AN EXAMINATION OF THE CYTOTOXIC EFFECTS OF SILICA ON MACROPHAGES*

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Plates 10 to 13

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The fibrogenic effects of silica in man and experimental animals are not yet satisfactorily explained. There is general agreement that in the first instance silica particles deposited in the lung are engulfed by phagocytic cells, which are rapidly destroyed. The cytotoxic effects of silica can be conveniently reproduced in cultures of peritoneal or alveolar macrophages (1), and the relative toxicity of different forms of silica, and of different dusts, on cell cultures agrees with the pathogenicity and fibrogenic potentialities of the dusts in vivo (2, 3).

Silica provides, in fact, a remarkable example of selective toxicity for one cell type by a substance of simple chemical composition and low chemical reactivity. Why particles of silica rapidly kill macrophages whereas other particles of comparable size and surface area (such as carbon particles or diamond dust) are ingested by the cells without harmful effects is a problem of academic interest as well as practical importance in relation to the pathogenesis of silicosis.

Earlier theories of silica toxicity were reviewed by Nagelschmidt (4) and Harington (5). King in his well known "solubility" theory suggested that silicic acid liberated into the tissues from silica particles brings about the deposition of collagen. Later observations did not support this view, as King himself (6) and Nagelschmidt (4) have pointed out. Curran and Rowsell (7) showed that silica particles implanted into the peritoneum in diffusion chambers do not induce any fibrogenic reaction, although silicic acid is liberated from the chambers. Suggestions have also been made that silica dust might damage macrophages as a result of protein adsorption or denaturation, oxidative changes, or interference with enzyme activity such as that of esterases in the cell.

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None of the evidence in support of these suggestions has been very convincing, and Vigliani and Pernis (8) were led to formulate an autoimmune causation of silicosis. However, extensive investigations have failed to provide any evidence that silica is directly involved in immune reactions, either cellular or humoral, or that an autoimmunity is responsible for the fibrogenesis (9). It now seems likely that if any immune responses occur in silicosis they may be incidental; for example, secondary to infection of the damaged lungs.

Hence the mechanism by which silica particles kill macrophages is still an open question. Recognizing the similarities between the uptake of silica dust and other particles, e.g. bacteria (10), or inert materials, for example Triton WR 1339 (11), we have been led to an alternative explanation of the phenomenon. According to this view, all particles are taken into phagocytic cells in an essentially similar fashion. The cell membrane is invaginated and the particles are enclosed in phagosomes. Primary lysosomes become attached to the phagosomes and discharge their enzymes into them. Provided that the membranes of the secondary lysosomes so formed remain intact, the cells are unharmed. However, if the enclosed particles can react with the lysosomal membranes and make them permeable, enzymes and particles will rapidly escape into the cytoplasm and damage the cells. Thus attention is focused upon interaction of the particles with lipoprotein membranes of phagosomes and of other biological systems. We have elsewhere (12) presented evidence that silica can interact with proteins and phospholipids and lyse red blood cells, whereas nontoxic particles do not have these effects. An analysis of the underlying physicochemical processes was also given.

In this paper cytochemical and electron microscopial observations of macrophages after uptake of silica are described. Conventional biochemical methods involving homogenization and assay of enzymes in particulate and supernatant fractions cannot be applied satisfactorily in the presence of silica. Even if added to normal cells in the cold, silica particles produce damage during the course of homogenization. However, Cohn and Wiener (13) have presented evidence that when lysosomal enzymes are released into the cytoplasm a proportion escapes from the cells into the surrounding medium. We have therefore assayed lysosomal enzymes in the medium of macrophages exposed to silica and other substances, and assume that these reflect, though not quantitatively, release of enzymes from lysosomes.

Material and Methods

Cultures of Macrophages.—Most experiments were carried out with mouse peritoneal macrophages. These were obtained by inoculating 3 ml of culture medium containing 10 IU heparin per ml into mice of strain VSBS/NIMR weighing 18 to 20 g. After brief massage the cells were recovered and dispensed in 1 ml volumes, containing 2×10^6 cells, into tubes with small cover slips. Cells were incubated in culture medium (medium 199, Glaxo Laboratories Ltd., Greenford, England, and 0 to 20% calf serum inactivated at 56°C for 1 hr) overnight before exposure to silica. In some experiments rabbit lung, alveolar macrophages prepared as described by Cohn and Wiener (14) were used.

Silica.—Four types of silica, kindly provided by Dr. I. Bergman of Sheffield were used: (*a*) "Snowit" silica particles (× 9947) of size range 15% 2.5 μ, 49% 2.5 to 5 μ, 30% 5 to

7.5 μ, and 5% 7.5 to 10 μ; (*b*) "Fransil" silica (× 5663), uncompressed, consisting of silica particles of diameter up to 0.3 μ; (*c*) Na-OH-etched Fransil (× 5843 from × 5663) of size range 0.1 to 0.5 μ; and (*d*) silica particles coated with aluminium according to Dale and King (15). All samples were heat-sterilized before being added as fine suspensions to culture media. Living cells were examined by time-lapse, phase-contrast cine-micrography in a warm chamber, and also by dark-ground microscopy.

Control Materials.—(*a*) Diamond dust, particle size 2 to 4 μ (Diamond Products, Sales, Ltd., London). This is not fibrogenic. (*b*) Highly purified carrageenan (Seaplant Corporation, New Bedford, Massachusetts). This sulfated polygalactase is fibrogenic (see reference 16, Williams). Polyvinylpyridine-*N*-oxide (P204, U33) was obtained through the courtesy of Professor H. W. Schlipköter, Düsseldorf.

