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Morphological Changes Induced by Dextran Sulfate 500 in Mononuclear Phagocytes of *Listeria*-Infected Mice

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Morphological changes involving mononuclear phagocytes in *Listeria*-infected mice after treatment with dextran sulfate 500 were investigated. Mononuclear phagocytes in livers and spleens, both circulating monocytes and fixed macrophages, showed uptake of electron-dense material. Mononuclear phagocyte changes were most pronounced within granulomatous lesions, where many phagocytes showed large membrane-bound inclusions and extensive cellular damage. It is concluded that dextran sulfate 500 selectively damages mononuclear phagocytes and that, in listerial infection, dextran sulfate 500 renders mononuclear phagocytes unable to express cellular resistance.

Dextran sulfate (DS) 500 (molecular weight, 500,000), a widely used immunological model substance (3, 4, 6, 8, 15), has been shown in mice to cause increased susceptibility to infections with the facultatively intracellular bacterium *Listeria monocytogenes* (7). Findings reported there suggested the main site of attack of DS 500 to be the mononuclear phagocyte.

In our experiments, morphological changes occurring within mononuclear phagocytes of DS 500-treated and *Listeria*-infected mice were analyzed by electron microscopy. Striking alterations in mononuclear phagocyte morphology were found, especially within granulomatous lesions, and many cells displayed overt signs of destruction, suggesting that damage to mononuclear phagocytes caused by DS 500 in vivo is the main reason for the decreased resistance to intracellular bacterial infection found after DS 500 treatment.

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MATERIALS AND METHODS

Mice, bacteriological techniques used, and administration of DS 500 have been described in (7). In short, CD-1 mice weighing 20 to 22 g were divided into two groups. One group received DS 500 (50 mg/kg) intraperitoneally, and the control group received 0.2 ml of an NaCl solution. After 24 h, both groups of mice were infected with about 5×10^3 viable *Listeriae*. Three animals each from the control and DS 500-treated groups were killed 15 min and thereafter every 24 h postinfection. Livers and spleens were removed aseptically, small sections were cut from each of the removed organs for electron microscopy, and the remaining portions were processed for bacterial counts. The sizes of the sections removed for electron microscopy were so small that correction for weight seemed unnecessary when bacterial counts were determined. The procedure for determination of bacterial counts has been described (17).

Electron microscopy. The sections removed for electron microscopy were fixed immediately in ice-cold glutaraldehyde solution buffered with cacodylate buffer at pH 7.4 (16). After fixing for 4 h, the specimens were washed in cacodylate buffer and subsequently put into Dalton's solution (5) for 2.5 h. After a brief washing step in buffer, the specimens were dehydrated in 30, 50, 70, 95%, and absolute ethanol, and, finally, propylenoxide, and embedded in Durcupan (Fluka). For preliminary orientation, sections 0.5 μm thick were cut with a glass knife on a Reichert ultramicrotome and stained with toluidine blue. Blocks were trimmed to size, and ultrathin sections were cut and collected on carbon-supported copper specimen grids. Sections were examined on a Zeiss EM GS 2 electron microscope using a 50-μm objective aperture.

RESULTS

Morphological changes involving mononuclear phagocytes of Listeria-infected mice. (i) Mononuclear phagocyte activation. In accordance with published evidence on peritoneal macrophages, we found changes typical of activation (2, 13) in the macrophage population of infected mice. Figure 1 shows a comparison between normal Kupffer cells (Fig. 1A) and Kupffer cells in the liver of a mouse with a 4-day-old listerial infection (Fig. 1B). The latter cells were distinguished by a contrast-rich cyto-

plasm, large vacuoles situated close to the cytoplasmic membrane, and development of a ruffled cytoplasmic membrane. Occasionally, phagocytosed *Listeriae* were recognizable (Fig. 2). Membrane activity appeared greatly increased as the cells protruded towards the vascular lumen, exposing a large surface area to the blood stream. The number of lysosomes was increased, the membranes of the endoplasmatic reticulum and the number of mitochondria were increased, and the Golgi apparatus appeared more pronounced than in control cells.

