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Enteric vascular endothelial response to bacterial endotoxin

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Summary. The response of enteric vasculature to endotoxin was examined at the ultrastructural level using a murine model of endotoxin-induced acute diarrhoea. Morphological changes indicative of endothelial damage were evident as early as 15 minutes following endotoxin challenge. These changes, characterized by widening of intercellular spaces, increased microvillous projections and the appearance of stress fibres, preceded the leucocytic response. Endothelial damage increased with time, being associated with progressive degenerative changes in the plasma membrane, cytoplasm and organelles, ultimately leading to desquamation. These latter changes were temporally associated with margination of neutrophils and platelet adhesion to the denuded subendothelium. The venules were the primary site of these changes while the capillaries were the least affected. The arterioles were markedly constricted with minimal endothelial damage. These changes suggest that the enteric vascular endothelium may be an important target organ, and the resultant endothelial injury may have implications in host responses to endotoxin.

Keywords: vascular endothelium, bacterial lipopolysaccharide, endotoxin, microvessels, intestinal mucosa, murine

Endothelial lesions of the intestinal microvasculature whose morphological features resemble that of the local Schwartzman reaction have been demonstrated in patients with acute diarrhoea (Mathan & Mathan 1985). The prevalence of the lesions was unrelated to the type of aetiological agent, but correlated with clinical severity of diarrhoea (Choudari *et al.* 1985). Similar lesions of the gut microvasculature were also observed in a murine model of endotoxin-induced diarrhoea (Mathan *et al.* 1988), which could be abrogated with type-specific anti LPS antibodies or by polymyxin B, a lipid A antagonist, suggesting a causal role for LPS in the pathogenesis of diarrhoea. These earlier studies also suggested that the diarrhoea was mediated, at least in part, by endothelial response to endotoxin. We now report a detailed ultrastructural study of the changes in the endothelium of the intestinal mucosal microvasculature in the murine model of endotoxin-induced diarrhoea.

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Materials and methods

The murine model of endotoxin-induced diarrhoea and the light microscopic changes in the enteric microvasculature have been described in detail elsewhere (Mathan *et al.* 1988). Six to 8-week-old specific pathogen free LAC strain mice were infected with *Salmonella* 885, a *Salmonella typhimurium–Escherichia coli* hybrid strain which expressed *E. coli* 08 antigens. Seven days after oral infection with *Salmonella* 885 the mice were challenged subcutaneously with 50 μg of *Salmonella typhimurium*