Histochemical Methods.—Acid phosphatase staining by a modified Gomori method (17) was carried out on unfixed cells and cells fixed in 2% glutaraldehyde buffered to pH 7.0 with 0.1 M cacodylate for 10 to 20 min at 4°C. Controls were treated with sodium fluoride (10^{-2} M) to inhibit the enzyme. Indoxylacetate esterase staining was carried out as described by Holt (18). Fluorescence microscopy of cells (17) was performed after incubation of cells with 1 in 10^5 (w/v) acridine orange in culture medium for ($\frac{1}{2}$) hr at 37°C, incubation in fresh medium without acridine for 15 min, mounting in fresh medium, and activation with blue-violet light (460 to 500 mμ). Metachromatic staining for intracellular carrageenan was carried out by washing the cells with saline, fixing in 70% ethanol for 5 min, staining in 0.1% toluidine blue in 30% ethanol for 10 min, and mounting in buffered glycerol.

For histochemical recognition of polyvinylpyridine-*N*-oxide, the method of Jancsó (see reference 47, Pearse) has been used.

Electron Microscopy.—Macrophages were cultured in Petri dishes overnight and then treated with Fransil (100 μg/ml) to avoid difficulty in cutting sections, which was experienced when quartz was used. After time intervals from 2 to 24 hr treated cells and untreated controls were gently scraped off the glass with a silicone rubber "policeman", centrifuged, and fixed in Palade's solution (19) at 4°C. For histochemistry, cells were fixed in 2% cacodylate-buffered glutaraldehyde for 10 min at 4°C, washed, incubated with the Gomori reagent for 15 min at 37°C, washed, and postfixed in Palade's solution. Embedding was in Araldite, and sections were stained with lead according to Karnovsky (20).

Enzyme Determinations in Medium.—Cells were incubated in Petri dishes in standard medium which was replaced by medium lacking serum 2 hr before assay. This can maintain healthy cells for at least 12 hr. Acid phosphatase and β-glucuronidase were measured by conventional techniques (21, 22) using *p*-nitrophenylphosphate and phenolphthalein glucuronide as substrates, incubating 0.2 ml samples of medium in 0.1 M sodium acetate buffer, pH 5.0, at 37°C for 1 hr.

OBSERVATIONS

Light Microscopy.—Macrophages were exposed to silica of various types (10 to 100 μg/ml) in culture media and observed by time-lapse, phase-contrast cine-micrography and dark-ground microscopy. Control cells moved about, preceded by ruffled membranes, and passed freely alongside and sometimes over one another. Phase-contrast dense bodies, identified as lysosomes, moved about in a random fashion in the cytoplasm. Only the larger granules were seen in motion pictures, the movement of the smaller granules being so rapid that it was not recorded. When silica particles were added, they were rapidly engulfed. The particles remained in phagosomes in the cells, often in a paranuclear posi-

tion but sometimes in the more distal part of the cytoplasm. Cells that were engorged with large amounts of silica remained fully extended and showed apparently normal mobility. Within 2 hr nearly all the lysosomes were attached to phagosomes containing silica, and by 10 hr very few granules were left intact, although cell movement was unimpaired. When moving pictures were made after 15 hr, some cells were already dead, and the events associated with cell death were recorded. Some cells that were moving suddenly stopped and remained immobile for a while before the processes were withdrawn and the cells rounded up. In other cells there were violent threshing movements of the cytoplasm while the cells became rounded and eventually immobile. During the later stages there was a tendency for the cells to stick to another, and occasionally they fused to form giant cells.

Histochemical Changes.—Control, unfixed macrophages showed virtually no phosphatase staining (Fig. 1). This corresponds with previous observations (17, 23) that if the membranes of lysosomes are intact the substrate does not penetrate and give a reaction. In fixed macrophages, numerous lysosomes of varying size showed phosphatase and indoxylacetate esterase in the cytoplasm (Fig. 2). After uptake of silica, the particles were readily seen in the cytoplasm by phase-contrast microscopy or dark-ground illumination (Fig. 3). In unfixed cells within a few hours numerous lysosomes were seen clustered around phagosomes containing the dust (Fig. 4). Later the reaction product accumulated in dense masses around the silica particles, and by 15 to 18 hr enzyme had diffused into the cytoplasm of many cells, which were often rounded up (Fig. 5). This change progressed until by 30 hr virtually all cells were rounded up, diffusely stained, and dead. Similar changes, somewhat less marked, were seen after 100 μg/ml Fransil.

Vital staining with acridine orange confirmed this picture. In normal macrophages intense orange fluorescence of lysosomes of varying size was seen throughout the cytoplasm. These were sensitive to light and readily coalesced into large masses while under observation. 5 hr after uptake of silica, many of the lysosomes were clustered around the phagosomes containing silica, where they still showed orange fluorescence. Thereafter there was progressively less discrete orange fluorescence and more diffuse green fluorescence around the silica particles and later in the cytoplasm of the cells.

Carrageenan (20 to 200 μg/ml) was rapidly taken up, even in the absence of serum or basic protein, and readily demonstrated in the phagosomes by its metachromatic reaction (Fig. 7). After 4 hr the phagosomes in unfixed cells showed discrete granules of phosphatase reaction product, and by 6 hr there was marked and more or less homogeneous staining of the phagosomes. This picture remained unchanged for about 18 hr, most of the cells still being fully extended. By 30 hr many cells had rounded up and showed diffuse phosphatase staining. The cells were often stuck together, sometimes bridged by metachromatic-staining material.

Cells took up aluminium-coated silica or diamond dust in essentially the same fashion as silica, and phosphatase was discharged into phagosomes in the usual way. However, the great majority of the cells remained fully extended and mobile for at least 30 hr (Figs. 10 and 11). The phosphatase reaction product closely enveloped the particles in the phagosomes (Fig. 6) and did not apparently escape into the remainder of the cytoplasm or nucleus.

Electron Microscopy.—Normal macrophages showed the cytoplasmic structures that have been described by others (14, 24). These include short, filamentous mitochondria with well defined cristae and numerous vesicles and cisternae lined by smooth membranes extending outwards from the Golgi region to the periphery of the cell (Fig. 9). Many lysosomes were present, and could be identified by the accumulation of acid phosphatase reaction product, though it was never possible to stain all of them in any cell. The lysosomes varied in size and form, usually being surrounded by single membranes and having fairly dense, granular contents. Occasional multivesicular bodies were also seen. Ribosomes were for the most part free, although some were membrane associated.