(ii) Granuloma formation. At the time macrophage activation became demonstrable in Kupffer cells of *Listeria*-infected animals, small cellular accumulations appeared in both livers and spleens that consisted of lymphoid cells and of cells that bore the characteristics of mononuclear phagocytes: a contrast-rich cytoplasm, a large indented nucleus that was often situated eccentrically, and large nucleoli (Fig. 3). In the liver, these cell accumulations—obviously microgranulomas—were situated within parasinusal areas and occupied spaces formerly occupied by parenchymal cells. Lymphoid cells and mononuclear phagocytes were often seen close together (Fig. 4).

Cellular changes in livers and spleens of DS-treated mice. A toluidine blue-stained, semithin sectioned preparation for light microscopy of livers in uninfected mice pretreated with DS 500 4 days previously showed that Kupffer cells had selectively taken up metachromatically staining material, which made them stand out in survey preparations.

A similar picture was obtained in livers of DS-treated animals with a 4-day-old listerial infection. However, in addition, numerous mononuclear phagocytes within granulomatous lesions displayed metachromasia. By electron microscopy, the cytoplasm of these cells showed inclusions containing electron-dense material. These inclusions were surrounded by membranes (Fig. 5). Lymphocytes, on the other hand, did not show such inclusions. Inclusions gave rise to vacuoles that could become so large as to take up most of the cytoplasmic space, or, on the other hand, inclusions led right on to the destruction and disintegration of the cell (Fig. 6). In the spleen, too, mononuclear phagocytes showed electron-dense inclusions and degenerative changes similar to those described in liver granulomas (Fig. 7). Occasionally, circulating monocytes were seen showing uptake of electron-dense material similar to that seen in Kupffer cells and mononuclear phagocytes within granulomatous lesions.

DISCUSSION

Cell-mediated immunity against infections with facultatively intracellular bacteria depends on collaboration between specifically reactive T-lymphocytes (9) and mononuclear phagocytes derived from bone marrow precursors (H. Hahn, submitted for publication). Mononuclear phagocytes express immunity after activation (2) and through granuloma formation (11, 14). Any interference with either modality would be expected to lower the efficiency of the antibacterial defense. DS 500 was shown in a companion paper (7) to interfere with this bicellular mechanism so as to render *Listeria*-infected mice more susceptible to the infection. The present paper describes an attempt to pinpoint the mononuclear phagocyte as the site of attack of DS 500.

Uptake of metachromatically staining, electron-dense material by circulating monocytes, Kupffer cells, and macrophages within granulomatous lesions of *Listeria*-infected mice as a consequence of DS 500 injection and the resulting extensive damage observed in these cells make it highly probable that mononuclear phagocyte damage is the reason for the inability of DS 500-treated animals to defend themselves against listerial infection.

In contradistinction to this interpretation, work performed both in vitro (4) and in vivo (3) has shown that DS 500 is taken up by peritoneal macrophages and Kupffer cells, respectively, without obvious ill consequences. Since, on the other hand, the main load of antibacterial resistance in the early preimmune phase of the infection rests with fixed mononuclear phagocytes of liver and spleen, and a weakening of the antibacterial resistance was observed by us already in the early stages of the infection, fixed macrophages in the livers and spleens of DS-treated and infected mice were probably rendered less able to kill bacteria without actually being themselves killed. This suggests that DS 500 interferes with antibacterial mechanisms in a more subtle way, possibly by interfering with the activity of lysosomal enzymes.