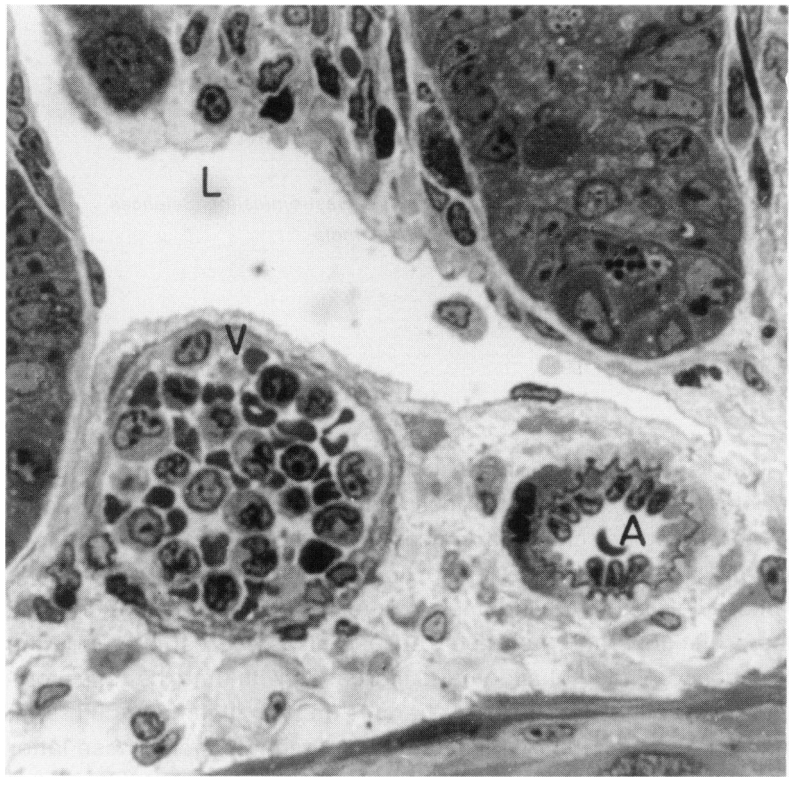

Figure 1. Lamina propria of upper small intestines (USI) 8 hours after LPS challenge—base of the crypt shows markedly dilated venule (V) and lymphatic (L) with endothelial denudation and constricted arteriole (A). Venule contains stagnated RBCs, WBCs and desquamated endothelial cells. × 370.

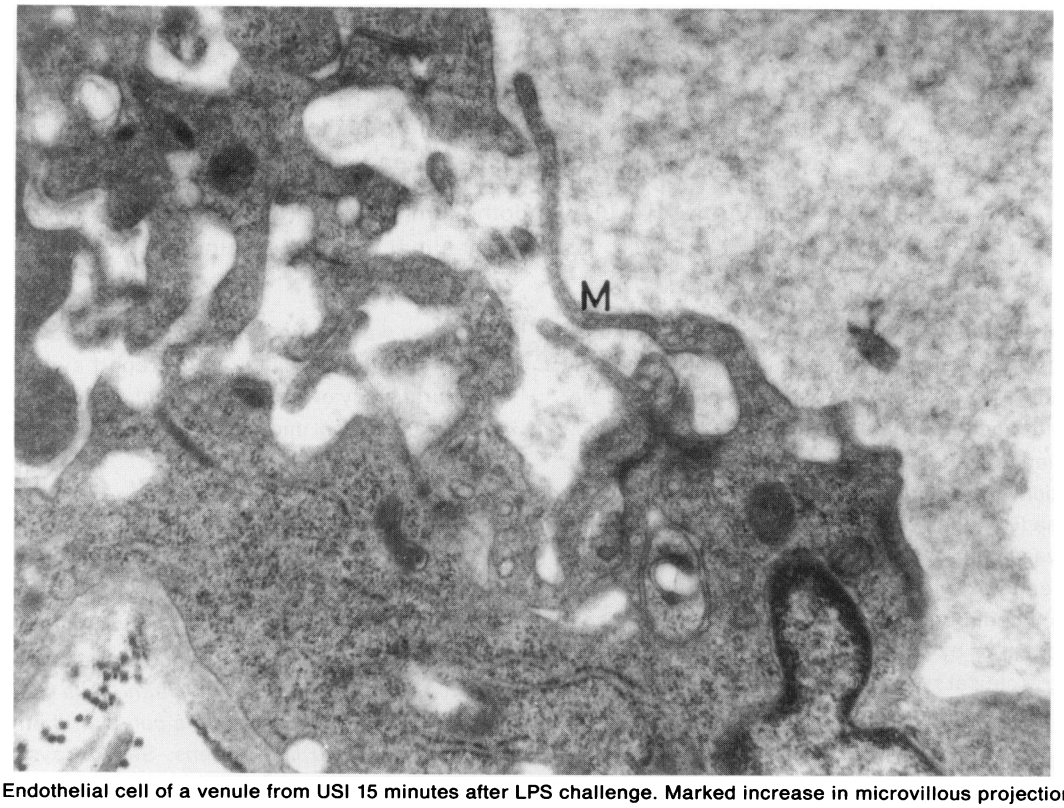

Figure 2. Endothelial cell of a venule from USI 15 minutes after LPS challenge. Marked increase in microvillous projections (M). × 28 000.

strain C5 LPS. Four hours after the LPS challenge changes were apparent in the venules of the lamina propria of the small and large intestine by light microscopy and 8–24 hours after the LPS challenge the animals developed diarrhoea. For detailed ultrastructural studies tissue was collected from control mice with subcutaneous saline injection and three LPS challenged animals each at fixed time points (15, 30, 60 and 90 minutes and 2, 3, 4, 8 and 24 hours post-LPS challenge) from the first loop of jejunum, the distal small bowel and the midtransverse colon, immediately fixed in 2% glutaraldehyde, post-fixed in osmium tetroxide, embedded in Araldite and 1-μm thick survey sections cut with a glass knife. Selected areas were thin sectioned on an LKB UM4 ultramicrotome with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a Philips EM 201C electron microscope.