After addition of silica particles (Fig. 8) these were seen in invaginations of the cell membranes and in phagosomes lined by single, or occasionally double membranes (Figs. 12 and 13). Lysosomes were seen closely associated with the phagosomes (Fig. 13), and possibly discharging their contents into the phagosomes. At this stage the cytoplasmic detail was well preserved. By 14 hr in a number of cells silica particles were seen apparently free in the cytoplasm and mitochondria were swollen and rounded (Fig. 14). By 25 hr many of the cells were severely damaged, with silica particles widely scattered through the cytoplasm and organelles no longer clearly identifiable (Fig. 15). In one preparation silica particles were seen in the nucleus. It is worth emphasizing that there were considerable differences in the time taken to induce cytotoxic effects. Some cells showed obvious damage by 14 hr, but even at 24 hr some cells remained in good condition.

Appearance of Lysosomal Enzymes in the Culture Medium.—Control cultures released only very small amounts of lysosomal enzymes into the culture medium even after 3 days, whereas the enzyme content of the cells increases in culture. Within 14 hr of uptake of silica particles, release of acid phosphatase and β-glucuronidase into the medium was demonstrable. Much more was released at 24 hr and less at 30 hr. The latter may reflect destruction or inactivation of enzyme. With diamond dust only small amounts of enzyme were released in the medium at 30 hr. With carrageenan there was some enzyme release at 24 hr but more at 30 hr.

Protective Effects of Polyvinyl-Pyridine-N-Oxide (PVPNO).—Schlipköter and his colleagues (25) found that PVPNO diminishes the fibrogenic effects of quartz in animals and protects cultures of L cells against cytotoxic effects of silica. This protection was confirmed in macrophages, using biochemical

methods of assessing cell integrity (26). We have confirmed that if mouse macrophages are treated with PVPNO considerable protection against cytotoxicity at 24 hr is found. Even if cells are pretreated with 1% PVPNO for 6 hr, followed by exposure to silica in fresh medium lacking PVPNO, considerable protection is seen. The fate of PVPNO and silica in the cells was therefore of interest. Cells were incubated for 6 hr at 37°C with 4% (w/v) PVPNO, washed with fresh medium, and examined with the histochemical technique for PVPNO. Numerous round cytoplasmic bodies containing PVPNO were seen, which suggests that there is uptake of this polymer into macrophage phagosomes in a manner analogous to the previously described uptake of Triton WR 1339 in liver lysosomes (11). If silica was added afterwards in the absence of PVPNO and the cells incubated for a further 2 hr at 37°C, then silica particles were seen in cytoplasmic vacuoles together with PVPNO. Acid

TABLE I

Release of Lysosomal Enzymes from Macrophages into Culture Medium

Measurements of enzyme activity as described in the text at different times after addition of silica or carrageenan.

Time after addition, *hr*	14		24		30	
Enzyme units.....	Phosphatase	Glucuronidase	Phosphatase	Glucuronidase	Phosphatase	Glucuronidase
Control.....	0.11	0.04	0.13	0.12	0.16	0.10
Silica, *100 μg/ml*.....	2.75	2.98	6.58	5.32	1.80	1.13
Silica + PVPNO.....	0.52	0.33	0.83	0.77	1.34	0.82
Carrageenan, *100 μg/ml*	0.12	0.09	1.03	0.97	3.6	2.76

phosphatase staining of unfixed cells treated with PVPNO alone, or PVPNO and silica, showed staining of the secondary lysosomes, but very little diffuse staining of cytoplasm even at 24 hr. Leakage of lysosomal enzymes into the cytoplasm was also much less marked after exposure to silica and PVPNO than with silica alone (Table I).

DISCUSSION

It is clear that the initial uptake by macrophages of toxic particles or substances (such as silica or carrageenan) is not noticeably different from that of nontoxic particles (diamond dust or aluminium-coated silica). In both cases engulfment and formation of phagosomes, with discharge of lysosomal enzymes into phagosomes, follows essentially the same pattern. However, the subsequent events are markedly different. Cells that have taken up diamond dust remain healthy for at least 30 hr, whereas cells that have taken up silica quite rapidly succumb. Histochemical studies of the cells with engulfed nontoxic particles

show that lysosomal enzymes remain confined within the phagosomes, and only very small amounts of these enzymes are released into the medium. After uptake of silica, diffuse cytoplasmic staining of lysosomal enzymes is seen and the enzymes are detectable in the surrounding medium. Electron micrographs show that silica particles are at first confined within phagosomes whereas later they are distributed throughout the cytoplasm. About the time that silica particles are seen in the cytoplasm, other morphological changes are apparent. Early changes include swelling and rounding of mitochondria and eventually there is complete disintegration of cytoplasmic structure.

These observations suggest that the integrity of the lysosomal membrane plays a large part in determining whether an ingested particle is toxic. Any particle that can react with constituents of the membrane so as to make it permeable may bring about release of lysosomal enzymes into the cytoplasm. There is evidence that other procedures which make lysosomal membranes permeable, such as exposure to bacterial toxins (27) or treatment with visible light after dyes are taken up by lysosomes (28), can have damaging effects on cells. These may be aggravated by direct interaction of the released particles with cytoplasmic constituents.

This interpretation of silica toxicity is supported by observations that silica can react with protein, phospholipid, and artificial or natural biological membrane systems whereas nontoxic particles such as diamond dust do not react in this way. We have recently presented evidence that the main reaction likely to occur under physiological conditions is hydrogen donation from the phenolic hydroxyl groups of silicic acid in the formation of complexes with hydrogen acceptors such as suitable oxygen or nitrogen atoms in living tissues. Such reactions with secondary amide (peptide) groups of proteins are demonstrable experimentally (12), and these could account for interaction of silicic acid with protein films (29) and the denaturation of proteins and rendering them susceptible to tryptic digestion as described by Scheel and his colleagues (30). However, stronger interactions are demonstrable between hydrogen donors and phosphate ester groups of phospholipids (12). The fact that silicic acid and silica particles are hemolytic (12, 31), whereas nontoxic particles are not, shows that silica can induce permeability changes in biological membrane systems, presumably as a result of interaction with phospholipid or protein.

