 g were given a single dose of a suspension of a quartz dust fraction with a calculated particle size of under 5 μ, in a dose of 20 mg in 0.6 ml physiological saline, by the intratracheal route. Lung tissue sections were examined in the JEM-100B electron microscope and pieces of lung tissue in the Qwikscan/100 scanning electron microscope. After fixation in glutaraldehyde and dehydration, the lung fragments were crushed in liquid nitrogen and sprayed with metallic gold. The presence of phagocytosed quartz particles in the macrophages was determined by the use of the Cameca MS-46 probe electron microscope.

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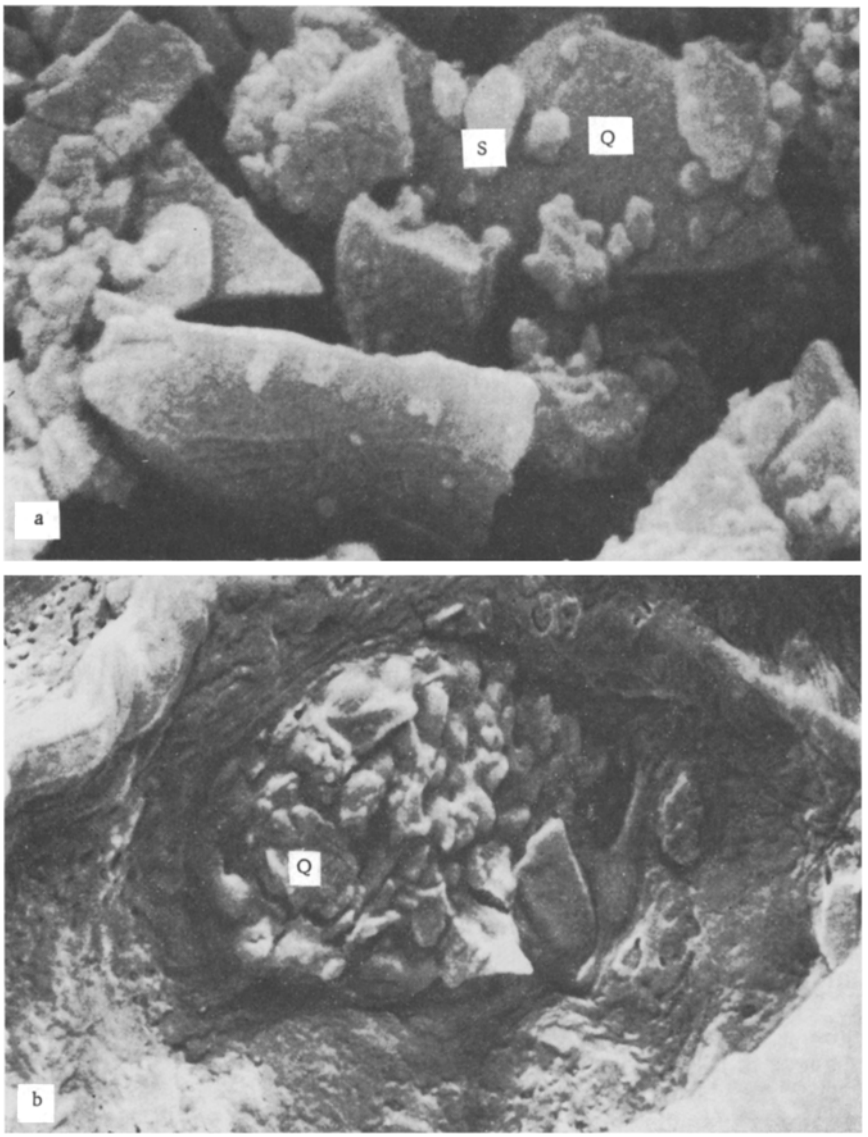

Fig. 1. Morphology of quartz particles (3-dimensional picture): a) polydispersed particles with tiny satellite subunits on their surface (30,000 ×); b) conglomerate of quartz particles in lumen of alveolus (10,000×). Q) Quartz particles; S) satellites.

