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Effect of macrophage inactivation on the neuropathology of lysolecithin-induced demyelination

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Summary. The effect of macrophage inactivation on lysolecithin-induced demyelination was studied. Adult rats were pretreated with a single intraperitoneal dose of 200 mg silica quartz dust, which selectively blocks the activity of circulating monocytes. Surgery was performed the following day and 2 μl of 1% lysolecithin were injected intraspinally. The animals were perfused with aldehydes at 8 to 60 days postoperatively. Tissues were examined by optical and electron microscopy. The injection of lysolecithin produced a localized demyelinating lesion. Myelin debris in controls was phagocytosed by invading macrophages. Axons within the lesion appeared denuded. At later stages remyelination occurred. When animals were pretreated with silica, the macrophage response was remarkably reduced. The clearance of collapsed myelin was delayed. Accumulation of fluid within the sheaths resulted in tissue oedema and persistent spongiform changes. At 28 and 60 days postoperatively, increased fibrillary astrogliosis was observed but did not appear to interfere with remyelination. The findings of this study suggest that (a) active participation of macrophages is essential for the attainment of demyelination in the lysolecithin model and (b) treatment with silica prevents complete demyelination but has no apparent effect on remyelination.

Keywords: demyelinating diseases, lysophosphatidylcholines, macrophages, silica

The injection of lysolecithin into the spinal chord of adult animals has served as a very useful model for studying demyelination and remyelination in the central nervous system (Hall 1972; Blakemore 1976). In a previous study on this model, dexamethasone was shown to reduce demyelination but delay remyelination (Triarhou & Herndon 1985). In order to evaluate the contribution of macrophages in the mechanisms underlying the phenomena observed, we pretreated adult rats with silica quartz dust, which is known to be selectively cytotoxic for mononuclear phagocytes (Allison *et al.* 1966), and then induced demyelination with lysolecithin. Such a methodology would allow us to examine the effects of macrophage depletion upon the degeneration and regeneration of myelin in a chemically induced demyelinating lesion and, also, to estimate the benefits of reticuloendothelial blockade for the clinical management of demyelinating conditions.

Materials and methods

Animals. Six weeks old male Sprague–Dawley rats were obtained from Charles River

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Breeding Laboratories, Wilmington, Massachusetts. The animals were individually housed in plastic cages. Rat chow and water were provided *ad libitum*.

Surgery. The animals were anaesthetized with pentobarbital sodium (50 mg/kg injected intraperitoneally). Dorsal laminectomies were performed at the lower thoracic region of the spinal chord. A glass micropipette attached to a 10-μl Hamilton syringe was inserted into the chord using a stereotaxic micromanipulator. Two microlitres of 1% (w/v) solution of lysolecithin (L-α-lysophosphatidyl choline, Sigma Chemical Company, St Louis, Missouri) in sterile isotonic saline, marked with a colloidal carbon suspension, were slowly injected under manual control. The pipette was withdrawn, the wound sutured and the animals allowed to recover. Five milligrams of tylocine were administered intramuscularly for prophylactic antibiotic coverage. The day of operation was taken as day zero.

Treatment. Silica quartz dust (approximate particle size 5 μm) was sterilized with hot air at 100°C overnight and then suspended in sterile saline and sonicated for dissolution. A single dose of 200 mg silica was administered intraperitoneally on day -1. Control animals were injected intraspinally with lysolecithin, but not treated.

Histopathologic technique. Groups were taken at days 8, 12, 28 and 60. Each group consisted of two control and four silica-treated animals. Perfusion was carried out through the left ventricle of the heart, first with McEwen's saline solution (McEwen 1956) and then with a 2% glutaraldehyde–1% formaldehyde fixative in 0.1 M phosphate buffer (pH 7.4). Spinal chords were dissected and tissue blocks containing the lesions were immersed in the same fixative at 4°C overnight and subsequently impregnated with 2% osmium tetroxide in 0.1 M phosphate buffer for 2 h, stained *en bloc* with a 2% aqueous solution of uranyl acetate, dehydrated in graded ethanols and embedded in Epon. One-micrometre-thick sections were stained with Stevenel's blue for light microscopy (del Cerro *et al.* 1980). Thin sections were obtained with a diamond knife in a Reichert OmU2 microtome (Reichert Optische Werke A.G., Vienna, Austria), mounted on copper grids and stained with lead citrate (Venable & Coggeshall 1965). Electron microscopic observations were carried out at 60 kV in a JEM-T7 scope (Japan Electron Optics Laboratory Co., Ltd, Tokyo, Japan).