The protective effects of PVPNO are readily explained on the same basis, because PVPNO is a powerful hydrogen acceptor and readily forms hydrogen bonds with silicic acid (12). Silicic acid or silica particles in the presence of PVPNO are much less hemolytic than in the absence of the polymer (12, 31). Hence it is not surprising that if silica particles and polymer are ingested together by macrophages, the cells are protected. When erythrocytes are treated with PVPNO and washed, there is only slight protection against silica hemolysis (12, 31), so the polymer does not remain associated with red cell membranes.

However, macrophages, unlike red cells, ingest PVPNO, so that the substance can remain in phagosomes which fuse with those containing silica. This is not surprising, since Gordon et al. (32) found that lysosomes prelabeled with colloidal iron fused with others enclosing DNA-protein coacervates labeled with colloidal gold. Likewise, we have observed that if macrophages are exposed first to asbestos and then after some hours to silica, the asbestos and silica particles are demonstrable within the same phagosomes. Thus PVPNO can coat the silica particles before they have time to damage phagosomal membranes. It is worth noting that the silica particles when first ingested are covered with a layer of denatured plasma protein (as can be shown by agglutination with appropriate antisera, see reference 8). Presumably this layer has to be digested away before the silica particles can react with the phagosomal membrane. Silica particles coated with lipid or protein show less interaction with membranes than free particles (31). If silica is added to cells in the presence of high concentrations of serum, its cytotoxic effect is significantly retarded.

The effects of carrageenan will be discussed elsewhere, but it appears that they are similar to those of silica although the cytotoxic effects on macrophages are more slowly exerted than those of silica. The highly acidic groups of the polymer may not be involved in their toxicity (33), but the mechanism of action remains to be fully analyzed.

We have found that silica particles released by death of macrophages have virtually undiminished cytotoxicity when added to fresh cultures of macrophages. Hence in vivo a cyclical killing of these cells would be expected. The relationship between death of macrophages and the fibrogenic effects of silica also remain open to analysis. Suggestive results were obtained by Curran and Clark (34), who implanted agar or egg white into the peritoneum of rats. These brought about death and dissolution of phagocytic cells in the vicinity, and within 4 days there was a well defined fibroblastic reaction around the dying phagocytes with laying down of connective tissue fibres. We have found that repeated subcutaneous injection of frozen and thawed macrophages from the same inbred mouse strain induces local deposition of fibrous tissue. It would thus appear that death of macrophages provides sufficient stimulus for mobilization of fibroblasts and fibrogenesis, although which components of macrophages are involved, and whether there is local transformation of connective tissue cells into fibroblasts or invasion from outside, remain to be determined.

Observations of other investigators, though not primarily concerned with lysosomes, are consistent with our interpretation of silica toxicity. Rilke and Kessel (35) found no detectable change in fixed preparations of acid phosphatase distribution in cells shortly after uptake of silica, whereas Webster et al. (36) found a decrease in macrophages 48 hr after exposure to silica. The methods used would be unsatisfactory for demonstration of redistribution of enzymes such as we have described, and the late changes could be due to loss of enzymes

through intracellular destruction or passage into the medium. Policard et al. (37) following the cellular reaction after injection of silica into rats, showed uptake into phagocytic cells, including neutrophils. They drew attention to disappearance of neutrophil granules in the process. Since neutrophil granules are lysosomes (38), these findings provide additional evidence of lysosomal changes in cells exposed to silica.

There is evidence that acid phosphatase activity increases in vivo in response to silica administration, both acutely in mesenchymal cells of liver and spleen (39) and in developing silicotic nodules (40). The former could be due to lysosomal enzyme activation, the latter to immigration of cells with high acid phosphatase activity and activation.

If lysosomal enzymes play an important part in silica cytotoxicity and the consequent fibrosis, agents which stabilize lysosomal membranes would be expected to diminish these effects. Cortisone and chloroquine are known to stabilize lysosomal membranes and prevent release of lysosomal enzymes under a variety of conditions (41). There is good evidence that cortisone administration diminishes the fibrogenic effects of silica (42, 43) although it is without effect on established lesions (44). Inhibition of the proliferative response to silica by chloroquine has also been described (45).

Thus the available evidence suggests that lysosomes play an important part in silica cytotoxicity and fibrogenesis, and that it is unnecessary to invoke either an immune response by the host or direct stimulation of fibrogenesis by silicic acid to explain the pathogenesis of silicosis. Clearly other factors, such as infection, will often supervene and complicate the pathological picture. The pathological effects of other substances or particles may be explained on a similar basis, although the detailed manifestations and long term effects may be different. We have found, for instance, that the initial fate of asbestos in macrophages is similar to that of silica, and this is one of the substances apart from silica and carrageenan that is fibrogenic. In another situation, the uptake of microcrystals of sodium urate by phagocytic cells, and the inflammatory response so induced, appears to play an important part in the acute exacerbations of gout (46). These inflammatory effects are likewise reduced by prior administration of cortisone or other drugs. Similar amounts of sodium urate in solution do not damage phagocytic cells or induce inflammation and it seems in the case of both silica and urate that having the offending material in particulate form ensures its uptake by phagocytic cells and concentration in a vulnerable region, the lysosome. A great deal of attention has been given to effects of endotoxin and other substances on lysosomes (see reference 41) but relatively little to the importance of these organelles in the pathogenesis of disease by toxic particles or substances. Further work on this aspect of the problem seems to be warranted.