On the other hand, mononuclear phagocytes within granulomatous lesions appear to succumb as a result of DS 500 injection. This suggests that circulating monocytes (which are the cells eventually making up a granulomatous lesion [14]) might be more susceptible than fixed macrophages to the action of the chemical, either before entering the granulomatous lesion or afterwards. This notion, if true, would serve to explain the relatively short duration of DS action as compared with its prolonged

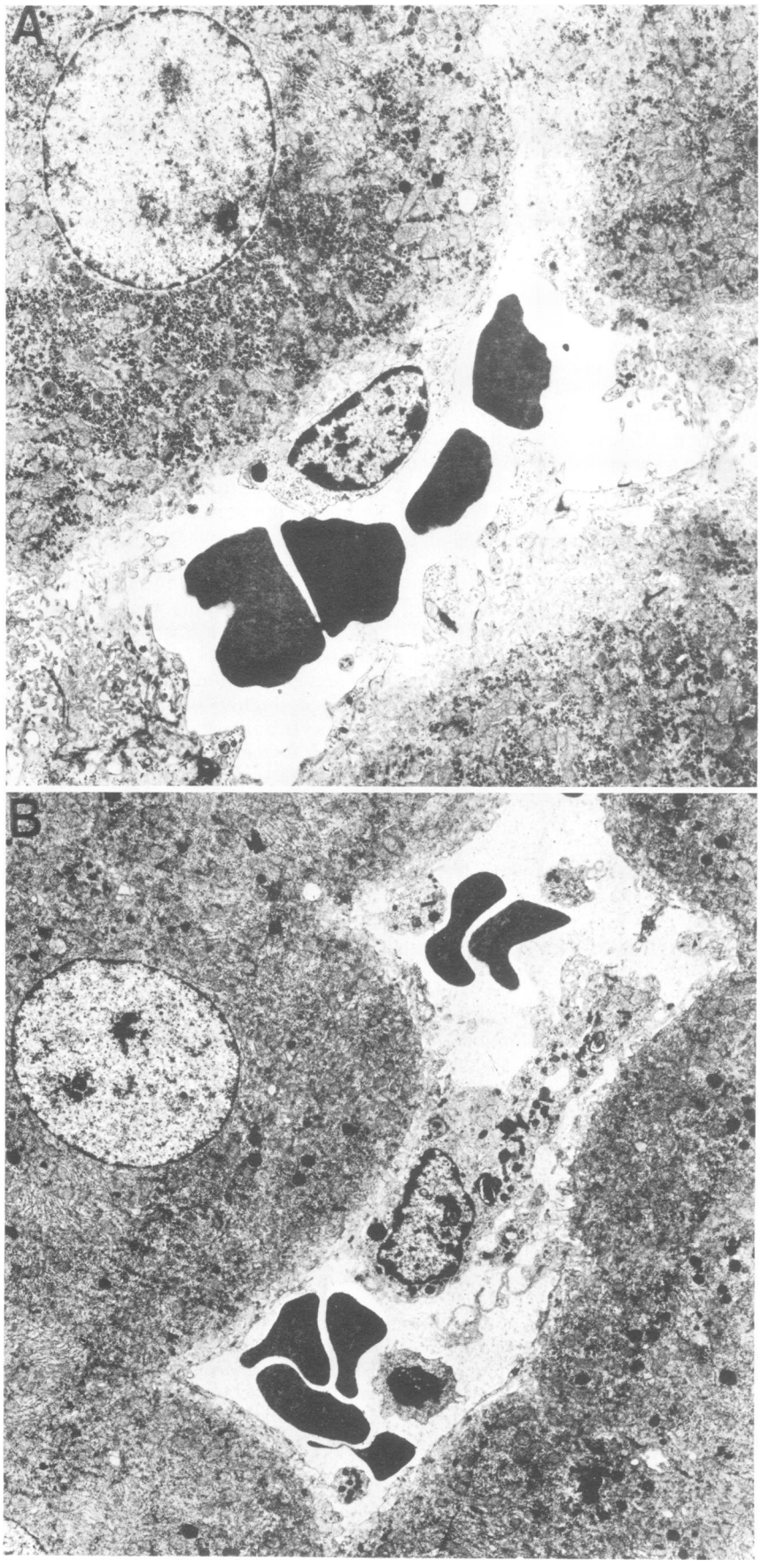

FIG. 1.