Results

The endotoxin challenge resulted in lesions in the postcapillary venules, arterioles and lymphatics of the intestinal mucosal lamina propria (Figure 1). The extent and evolution of the lesions were different in these vessels in the jejunum, ileum and colon.

Venules

Fifteen minutes after the endotoxin challenge the venules in the upper small intestine were dilated, with widening of intercellular spaces and increase in microvillous projections on the luminal membrane of endothelial cells (Figure 2). By 30 minutes from challenge there was thinning of the endothelial cytoplasm and more marked widening of the intercellular spaces. Platelets were increased in the vessel lumen and they were found adjacent to the widened intercellular spaces. Early degenerative changes were present in the endothelial cells with blebbing of the luminal cell membrane of the endothelial cells. Occasionally endothelial cells were detached from the wall of the venule. Forty-five to 60 minutes after the challenge the damage in the endothelial cells was more marked with focal rarefaction of cell cytoplasm, swelling of mitochondria and dilatation of rough endoplasmic reticulum and the Golgi apparatus. Sub-endothelial oedema led to lifting of the endothelial cells and denudation (Figure 3). Platelet clumping, especially over widened intercellular spaces, and polymorphonuclear neutrophil margination were quite marked at this time. Fragments of endothelial cells were

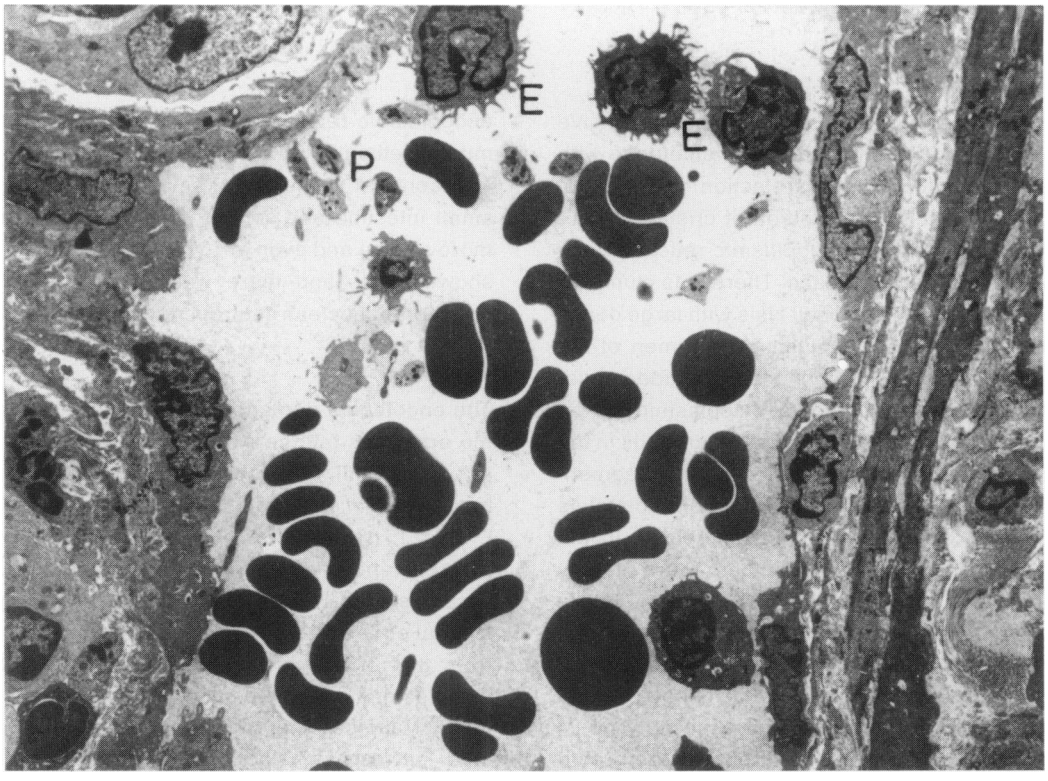

Figure 3. Longitudinal section of a venule from USI 60 minutes after LPS challenge. Denuded endothelial cell (E), platelet (P) and RBCs are seen in the lumen. × 2500.