In the case of silica and urate the major uptake is by phagocytic cells (macro-

phages and polymorphonuclears, respectively), the effects on other cells being secondary and indirect. However, we have indications that other substances such as carrageenan are also taken up by fibroblasts, so that the possibility of direct stimulation of cell multiplication and fibrogenesis, perhaps by a lysosomal mechanism, must be considered. This interpretation is different from the previously held view that silicic acid or other substances act merely as templates on which fibrous tissue is deposited.

SUMMARY

Effects of silica, diamond dust, and carrageenan on mouse macrophages were studied by phase-contrast cine-micrography, electron microscopy, histochemical techniques for lysosomal enzymes and measurements of the release of lysosomal enzymes into the culture medium.

All added materials were rapidly taken up into phagosomes, to which lysosomes became attached. In all cases lysosomal enzymes were discharged into the phagosomes to form secondary lysosomes. Within 24 hr most of the silica particles and enzyme had escaped from the secondary lysosomes and lysosomal enzymes were found in the culture media. Most macrophages were killed by this time. With nontoxic particles (diamond dust, aluminium-coated silica, or silica in the presence of the protective agent polyvinyl-pyridine-N-oxide, PVPNO) ingested particles and lysosomal enzymes were retained within the secondary lysosomes for a much longer time, and cytotoxic effects were considerably delayed or absent altogether.

It is concluded that silica particles are toxic because they are efficiently taken up by macrophages and can then react relatively rapidly with the membranes surrounding the secondary lysosomes. The particles and lytic enzymes can then escape into the cytoplasm, producing general damage, and thence into the culture medium. It is suggested that hydrogen bonding of silicic acid with lipid and protein constituents of the membrane accounts for the induced permeability. Protective agents such as PVPNO are retained in lysosomes and preferentially form hydrogen bonds with silicic acid.

Carrageenan is demonstrable within macrophages by its metachromatic reaction. It brings about release of enzymes from secondary lysosomes, but much more slowly than does silica. Silica released from killed macrophages is as cytotoxic as the original preparation. It is suggested that repeated cycles of macrophage killing in vivo leads to the mobilization of fibroblasts and fibrogenesis characterizing the disease silicosis.

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EXPLANATION OF PLATES

PLATE 10

FIG. 1. Unfixed macrophages in culture, Gomori acid phosphatase reaction. No lysosomal staining is seen. × 1000.

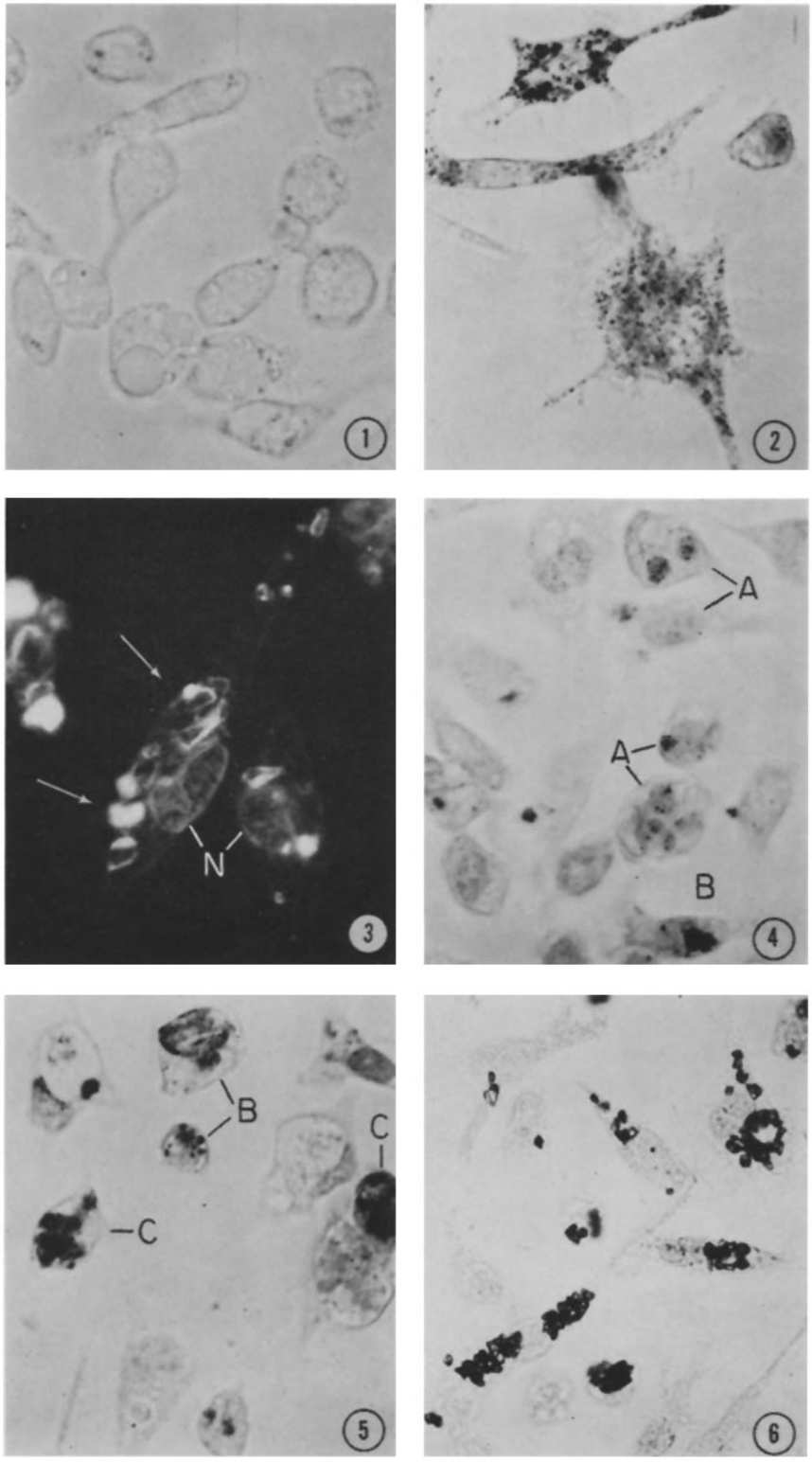
FIG. 2. Fixed macrophages in culture, Gomori acid phosphatase reaction. Numerous lysosomes of various sizes are seen. × 1000.