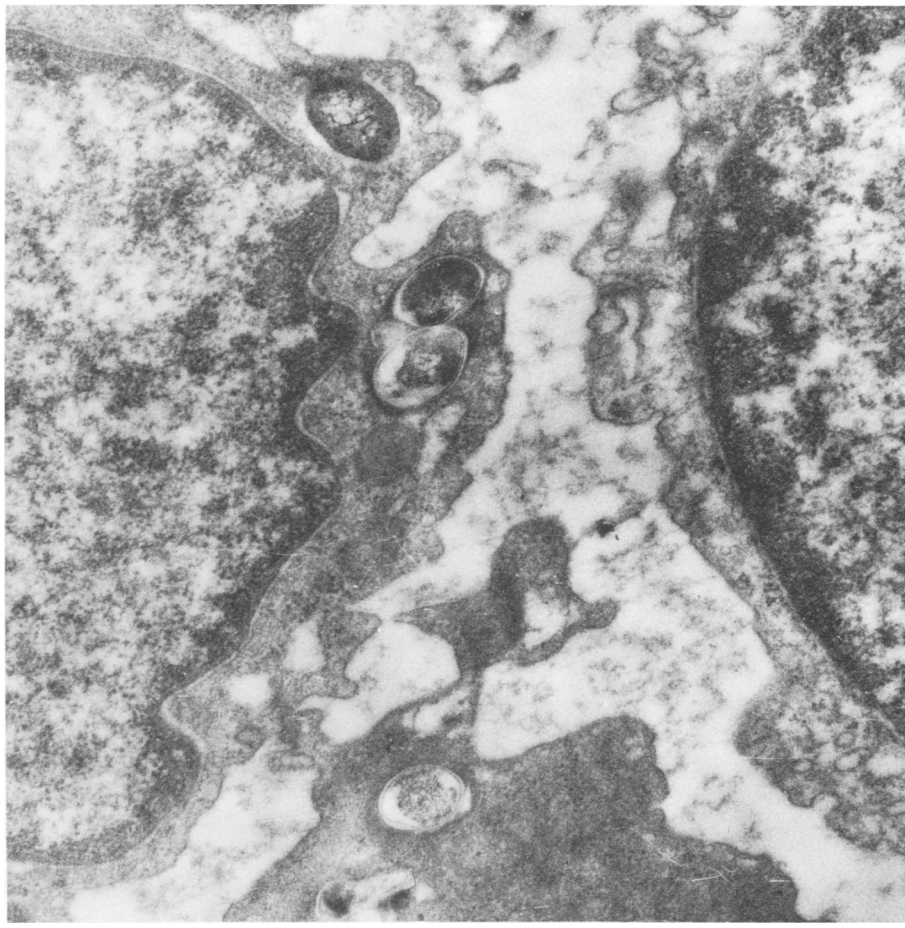

FIG. 2. *Kupffer cells with phagocytosed Listeriae, 4th day of a listerial infection.* ×*22,000.*

FIG. 1. *Kupffer cell in a normal control mouse (A) and in a mouse with a 4-day-old listeral infection (B).* ×*3,600.*