Liver and spleen blocks were also processed and embedded. One-micrometre-thick sections were stained with methylene blue—azure II—basic fuchsin (Humphrey & Pittman 1974) for comparative histopathology.

Peripheral white blood cell counts. Peripheral blood smears were stained with Wright's stain (Begemann & Rastetter 1972). Total white blood cell counts were obtained in a Coulter Counter. Monocyte differential counts were based on absolute numbers of monocytes per 1000 leucocytes.

Results

Acute demyelination phase.

Eight days postoperatively, axons within the lesion appeared stripped of myelin in control animals (Fig. 1). The clearance of debris and colloidal carbon took place by invading macrophages. In silica-treated animals, collapsed loops of myelin were seen within the area of the lysolecithin injection without evidence of phagocytosis (Fig. 2). The invasion of macrophages and their activity in stripping myelin off the axons were remarkably reduced. The clearance of degraded myelin was delayed. Accumulation of fluid within the sheaths resulted in tissue oedema and persistent spongiform changes. At the electron microscopic level, the myelin sheaths appeared widely distended. Large vacuoles developed within the sheaths and displaced portions of compact myelin or individual lamellae (Fig. 3).

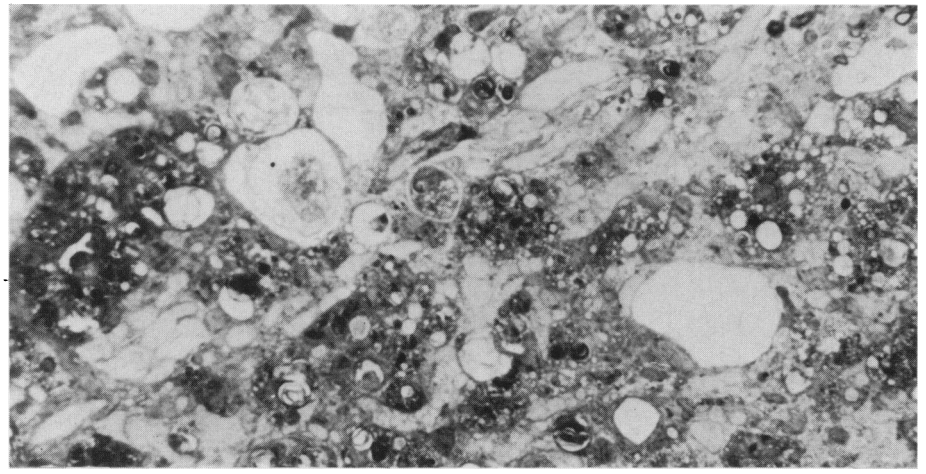

Fig. 1. Inflammatory infiltration and demyelination 8 days postoperatively in control. Invading macrophages have ingested all of the disrupted myelin within the lesion site and axons appear denuded. Stevenel's blue × 550.