FIG. 3. Living macrophages showing ingested silica (arrows) by darkground microscopy. Nuclei are marked (*N*). × 1500.

FIG. 4. Unfixed macrophages stained for acid phosphatase 4 hr after ingestion of silica particles. In cells marked (*A*) discrete lysosomes are attached to phagosomes containing silica. In the cell marked (*B*) a phagosome shows accumulation of enzyme around a silica particle. × 1140.

FIG. 5. Unfixed macrophages 18 hr after ingestion of silica, acid phosphatase reaction. Marked phagosomal staining is shown in cells marked (*B*) and diffuse cytoplasmic staining, with rounding of cells marked (*C*). × 1140.

FIG. 6. Unfixed macrophages 30 hr after ingestion of diamond dust, acid phosphatase reaction. The cells are still fully extended, and the reaction product closely envelops ingested particles, with no diffuse cytoplasmic or nuclear staining. × 750.



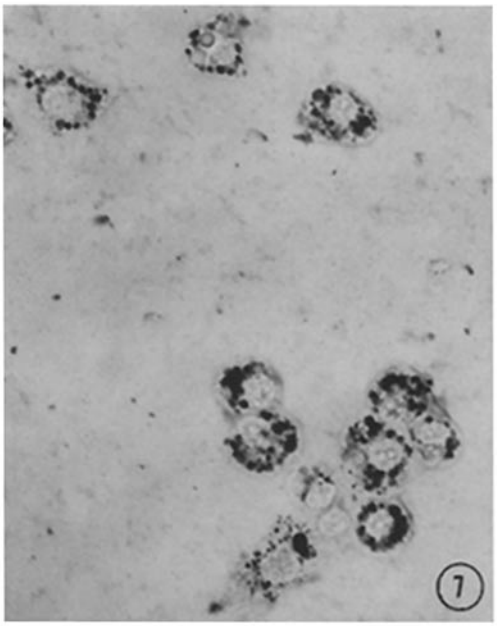
(Allison et al.: Cytotoxic effects of silica on macrophages)

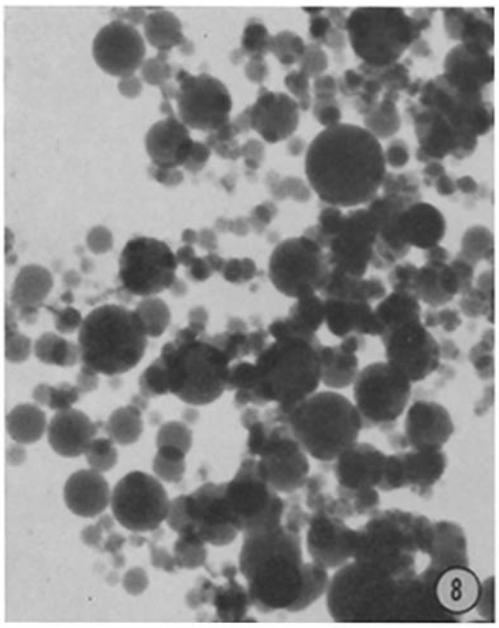
PLATE 11

FIG. 7. Macrophages 18 hr after uptake of carrageenan, toluidin blue. The ingested material is shown by the metachromatic reaction in secondary lysosomes. × 540.

FIG. 8. Electron micrograph of Fransil silica. × 72,000.

FIG. 9. Electron micrograph of an ultrathin section of a normal macrophage, showing nucleus (*N*), mitochondria (*M*), lysosomes (*L*), multivesicular body (*MB*), and elaborate smooth membrane vesicular and cisternal system. × 24,000.





