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Short Report

Ultrastructural observations on the peritoneum in the mouse

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ABSTRACT

The serous mesothelium of the serosa and mesentery of the small intestine in the mouse were examined by scanning and transmission electron microscopy. The serosa consisted of a single layer of flattened microvilli-bearing cells containing nuclei, caveolae and micropinocytotic vesicles. The observations in this study differed from previous reports on mesothelial surfaces in two respects. A surface layer of amorphous material was present over parts of the serosa. This layer probably represents serous fluid trapped by the mesothelial microvilli but is unaffected by prefixation rinsing in saline or ultrasonic cleaning. The layer is lost following osmication and routine processing for transmission electron microscopy. The possibility that a serous fluid layer may be preserved in this way may be useful in assessing changes in the peritoneum. Stomata were observed in the mesentery but there was no evidence of a connection with the lymphatic system. The presence of mesenteric stomata may explain the difference in permeability reported between parietal peritoneum and mesentery.

INTRODUCTION

There have been several studies of the peritoneal lining of the abdominal cavity. This is largely due to the fact that this membrane is an important element in peritoneal dialysis. Exchange between the body and dialysis solutions introduced into the peritoneal cavity takes place across the semipermeable peritoneum. In addition to this role in dialysis, there has been considerable interest in openings (stomata) which have been described on the diaphragmatic parietal peritoneum (Allen, 1936; Tsilibary & Wissig, 1977, 1987; Fukuo et al. 1990). These stomata are believed to be involved in the drainage of fluid from the peritoneal cavity and are connected to subperitoneal lymphatic channels. The role of the peritoneum in dialysis and in fluid drainage into the lymphatic system has sustained a continuing interest in the structure and function of this mesothelial membrane. Its fine structure has been described in several animal species including the mouse, rat, rabbit, pig and human (Odor, 1954; Baradi & Hope, 1964; Baradi & Rao, 1976; Pfeiffer et al. 1987; Slater et al. 1989). This report describes the peritoneum of the serosal surface and mesentery of the small intestine in the mouse and presents additional features which have not previously been described by others. Some of the results in this study have been presented earlier in abstract form (Ettarh et al. 1990).

MATERIALS AND METHODS

Twelve female BSVS mice aged 10–12 wk weighing 25–30 g were used. They were kept in cages with a 12 h:12 h light–dark cycle and allowed free access to food and water. Each animal was killed by cervical dislocation and the small intestine and its mesentery removed. Several segments 1 cm in length, with corresponding lengths of mesentery still attached, were cut and either fixed immediately in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer or rinsed briefly in 0.9% saline before fixation. Specimens were kept in fixative for 24 h. Some specimens were postfixed in 1% cacodylate buffered osmium tetroxide

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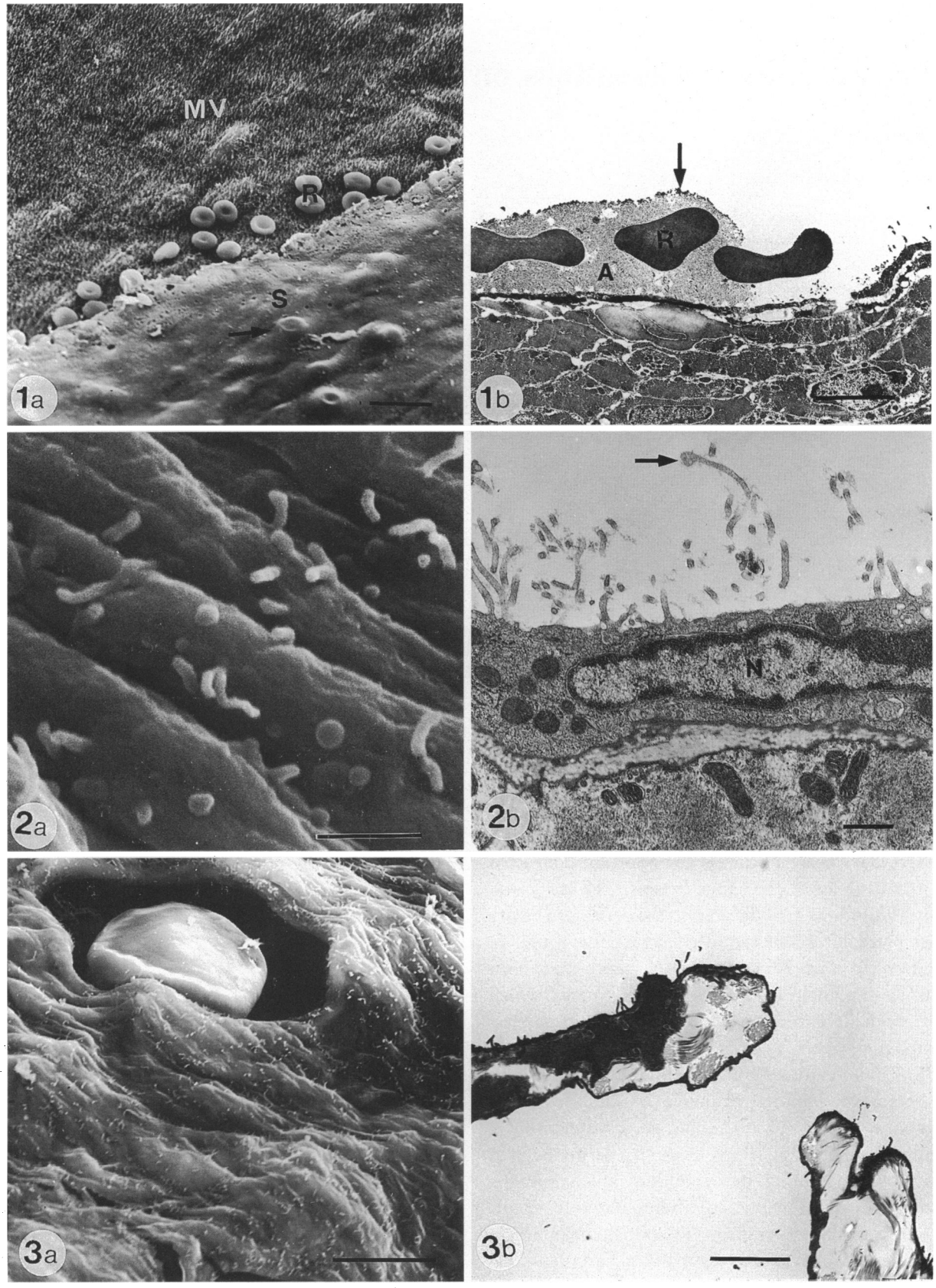