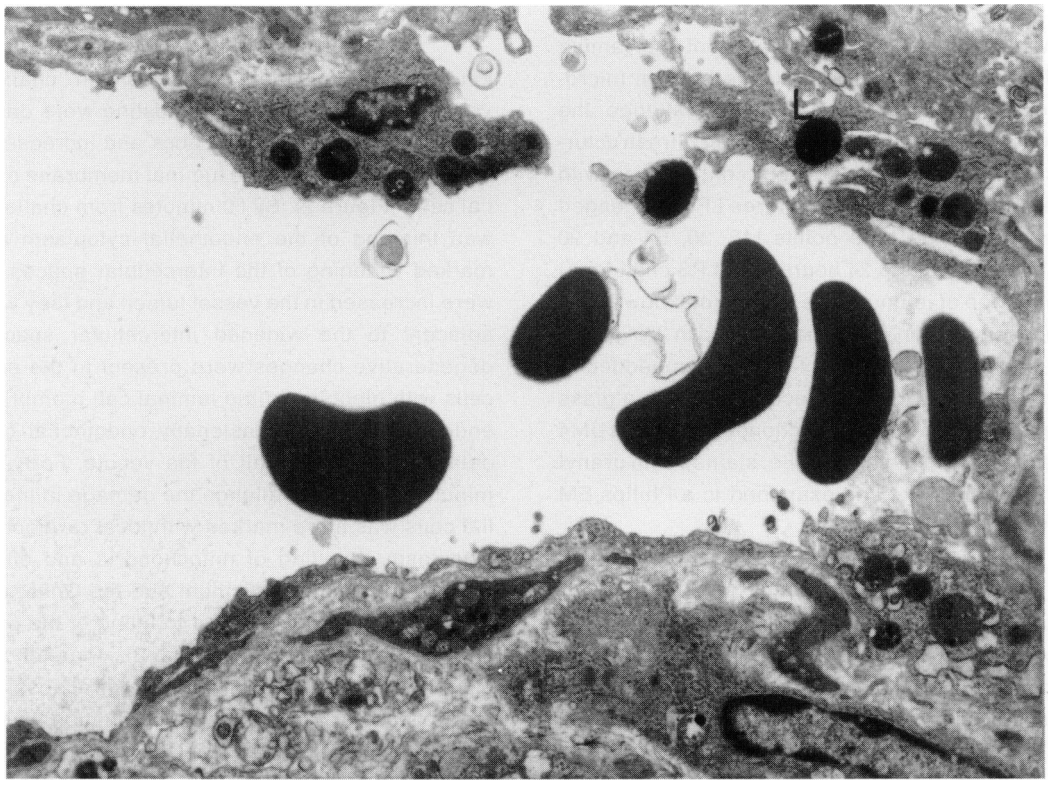

Figure 4. Venule from colon, 24 hours after LPS challenge. Endothelial cell shows marked degenerative changes and increase in dense lysosomal bodies (L). ×6000.

present in the lumen of the vessel. More extensive damage occurred after 90 minutes from challenge with more prominent cytoplasmic rarefaction, swelling of mitochondriae with loss of definition of cristae, prominent dilatation of the rough endoplasmic reticulum and widening of the perinuclear space. There was clumping of chromatin in the nucleii and cell lysis with large dense lysosomal bodies in the cytoplasm. The lumen of the vessel showed many cell fragments and denuded endothelial cells with prominent irregular cytoplasmic projections. Some of the polymorphonuclear neutrophils in the lumen showed signs of degeneration and hypersegmentation. There was an increase in the number of platelets and platelets were found to adhere to defects in the endothelial continuity. Some venules were totally denuded and lined only by pericytes (Figure 4). Stress fibres were present predominantly near the luminal border of the endothelial cells at all time periods. Even at 15 minutes an occasional endothelial cell showed mitosis but in the later time periods and especially by 24 hours attempts at regeneration and repair were prominent with cells showing hyperplastic changes indicated by increase in Weibel–Palade bodies, prominent rough endoplasmic reticulum and Golgi zones and increased mitotic activity (Figure 5).