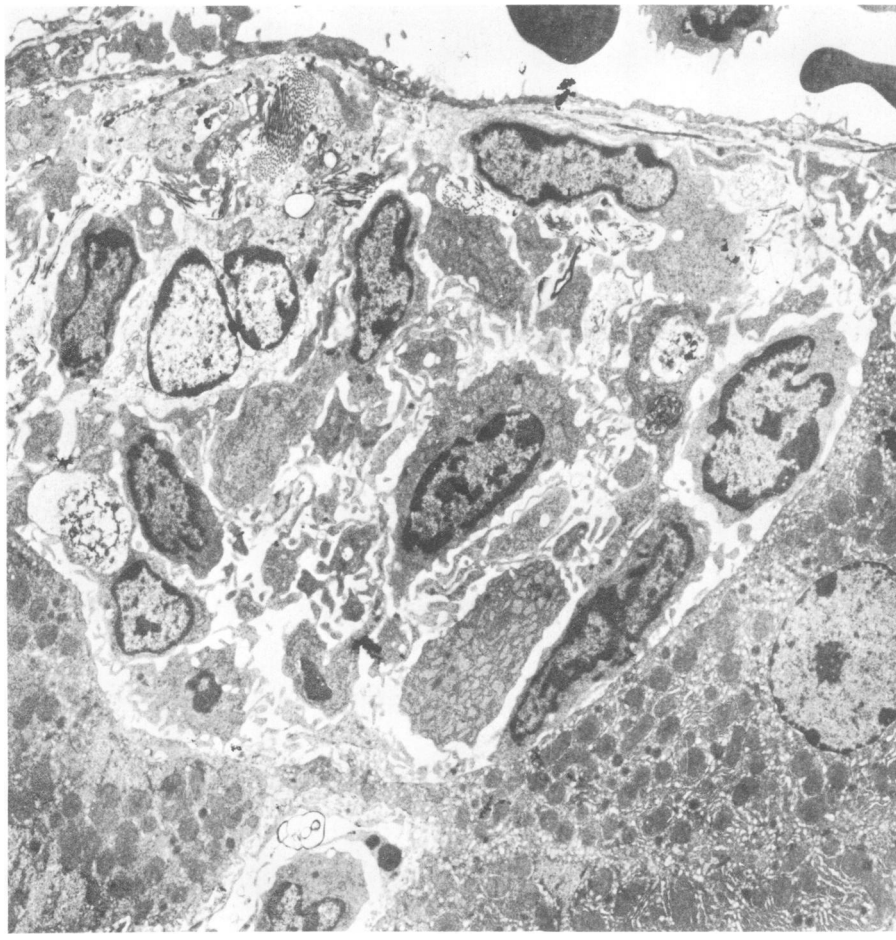

FIG. 3. *Beginning granuloma formation in the liver of a mouse with a 4-day-old listerial infection.* ×3,600.