Electron-microscopic study of the dust used revealed considerable polymorphism of particle size. About 30% of the particles were under 0.5 μ, chiefly 0.3-0.02 μ, from 63 to 67% of particles had a diameter of 0.5-3 μ, not less than 3-4% of particles a diameter of 4-5 μ, and 1-2% actually measured 6-7 μ. Despite careful fractionation, the dust injected was thus polydispersed in character. Numerous tiny satellite subunits were found on the surface of each isolated particle. Satellite particles could be found in the scanning electron microscope under high power (20,000×) (Fig. 1a).

Massive intraalveolar accumulations of dust particles of varied degrees of dispersion were detected by scanning electron microscopy 18 h after intratracheal injection of dust (Fig. 1b). Features of the cellular and tissue response to dust particles of different sizes could be distinguished 42 h after the beginning of the experiment. Three main variants could be definitely discerned.

The few largest particles which entered the deep respiratory passages, evidently because of the intratracheal method of injection of the dust, as a rule were not ingested by cells but "bored" through the alveolar wall in the free form (Fig. 2a). Their penetration was accompanied by the formation of a traumatic tissue defect. Necrosis of the tissue took place around the wound channel (Fig. 2b). Extensive foci of thrombosis developed in the pulmonary capillaries surrounding the region of trauma.