In contrast to the upper small intestine, in the distal small intestine and colon the endothelial damage was more severe and even at 15 minutes the endothelial cell showed lysis and there was marked margination of polymorphonuclear neutrophils and platelet clumping.

Arterioles

The endothelial damage and lysis were less marked in the arterioles but there was striking constriction of the arterioles in all regions of the intestine throughout the 24-hour period of observation. The constriction of arterioles even at 15 minutes from challenge was in contrast to the marked venular dilatation (Figure 1). Large subnuclear vacuoles of varying size were present in many of the arterial endothelial cells. These vacuoles ranged in size from that of a mitochondrion to occupying the entire cytoplasm, pushing the nucleus to one side, usually the luminal aspect. The vacuoles were mostly single and clear but occasionally contained small vesicular fragments or myelin figures (Figure 6). Occasional vacuoles had a double layered membrane. This change in the

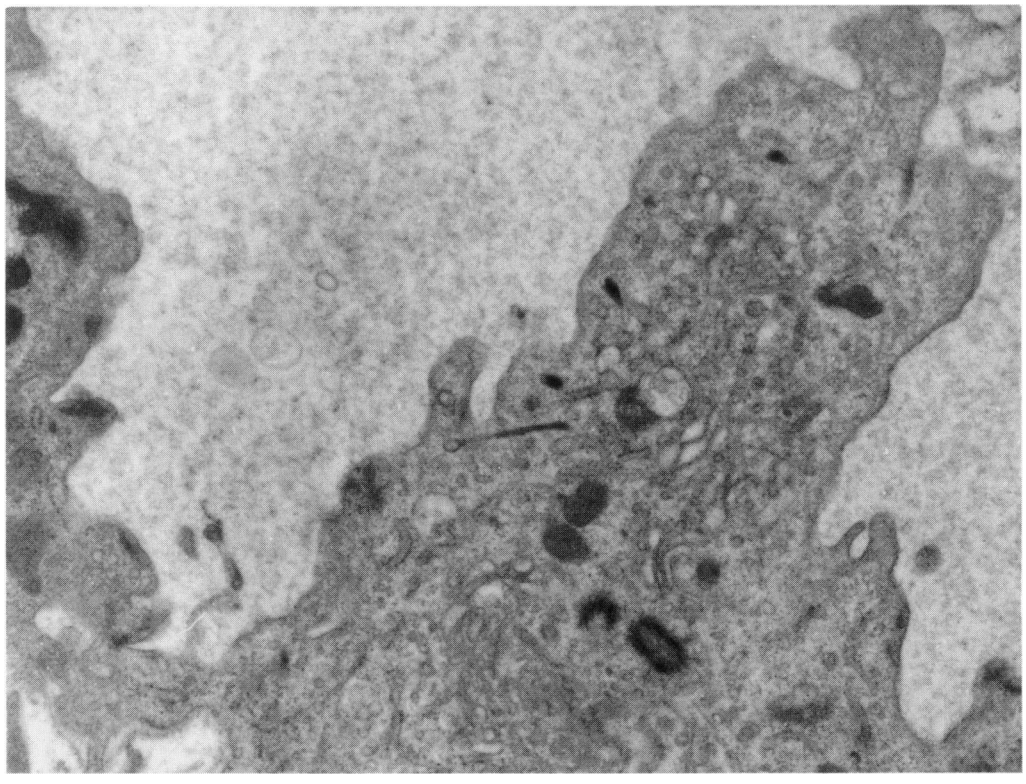

Figure 5. Venule from USI 8 hours after LPS challenge. Hyperplastic endothelial cell with increased Weibel–Palade bodies, prominent rough endoplasmic reticulum and Golgi apparatus. × 19000.

arteriolar endothelium was found in all the segments of the intestine from 15 minutes onwards and was maximal in the upper small intestine at 4–8 hours and in the other segments at 24 hours. In addition, actomyosin filaments appeared aggregated as long thick bundles, with periodic banding, organized parallel to the luminal cell membrane, both subluminally and towards the basal aspect of endothelial cells. These condensations were more frequent in cells with distorted shape (Figure 7). There was also accumulation of thinner vimentin-like fibres, forming parallel bundles in the centre of the endothelial cell cytoplasm. Frank endothelial cell degeneration similar to that in the venules was not found in the arterial endothelium, although an occasional cell showed mild degenerative changes.