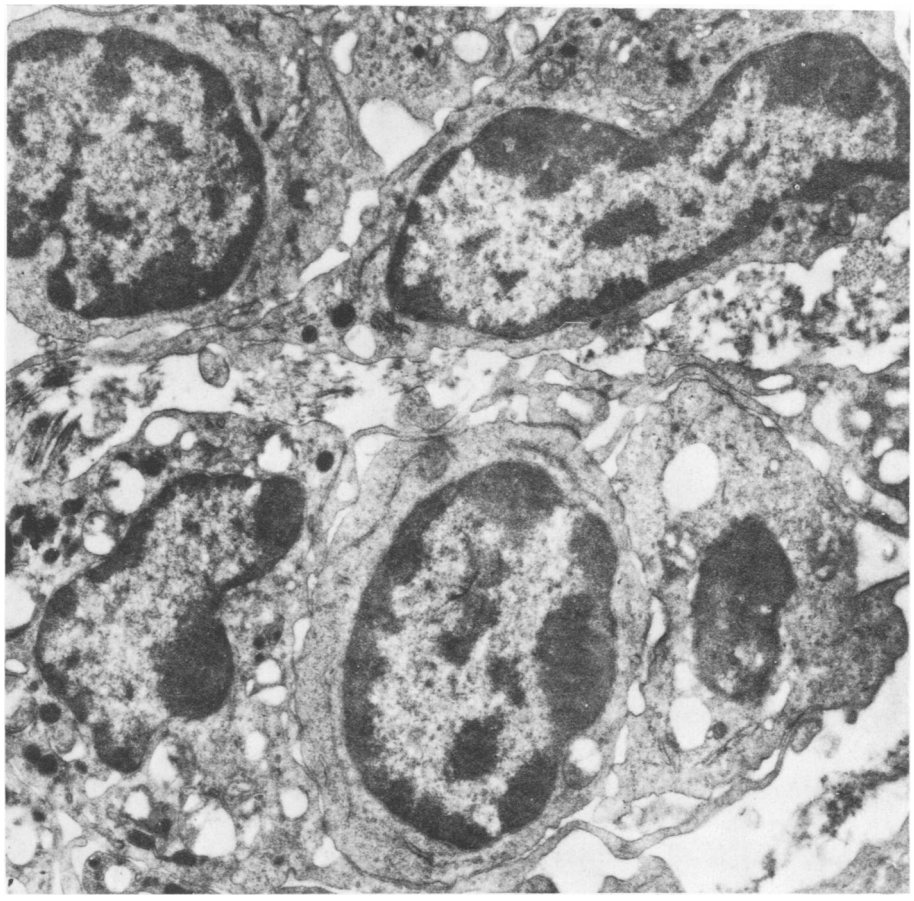

FIG. 4. *Detail from granuloma in a mouse with a 4-day-old listerial infection.* ×*9,000.*

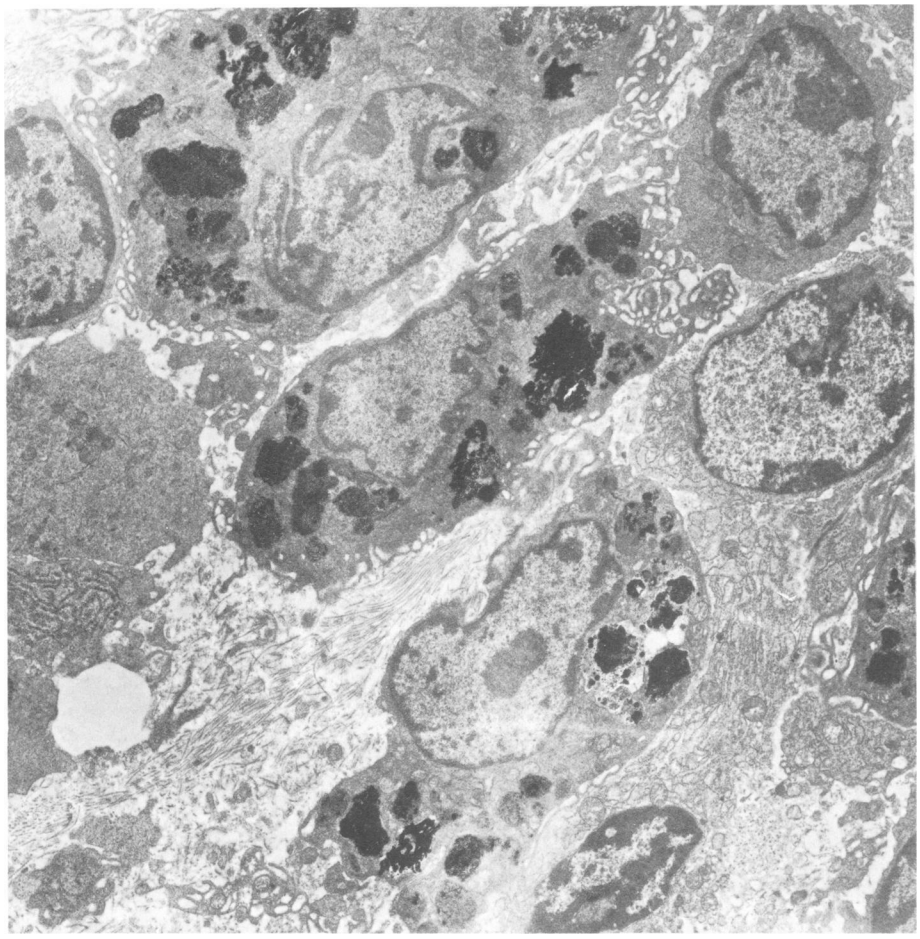

FIG. 5. *Macrophages in hepatic granulomatous lesion of a Listeria-infected mouse (4th day of infection) which was pretreated with DS 500 on the day of infection. Numerous electron-dense inclusions within macrophages.* ×*3,500.*