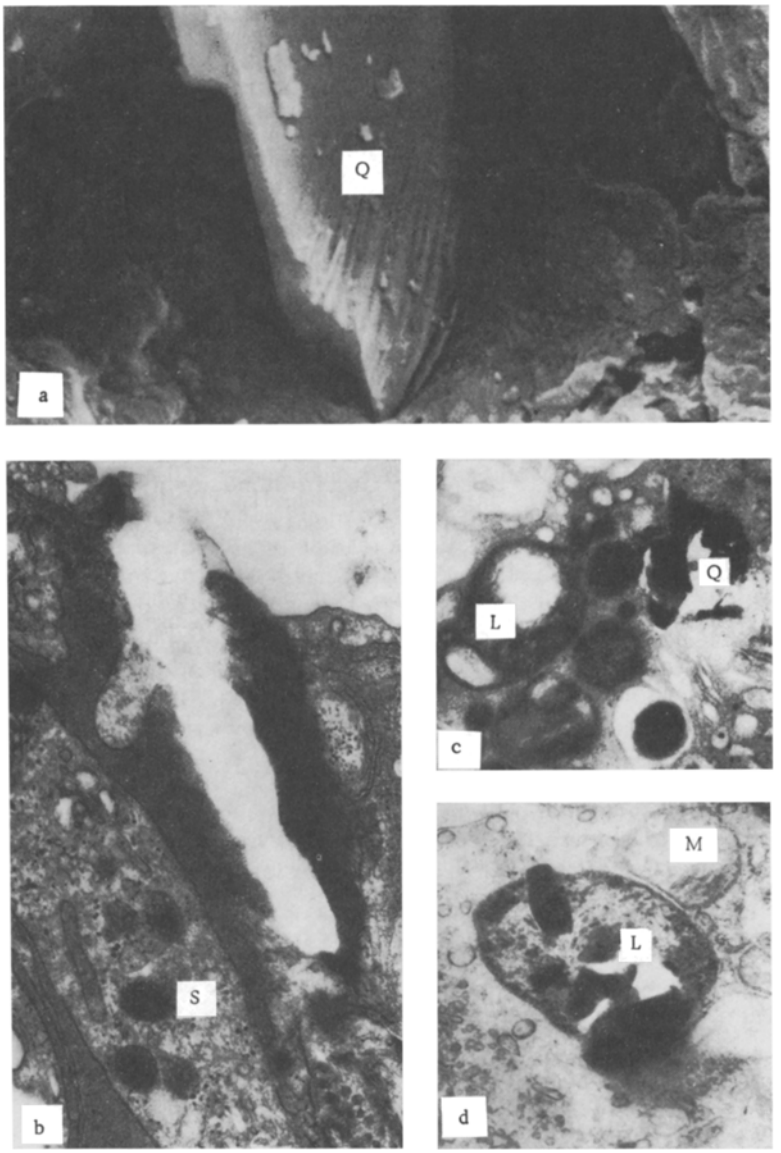

Fig. 2. Penetration of quartz particles of different sizes into lung tissue and their cytotoxic action: a) large quartz particle (8 μ), penetrating through alveolus (10,000×); b) necrosis of ultrastructures of air–blood barrier around insinuated quartz particle (removed during preparation of tissue section; 30,000×); c) condensation, lysis, and swelling of ultrastructures of alveolar macrophage in region of penetration of particles (12,000×); d) accumulation of highly dispersed particles in phagolysosome of macrophage (30,000×). Q) Quartz particles; L) lysosomes; M) mitochondria.

Quartz particles from 2 to 0.5 μ in diameter had a distinct cytotoxic action, leading to destruction of the macrophages responsible for their phagocytosis. Complete destruction took place when two to four such particles were present in the cytoplasm. After phagocytosis of the quartz dust by the alveolar macrophages, their lysosomes swelled (Fig. 2c). The lysosome membranes then gradually disappeared and cavities with necrotic contents were formed in the cytoplasm. Swelling of mitochondria, with shortening of the cristae and translucency of the structure of their matrix also took place regularly. Similar changes were observed when the action of quartz dust on a culture of macrophages was studied [5].

However, the two variants described above are by no means all the types of responses to dust particles of different degrees of dispersion. Frequently quartz particles with a maximal diameter of 0.02–0.3 μ were

found in the cytoplasm of the macrophages. It is interesting to note that these very highly dispersed particles produced no visible disturbances of ingesting macrophages for a long time. Moreover, a picture of accumulation of 10 to 15 tiny quartz particles in the numerous undamaged phagolysosomes of a macrophage could be observed (Fig. 2d). These cells were considerably enlarged. Consequently, "working" hypertrophy of the coniophages took place because of an increase in the number and size of their intracellular ultrastructures.

Depending on the degree of dispersion, particles of silica disintegration aerosols may thus differ in their effects. Particles with a maximal diameter of 5-7 μ have a mechanical traumatic action of the alveolar wall. Particles measuring 0.5-2 μ cause marked toxic destruction of macrophages, whereas particles with the highest degree of dispersion (0.02-0.3 μ) caused disintegration of the hypertrophied coniophages because of decompensation of the numerous hyperplastic organoids.

What causes the clear decrease in the cytotoxic properties of the smallest quartz dust particles? During mechanical grinding of silica disorganization of the outer layer of the crystal lattice takes place, and the higher the degree of dispersion of the dust the greater the disorganization. During very prolonged grinding of finely dispersed quartz, it may be completely converted into amorphous silica [8]. The latter is characterized by a higher degree of mobility of silicic acid tetrahedra in the boundary layer and, consequently, by fewer unsaturated bonds and, correspondingly, fewer hydroxyl groups per unit of surface. For instance, whereas crystalline silica contains on average 2.70-2.77 silanol groups per 10 nm of surface, in silica-gel there are only 1.16-1.26 [2]. To this it must be added that when the weight of silica in the boundary layer becomes commensurate with the weight of matter in the volume of the particle, the degree of mobility of the molecules rises stepwise, and closing of bonds broken during grinding is facilitated to an even greater degree. For instance, if the number of OH-groups per unit surface of quartz dust with a mean equivalent diameter of 1 μ is taken as 100%, on particles measuring 0.05 μ it is only 39-45% [3]. The principal role in the reduction of the cytotoxic effect of highly dispersed quartz dust is thus played by the low concentration of chemical active centers per unit surface of such particles.

What is the role of increased solubility of highly dispersed quartz particles in their elimination from the lungs? An indirect answer to this question can be obtained by studying changes in the fibrogenicity of silica condensation aerosols depending on the degree of dispersion of the dust particles.