Capillaries

Changes in the endothelial lining of the capillaries were minimal with occasional increase in luminal microvilli and no degenerative changes.

Lymphatics

The endothelial lining of the lymphatics also showed changes similar to that in the venules with prominent microvillous formation, cell degeneration, cytolysis and denudation which were maximal 2–8 hours after the endotoxin challenge.

The microvessels in the small intestine and colon of control animals were free from any change when examined by light or electron microscopy.

Discussion

The results of the present study confirm and extend our earlier observations of extensive enteric microvascular damage in a murine model of endotoxin-induced acute diarrhoea (Mathan *et al.* 1988). A temporal analysis of the progression of events leading to endothelial cell damage, and a comparison of regional differences at the ultrastructural level, are suggestive of specific pathogenetic processes.

The early onset of endothelial damage at 15 minutes post challenge which precedes the infiltration of monocytes or granulocytes is indicative of a primary effect of LPS on the enteric endothelium. Cytokines such as TNF-α and IL-1 secreted by activated monocytes and macrophages in response to LPS (Beutler & Cerami 1988;

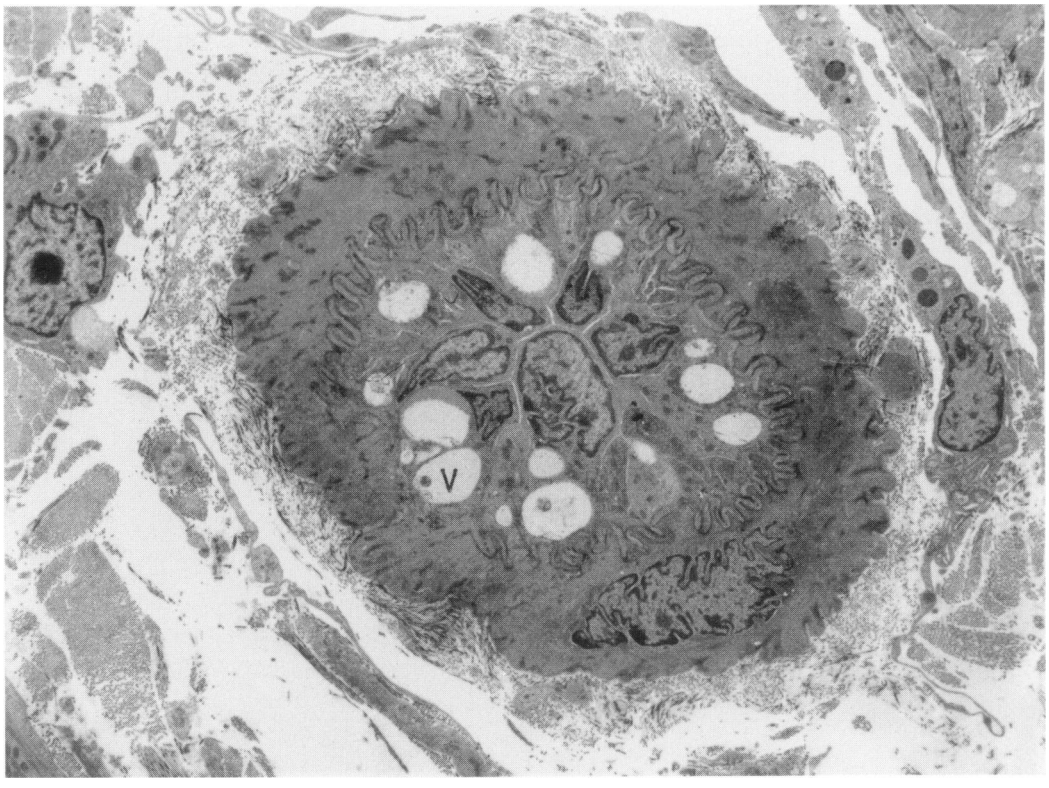

Figure 6. Cross-section of arteriole from LSI 15 minutes after LPS challenge shows marked constriction. Numerous subnuclear cytoplasmic vacuoles (V) are present in the endothelial cell. × 3800.