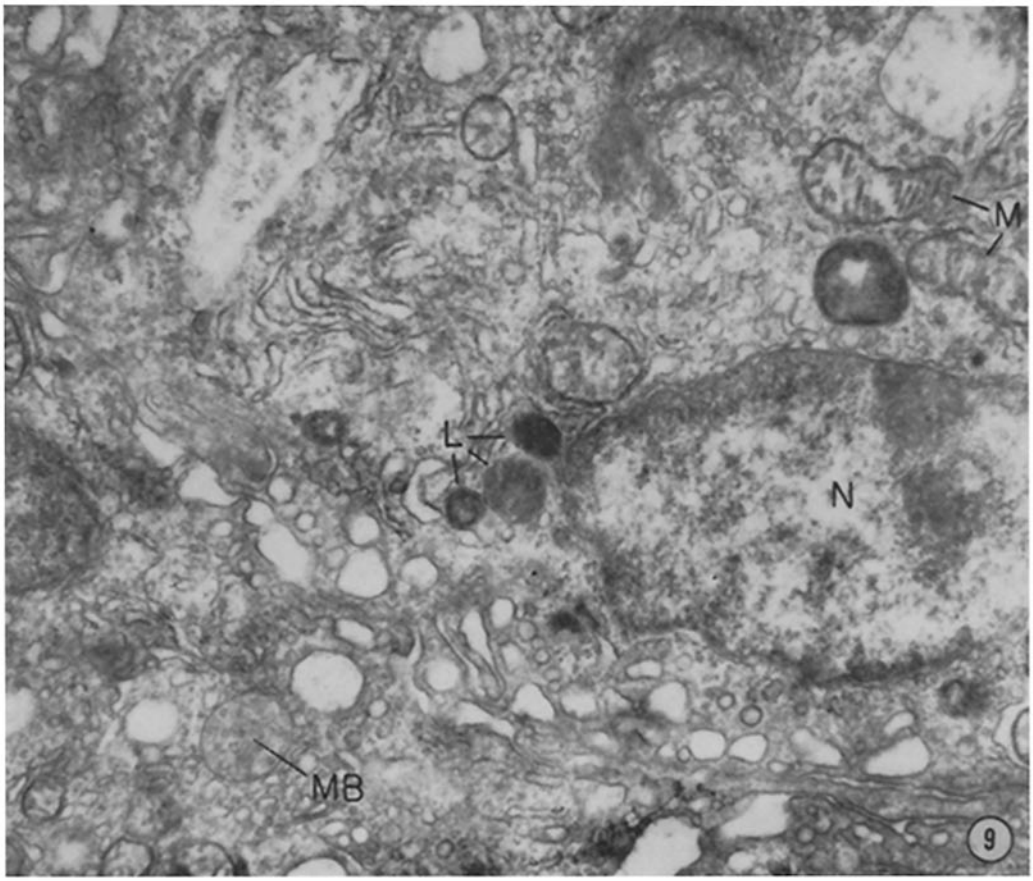


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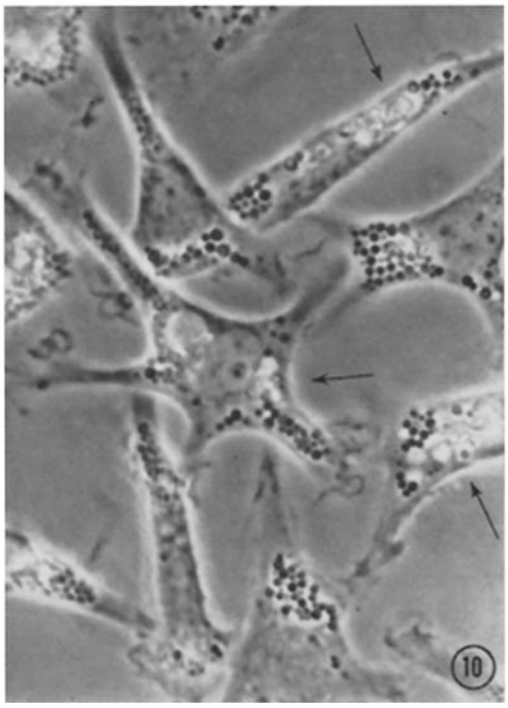
PLATE 12

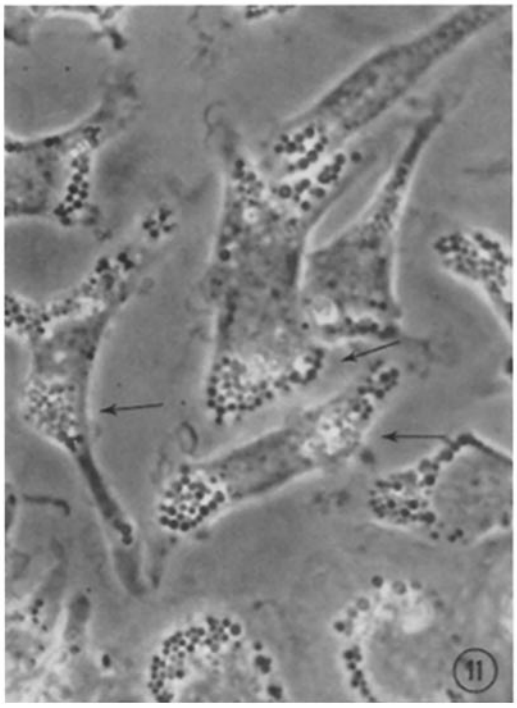
FIG. 10. Phast-contrast photomicrograph of living macrophages 24 hr after ingestion of diamond dust. Although lysosomes are clustered around ingested particles (arrows) the cells are fully extended and move about normally. × 1280.

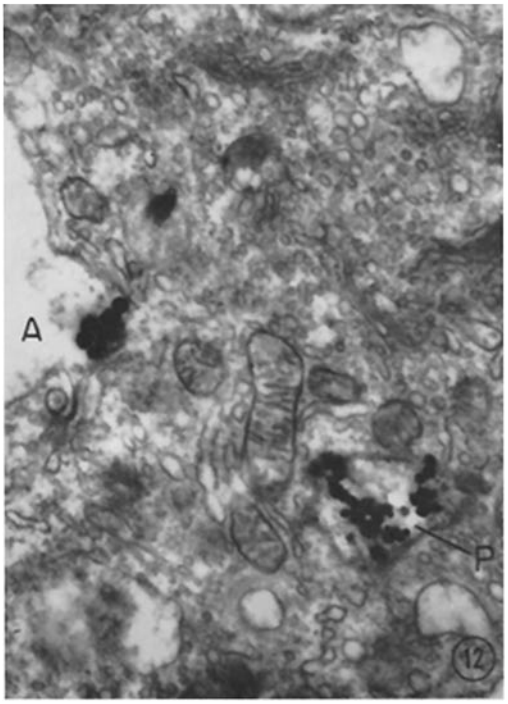
FIG. 11. As in Fig. 10 but after ingestion of aluminium-coated silica. × 1280.

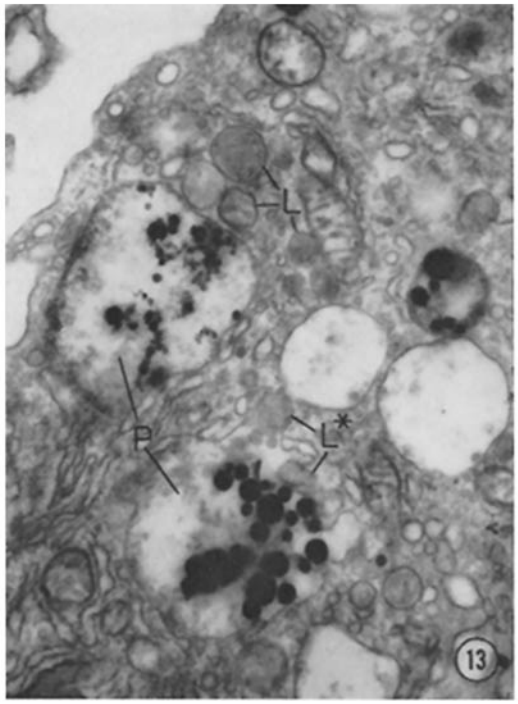
FIG. 12. Electron micrograph of thin section of a macrophage showing uptake of silica particles. These are taken in by endocytosis (*A*) and are found in phagosomes (*P*), in this case lined by a double membrane. × 24,000

FIG. 13. Electron micrograph of thin section of macrophage cytoplasm, showing ingested silica particles in phagosomes (*P*). Numerous lysosomes (*L*) are seen, some (*L**) attached to a phagosome and perhaps about to discharge their contents into it. Cytoplasmic structure is still well preserved. × 24,000.