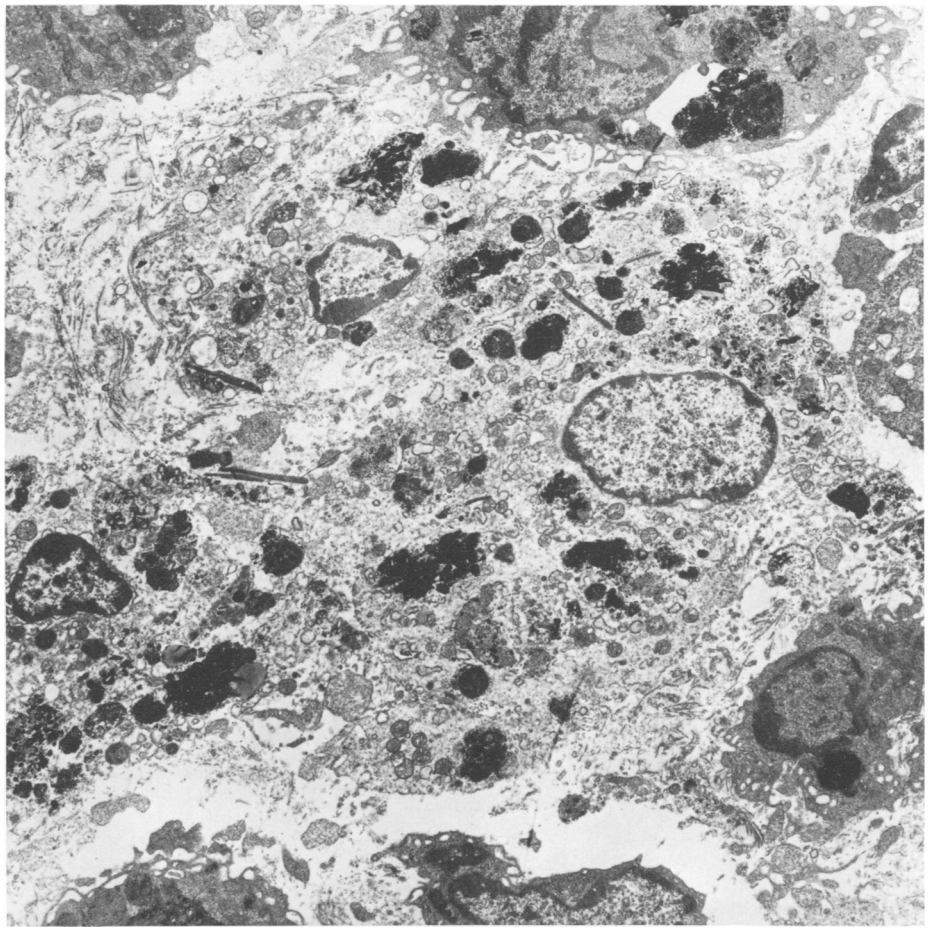

FIG. 6. *Disintegrating macrophages within the hepatic granulomatous lesion of a Listeria-infected mouse pretreated with DS 500 on the day of infection 4 days previously.* ×*3,500.*

persistence in the tissues. Presumably the injected material is quickly taken up by cells of the mononuclear phagocyte system and stays within fixed macrophages for prolonged periods of time without affecting the bulk of the remaining or newly formed mononuclear phagocytes. However, if DS 500 is injected at a time when granulomas are formed during bacterial infection and circulating monocytes become relevant to antibacterial defense as they are called upon to form granulomas, the DS load of circulating monocytes might, under the conditions of extravasal differentiation, prove a stress factor to these cells and cause cell damage.

Later on, as granulomas have consolidated, DS 500 might become less able to gain entry into lesions, since fewer freshly formed monocytes carry DS into the lesions. This would explain the fact that DS 500 given after the 3rd day of infection is much less effective in weakening cellular antibacterial resistance than when given during the first 3 days (7).