Dinarello 1989) exert important effects on both the endothelial cell (Mantovani & Dejana 1989) and the neutrophil (Zeck Kapp *et al.* 1990); LPS, TNF and IL-1 also modulate several polymorphonuclear neutrophil (PMN) functions such as the release of oxygen free radicals, proteases, arachidonic acid metabolites and platelet activating factor (Doebber *et al.* 1985; Bottoms *et al.* 1985; 1986; Camussi *et al.* 1987; Mantovani & Dejana 1989; von Asmuth *et al.* 1991), all of which affect the endothelium. However, the release of these pro-inflammatory mediators occurs over several hours, and thus is unlikely to be contributory to the early events of endothelial injury. This would be consistent with earlier studies which demonstrate the direct cytotoxic effect of endotoxin on endothelial cells in culture (Harlan *et al.* 1983; Raghu *et al.* 1986; Meyrick *et al.* 1986) and in neutropenic animals (Gaynor 1973). The marked increase in endothelial damage over time parallels leucocytic infiltration and probably reflects a synergism of the effects of LPS on the endothelium involving the expression of adhesion molecules (Gimbrone *et al.* 1989; Morzycki *et al.* 1990), enhanced procoagulant activity (Garner & Evensen 1974), leucocytic pro-inflammatory response, and other processes such as complement activation (Morrison & Kline 1977).

The vascular response of the lower small bowel and the colon was markedly more severe and occurred at earlier time-points when compared with the upper small bowel. These changes were also markedly attenuated in animals which were not presensitized with *Salmonella* 885, which colonizes the Peyer's patches without causing illness (Mathan *et al.* 1988). This would suggest that the enteric vasculature is sensitized, by the luminal exposure of LPS, to subsequent parenteral challenge with endotoxin, analogously to the local Schwartzman reaction (Mathan & Mathan 1985). Enhanced endothelial susceptibility as a consequence of sensitization to LPS occurring in an organ which normally contains very large amounts of LPS may have important implications in the pathogenesis of endotoxic shock (Nolan 1988).

The morphology of the cellular changes is closely similar to earlier studies which have examined large vessels (Gaynor *et al.* 1970; Stewart & Anderson 1971; Gaynor 1973; Gerrity *et al.* 1976; Pesonen *et al.* 1981; Bisio *et al.* 1983; Reidy & Schwartz 1983; Jones *et al.* 1986; Richardson & Parbtani 1987; Kang & Williams 1991;