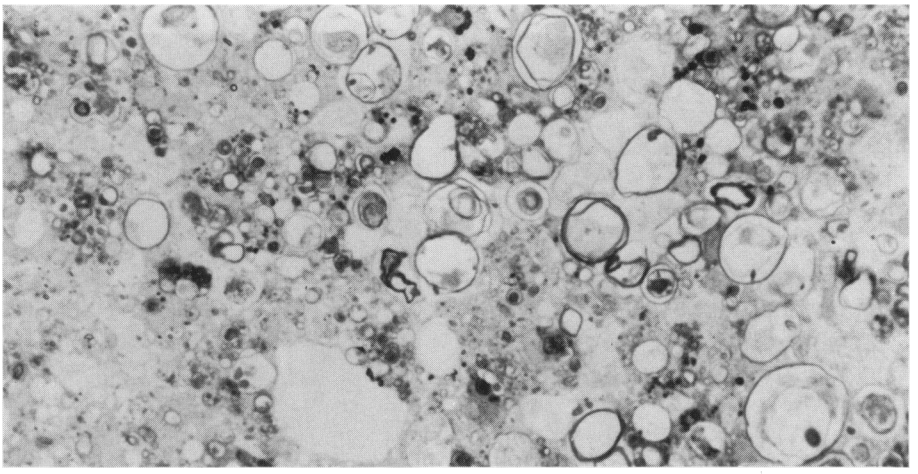

Fig. 2. Invasion of only a few macrophages compared to controls 8 days postoperatively in a silica-treated animal. Many loosened myelin sheaths have not been phagocytosed. The loops of myelin appear distended due to tissue oedema. Stevenel's blue × 550.

Remyelination phase

At 28 days, remyelination by oligodendrocytes and invading Schwann cells was a prominent feature of the lesions in control animals (Fig. 4). Myelin sheaths formed by Schwann cells were generally thicker than those formed by oligodendrocytes. In animals treated with silica, remyelination was evident as well (Fig. 5). However, colloidal carbon was removed from the lesion site slowly and astrocytic scarring was present. The thickness of remyelinated fibres increased with time (Fig. 6). At 60 days, all of the axons in the lesion were well remyelinated. Colloidal carbon was still present in the site of lysolecithin injection. Increased fibrillary gliosis was seen at this stage, but it did not appear to interfere with remyelination (Fig. 7).

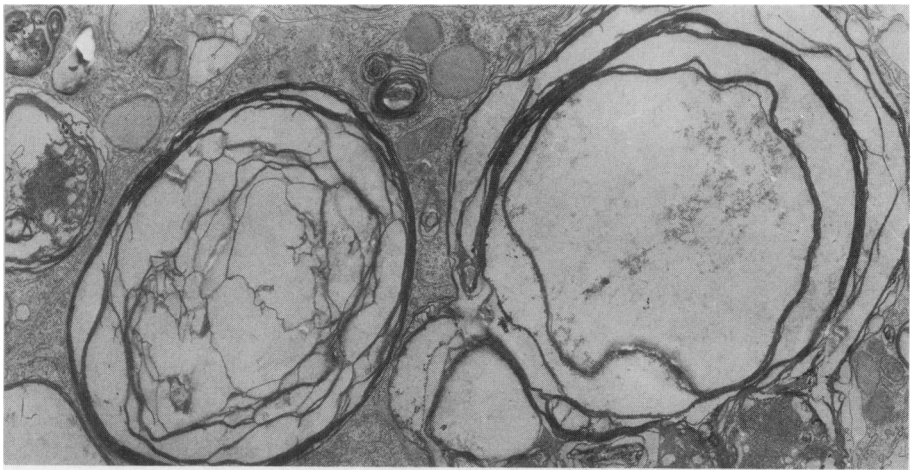

Fig. 3. Electron micrograph depicting the development of large intramyelinic vacuoles and the spongiform appearance of myelin 8 days postoperatively in a silica-treated animal. × 6630.