As to the mechanism of cellular damage, it appears that the cell is unable to contain the ingested material in membrane-bound vacuoles, possibly because DS 500 interacts with lysosomal membranes and damage might result from leakage of lysosomal enzymes. This conclusion rests on an observation by Allison et al. (1), who demonstrated uptake of carrageenan, a negatively charged polygalactose, by macrophages and subsequent leakage of lysosomal enzymes.

Our findings on the action of DS 500 serve to support the two-cell concept of cell-mediated antibacterial immunity (12) by demonstrating that selective damage to mononuclear phagocytes leaves the organism with no alternative in defending itself against facultatively intracellular bacteria. They also suggest that the effects

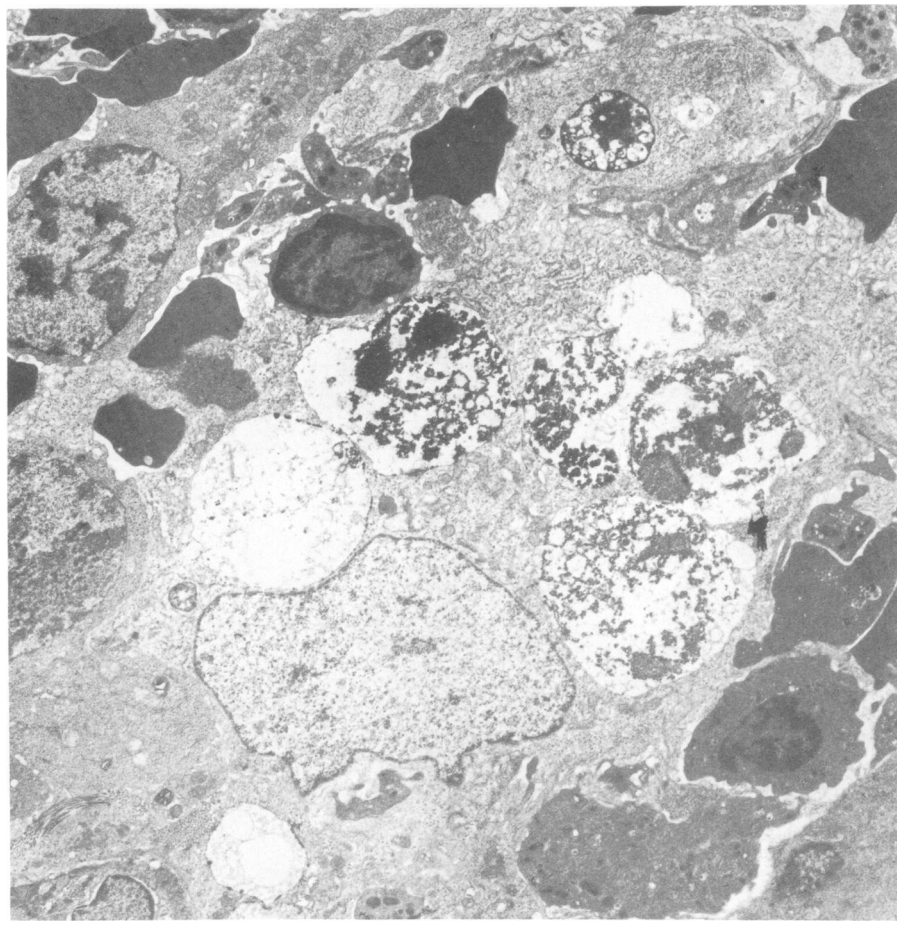

FIG. 7. *Phagocytic spleen cell with large heterogenous membrane-bound inclusions 4 days after DS 500 treatment and Listeria infection.* ×*3,500.*

of DS 500 on cells of the mononuclear phagocyte system should be remembered whenever the therapeutic use of DS 500 as an adjuvant is considered, since it appears that DS 500 might lead to decreased cellular resistance, not only against intracellular bacteria but possibly against infections with viruses as well, and possibly might even reduce antitumor resistance. The role of DS 500 in these experimental systems will be the subject of further investigations.

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