The mechanism of formation of silica condensation aerosols ensures that the surface properties of the dust particles remain unchanged over the whole range of their dispersion. The decisive role in the reduction of fibrogenicity of highly dispersed fractions of such dust is therefore played by increased solubility, leading to the more rapid removal of the particles from the lungs.

The boundary beyond which any further increase in the degree of dispersion of the particles leads to a decrease in the development of fibrotic changes in the lungs of experimental animals is 0.03-0.05 μ for silica aerosols.

It follows from what has been said that solubility begins to play the predominant role in the reduction of fibrogenicity of silica disintegration aerosols only when the particle size becomes analogous, i.e., 1.5 orders of magnitude below the "critical" diameter of quartz dust or, in other words, only for particles measuring 0.03 μ or less. The main limiting factor for larger submicroscopic particles remains a change in their surface properties.

These investigations suggest that characteristic clinical manifestations of dust pathology are largely connected with exposure to silica disintegration aerosols of predominantly one or another degree of dispersion. The largest quartz-containing dust particles mainly cause the development of dust bronchitis. The most cytotoxic "medium-sized" fractions lead to the development of nodular forms of silicosis, whereas highly dispersed quartz dust particles lead to the formation of diffuse-sclerotic changes in the lung tissue.

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POLYPLOIDIZATION OF HEPATOCYTES IN RATS FOLLOWING EXPOSURE TO CCl_4 UNDER DIFFERENT CONDITIONS

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A karyometric study was made of hepatocyte polyploidization in albino rats during continuous and interrupted poisoning with CCl_4. Polyploidization, as a response to exposure to chlorinated hydrocarbons, was shown to depend on the toxicity of the poison, which depends on the number of chlorine atoms in the molecule, and on the character of exposure (mode of administration, dose, regime).

KEY WORDS: karyometry; ploidy of hepatocytes; chlorinated hydrocarbons; CCl_4.

Studies of the effects of various chlorinated hydrocarbons (di- and trichloropropanes – DCP and TCP) [1, 2] and investigations of the action of CCl_4 on the rat liver [10] have shown that the response of the hepatocytes depends on the concentration of the harmful agent and the duration of exposure to it. Since it is assumed that the number of DNA-synthesizing hepatocytes depends on the rhythm of exposure to CCl_4 [8], the effect of this factor on polyploidization under the conditions of action of the hepatotoxin on rats must also be taken into account. The object of the investigation described below was to determine how polyploidization of the hepatocytes depends on the character of entry of CCl_4 into the body (continuous or interrupted exposure). Both the total (including intervals between exposures in the case of interrupted administration) period of exposure and the duration of exposure proper were estimated.

EXPERIMENTAL METHOD

The DNA content in the hepatocytes was studied in control (20) and experimental (51) noninbred male albino rats made to inhale CCl_4 continuously or interruptedly, and divided into 13 groups depending on the experimental conditions. In airtight chambers (capacity 200 liters) containing the experimental animals constant concentrations of CCl_4 (5, 100, and 300 mg/m^3) were maintained. Interrupted exposure was studied by two programs: inhalation of CCl_4 in a concentration of 300 mg/m^3 for 4 h with intervals of 20 and 8 h between exposures. The data on the length of exposure in the case of continuous and interrupted programs are given in Table 1. Rats kept in airtight chambers ventilated with atmospheric air for the same length of time as the experimental animals served as the control.

Films of hepatocytes for karyometric analysis were obtained by the method described previously [1], fixed in Carnoy's mixture, and stained with hematoxylin-eosin and gallocyanin. Karyometric analysis for each animal was based on determination of 500-2000 cells.

EXPERIMENTAL RESULTS

During constant inhalation of CCl_4 for 1, 3, and 7 days changes in polyploidization began after poisoning for 24 h (group 2). An increase in the length of exposure led to aggravation of the changes. After exposure for 3 days (group 3) the number of binuclear cells with diploid nuclei was significantly reduced ($P < 0.02$) and the number of tetraploid hepatocytes was increased ($P < 0.001$). After exposure for 7 days (group 4) the number of binuclear tetraploid hepatocytes was reduced even more ($P < 0.001$) whereas the number of mononuclear

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