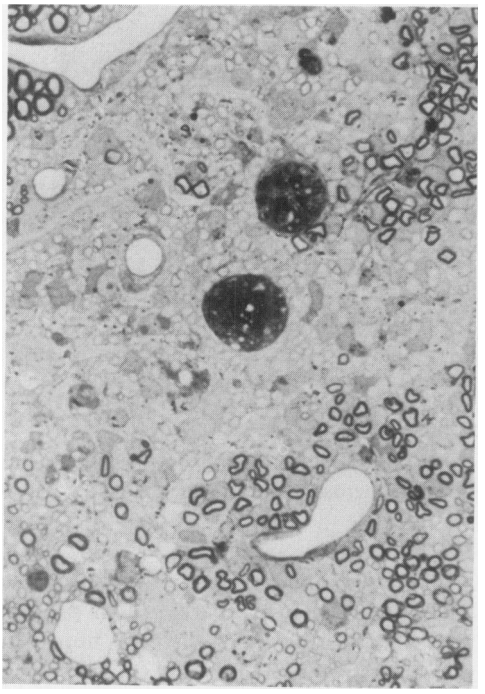

Fig. 4. Remyelination by oligodendrocytes (thinner myelin sheaths) and by Schwann cells (thicker myelin sheaths) 28 days postoperatively in a control. Stevenel's blue × 550.

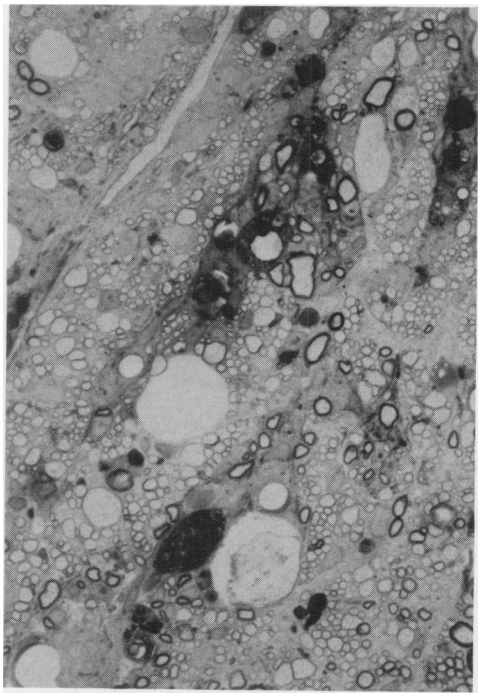

Fig. 5. Remyelination 28 days postoperatively in a silica-treated animal. Gliosis and persistence of colloidal carbon are seen within the site of lysolecithin injection. Stevenel's blue × 550.

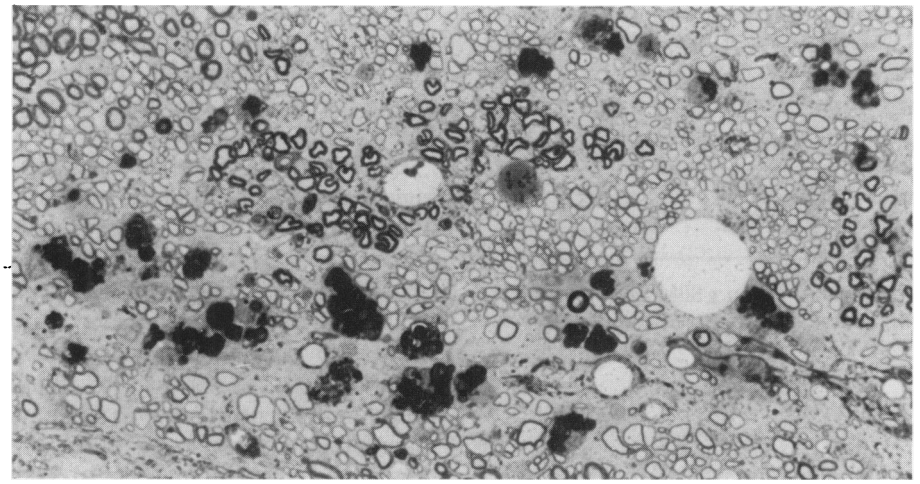

Fig. 6. Myelin thickness has increased by 60 days postoperatively in a silica-treated animal. Colloidal carbon has not been totally removed from the lesion as yet. Stevenel's blue × 550.