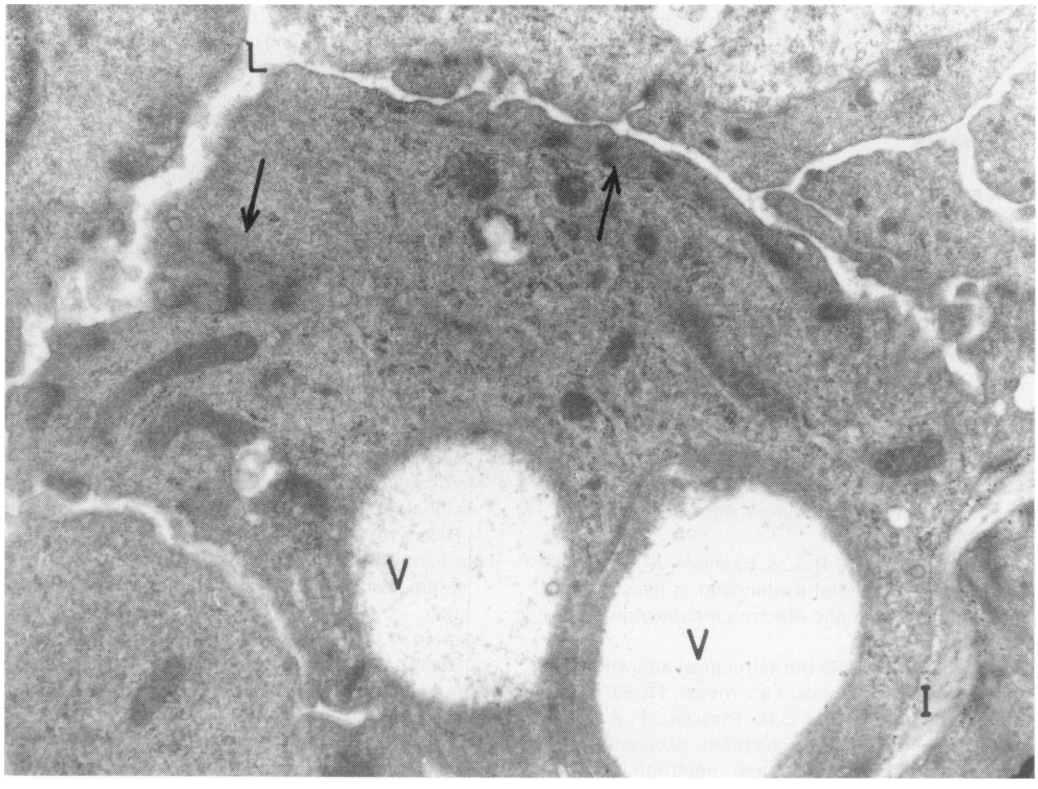

Figure 7. Cross-section of arteriole with markedly narrowed lumen (L). The endothelial cytoplasm shows vacuolization (V) and actomyosin filaments (arrow). Part of the internal elastic lamina (I) is also seen. × 19800.

Penn & Chisolm 1991), and microvasculature (McKay *et al.* 1966; Boler & Bibighaus 1967; Richman *et al.* 1980; Nayyar *et al.* 1989; Cho *et al.* 1991) in several animal models. However, the marked vasoconstriction observed in the arterioles which is unaccompanied by severe endothelial damage was an unexpected, but consistent, finding. Potent mediators with both pressor (endothelin (Sugiura *et al.* 1990)) and dilator (EDRF (Radomsky *et al.* 1990)) effects are known to be released in response to LPS. Vasoconstriction in the splanchnic bed has been observed in a rabbit model of endotoxic shock (Wright *et al.* 1992) and endotoxin has been known to impair the endothelium-dependent relaxation of the mesenteric vasculature in dogs (Wylam *et al.* 1990). This may be due to regional differences in the relative levels of expression of the vasoactive mediators, or differential endothelial responses. Further work is required to clarify the mechanisms underlying this observation. The prominent cytoplasmic filaments, stress fibres, actin and vimentin-like filaments, which represent the contractile apparatus of the endothelial cell, are likely to be responses to endotoxin-induced injury. These structures have been observed as early as 30 minutes after endotoxin administration and thus appear to be a component of the primary response to LPS.

These results, taken together, suggest that the intestinal microvasculature may be an important target organ for endotoxin. The endothelial damage in the early time period is less severe and probably the result of direct action of LPS. Subsequently, TNF and IL-1, which are secreted by monocytes, macrophages and possibly endothelial cells in response to endotoxin, may also contribute to continuing damage to the vascular endothelium. The endothelial damage in the early time period is less severe and probably the result of direct action of LPS. Subsequently TNF and IL-1, which are secreted by monocytes and macrophages in response to endotoxin, may combine to cause furthur damage to the vascular endothelium. A likely reason for the apparent enhanced sensitivity to LPS may be a consequence of its proximity to the distal intestinal lumen which harbours large quantities of endotoxin. The response of the intestinal vasculature may result in diarrhoea as a consequence of altered vascular permeability. The derangement of the epithelioluminal barrier function of the overlying mucosa may also lead to increased release of endotoxin into the

systemic circulation, thereby perpetuating the primary effects of LPS.

Acknowledgements

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