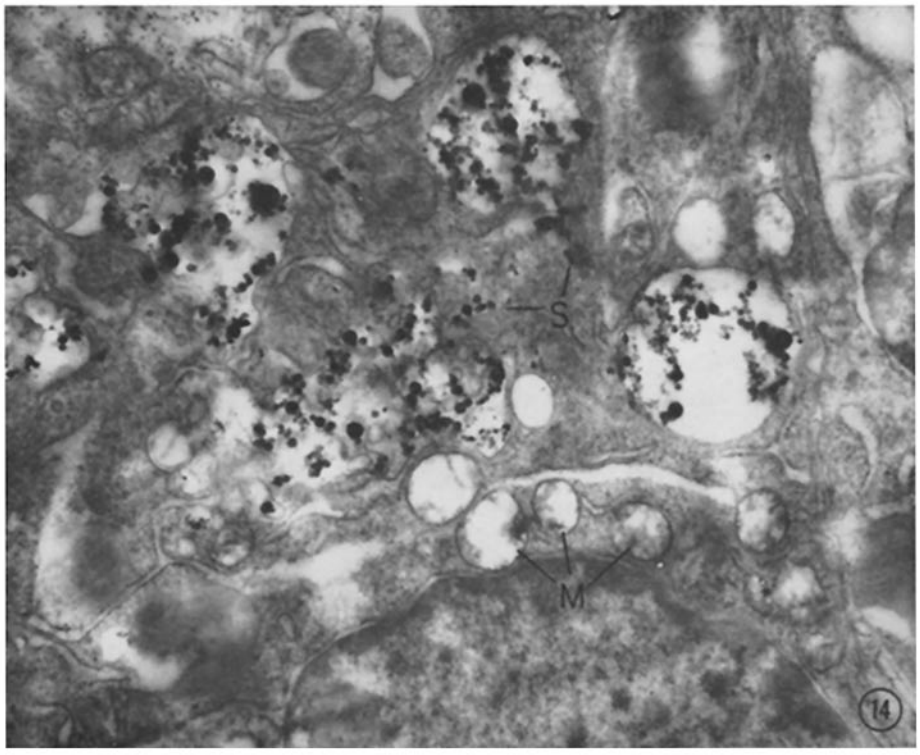


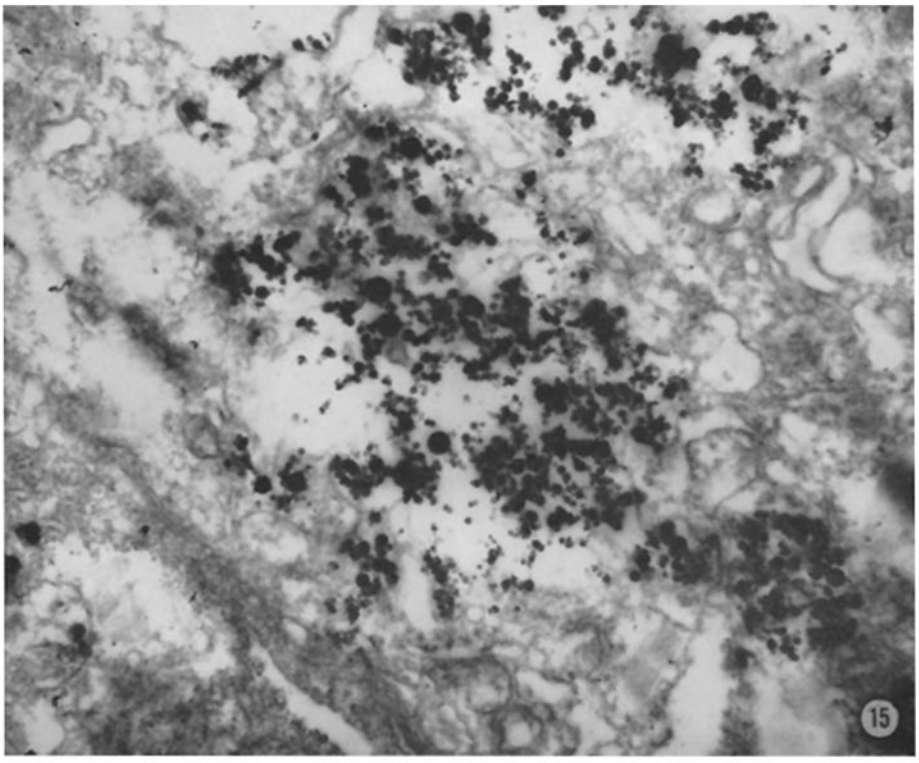
(Allison et al.: Cytotoxic effects of silica on macrophages)

PLATE 13

FIG. 14. Electron micrograph of a thin section of a macrophage 18 hr after ingestion of silica. Some silica particles are still in secondary lysosomes, others are apparently lying free in the cytoplasm (*S*). Mitochondria (*M*) above the nucleus are swollen and degenerating, and cytoplasmic detail is less clear than in the control (Fig. 9). × 24,000.

FIG. 15. Electron micrograph of a thin section of a macrophage 25 hr after ingestion of silica. The silica particles are scattered in the cytoplasm, the structure of which has been lost. × 24,000.





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