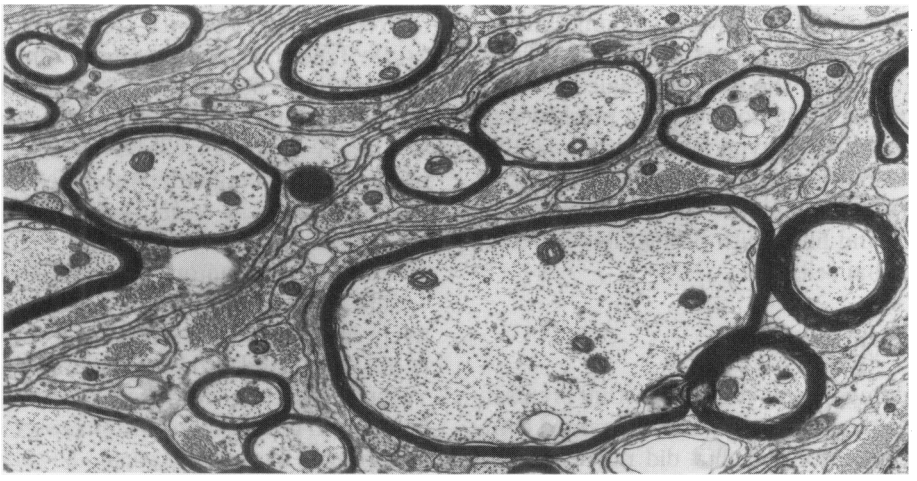

Fig. 7. Electron micrograph showing marked fibrillary astrogliosis in the space between remyelinated axons 60 days postoperatively in a silica-treated animal. × 7240.

The examination of peripheral blood from control and silica-treated animals revealed a reduction in the counts of circulating monocytes (Table 1). A quantitation of macrophages per area of damaged nervous tissue at each time point showed a marked reduction of their numbers in silica-treated groups (Table 2). Thus, the reduction of both circulating monocytes and of those gaining entry into the area of the lesion provided an index of the degree of macrophage suppression. Such a correlation seemed necessary, since the intraperitoneal injection of silica quartz as a procedure for macrophage suppression may not always be quantitatively predictable *in vivo*.

Table 1. Counts of peripheral white blood cells in control and silica-treated animals 24 h following treatment

Group	Total WBC/mm³	Monocytes (%)
Control	5650±1150	2.9±0.5
Treated	6950± 920	0.8±0.2

Numbers are mean±SEM from a group of two control and four treated animals.

Toxicity

Hepatosplenomegaly was found in treated animals at 28 and 60 days. The histologic picture of the liver was characterized by the eruption of multiple granulomata in the capsule of Glisson and in the hepatic lobules. Multinucleated foreign-body-type giant cells with ingested quartz were predominant inside the granulomatous lesions. Histologic examination of the spleen revealed reactive hyperplasia of the follicles and marked reticulo-endothelial hyperplasia with plentiful free macrophages in both the red and white pulp. The malpighian corpuscles were indistinct and filled with proliferated reticulo-endothelial cells.

Discussion

The findings of this study show that pretreatment with silica quartz dust prevented complete demyelination, but did not exert any apparent effect on remyelination. Some of the advantages of utilizing the lysolecithin model of demyelination in these experiments, as well as the implications of the findings, are discussed.

Lysolecithin favours the transition of biological membranes from the bilayer into the micellar phase, thus acting as a fusogen (Lucy 1970). However, the demyelinating activity of lysolecithin appears to be due not to its general detergent action, but to be triggered by specific biochemical interactions (Low *et al.* 1983). On the other hand, local injury to the central nervous system elicits a macrophage response of haematogenous origin (del Cerro & Monjan 1979). Thus, lysolecithin-induced demyelination represents a chemical and not an immune-mediated model. Furthermore, there is immunocytochemical (Prineas & Graham 1980), electron microscopic (Prineas & Kwon 1983) and neurochemical evidence (Cammer *et al.* 1978; Trotter & Smith 1984) supporting a primary lytic effect of macrophages on myelin membranes, in addition to the clearance of already existing cellular debris. Leukotrienes and prostaglandins secreted by macrophages (Rankin *et al.* 1982; Razin *et al.* 1980) may also be involved in the pathogenesis of inflammatory demyelination.

Silica quartz dust is known to be selectively toxic to macrophages *in vitro* (Allison *et al.* 1966) and *in vivo* (Von Behren *et al.* 1983). A toxic effect of silica particles on lipoprotein molecules of the phagolysosomal membranes has been implicated. Forty-eight

Table 2. Counts of macrophages per mm² of damaged area in control and silica-treated animals

	Survival time postoperatively (days)			
Group	8	12	28	60
Control	544.6±43.4	412.9±7.3	358.9±43.2	224.8±28.3
Treated	242.6±30.5	190.8±14.6	152.2±14.6	56.5±12.7

Each number represents the mean ± SEM of four to eight measurements in two to four animals.

hours after silica administration reticulo-endothelial function was shown to be still depressed (Nash *et al.* 1980). Given the fact that lysolecithin exerted most of its toxicity in the first 4 h following injection and that silica was administered on the previous day, the lytic activity of the lysophosphatide concurred within the temporal boundaries of reticulo-endothelial blockade.

The biological activity of the silica in the present experiments was demonstrated by a reduction in the numbers of both circulating monocytes and of those migrating into, and/or proliferating in, the area of demyelination. Morphological evidence for de-damaged tissue was provided. Moreover, the lesioned tissue was provided. Moreover, the neuropathology of silica-treated animals was characteristically different from controls during the acute phase to the point of being pathognomonic of the treatment. In a sense, the histologic picture in controls was produced by the action of lysolecithin and the participation of blood-borne macrophages as well, resulting in active demyelination. In silica-treated animals, the histologic appearance was produced by lysolecithin alone, since macrophages were suppressed by the quartz, leading to a spongiform-like myelinopathy. The latter may be either a reversible condition or proceed to demyelination on chronic exposure to toxic agents (Lampert 1983). Our findings lend further support to the idea that macrophages contribute to the myelin destruction in addition to clearing up debris, and that their active participation is essential for the attainment of demyelination in the lysolecithin model.

Treatment with silica has been used in other models of demyelination by other investigators. Intraperitoneal injections were shown to protect Lewis rats against both experimental allergic encephalomyelitis (Brosnan *et al.* 1981) and experimental allergic neuritis (Tansey & Brosnan 1982). Those effects were attributed to a direct toxic effect of silica on macrophages. However, inhibition of lymphocyte functions by silica has been reported, varying according to preparation, particle size or route of administration (Wirth *et al.* 1980). The results obtained in the lysolecithin model may be safely attributed to macrophage and not lymphocyte suppression, since allergic reactions are not involved in the pathogenesis of demyelination in that system.

Regarding the role of polymorphonuclear leucocytes in the induction of the inflammatory response, it is known that the initial migration of those cells into the lesion accompanies the onset of acute inflammation; subsequently, it is followed by a transition to a predominantly mononuclear phagocytic influx (van Waarde *et al.* 1977). In lysolecithin-induced demyelination, Blakemore (1976) observed a few polymorphonuclear leucocytes at 2 days postoperatively. However, 1 week postoperatively, the inflammatory infiltrate consists mainly of macrophages, and polymorphonuclear leucocytes are generally absent from the lesions.

During the phase of remyelination, astrocytic gliosis was not seen in controls to the extent that it was seen in treated animals. The reasons for this reaction remain incompletely understood. It could be due to stimulation of gliosis by the prolonged presence of myelin breakdown products in the lesion. On the other hand, silica did not appear to affect the process of remyelination, in contrast to azathioprine or cyclophosphamide, which were shown to accelerate it, and dexamethasone, which was shown to retard it (Triarhou & Herndon 1984). Since silica is not likely to have any direct effect on myelin-forming cells, a differential action of the other immunosuppressive agents upon oligodendrocytes was suggested.

The suppression of immune reactions by silica is very useful for experimental purposes, but its toxic actions preclude clinical applicability. Nonetheless, temporary reticulo-endothelial blockade with non-toxic agents, such as phospholipid vesicles (Gregoriadis 1977; Proffitt *et al.* 1983), could be of considerable importance in the therapeutic management of human demyelinating diseases.

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