Fig. 1. (*a*) Scanning electron micrograph of the serosal surface of the small intestine showing a mat of microvilli (MV). Part of the serosa is covered by a smooth surface layer (S) which is frequently associated with red blood cells (R). Note the outline of cells trapped beneath the layer (arrow). Bar, 10 μm. (*b*) Transmission electron micrograph of the specimen in (*a*). This specimen was reprocessed for transmission electron microscopy. Some of the gold coating is still present as a dark 'crust' on the left of the micrograph (arrow). Microvilli are visible

solution for 2 h. Following fixation, specimens were divided along their antimesenteric border and pinned onto pieces of cork with the serosal surface upwards. Mounted specimens were dehydrated through a series of graded strength ethanols, critical-point dried, mounted on aluminium stubs with Araldite, coated with gold in a Polaron sputter coater for 75 s and examined in a JEOL JSM 840A scanning electron microscope at an accelerating voltage of 10 kV. Scanned specimens were reprocessed for correlative transmission electron microscopy as follows. Specimens were immersed in mercury for 60 s to remove the gold coating (Dickson et al. 1989), placed in 100% ethanol for 30 min, cleared in 1, 2 epoxypropane and embedded in TAAB resin. Ultrathin sections (80–90 nm) were cut with a Reichert-Jung Ultracut-E ultratome and stained with uranyl acetate and lead citrate. Sections were examined in a JEOL JEM-I00 CX II transmission electron microscope at an accelerating voltage of 80 kV. Some specimens were subjected to ultrasonic cleaning as follows: a beaker containing 100% ethanol was placed in a sonicator which was half-filled with water. The specimens were then immersed, serosal side downwards, in the beaker for 15 s (Dickson et al. 1989).

RESULTS

Scanning electron micrographs of the serosal surface of the peritoneum showed a mat of microvilli in all specimens studied (Fig. 1*a*). The microvilli on the serosa measured between 0.7 and 1.5 μm in length but those on the mesentery were shorter and spaced out (Fig. 2*a*). No cilia were seen in any of the specimens. In addition, specimens which were processed for scanning electron microscopy without postfixation in osmium tetroxide showed a smooth surface layer over the microvilli (Fig. 1*a*). This layer of material obscured the mesothelial surface, was usually associated with red blood cells and was unaffected by ultrasonic cleaning or prefixation rinsing in saline. At very high magnifications, pores were visible between the microvilli in scanning electron micrographs of serosal specimens. Oval or irregularly shaped stomata with smooth microvilli-bearing margins, measuring between 10 and 20 μm in diameter, were present in the mesentery (Fig. 3*a*). Estimates, derived from calculations performed on scanning electron micrographs of mesentery taken at zero tilt, indicate that up to 400 stomata may occur in 1 mm^2 of mesentery.

Transmission electron microscopy (TEM) showed that the serosa consisted of a layer of mesothelial cells containing centrally placed nuclei (Fig. 2*b*). Nucleoli and microvilli with filamentous cores were also present. Some of the microvilli had bulbous expansions at their tips (Fig. 2*b*). There were numerous caveolae and micropinocytotic vesicles scattered throughout the cytoplasm of the cell; many of these vesicles opened on to the outer and basal surfaces of the cells. The vesicles opening onto the outer membrane correspond to the pores which were visible between microvilli in high-magnification scanning electron micrographs. Scanned specimens which were reprocessed for correlative TEM showed that the smooth surface layer, observed in nonosmicated specimens, consisted of amorphous material (Fig.1*b*). This layer was not seen in specimens which were processed directly for TEM (Fig. 2*b*). The mesentery was similar in appearance to the serosa but consisted of a double layer of mesothelial cells. This arrangement was interrupted by stomata, the margins of which showed a continuity between the two layers of mesothelial cells (Fig. 3*b*). Mesenteric mesothelial cells showed fewer caveolae and vesicles than cells in the serosa.

DISCUSSION

Current knowledge on the morphology of serous mesothelial surfaces has come from several ultrastructural studies on various animal species. Odor (1954) was the first to use the electron microscope in a study of the mesothelium of the rat oviduct. Subsequent investigations (Baradi & Hope, 1964; Andrews & Porter, 1973; Baradi & Rao, 1976; Pfeiffer et al. 1987; Abu-Hijleh et al. 1994) have established the ultrastructural appearance of serous mesothelium in various animal species, including man. The de-

on the right but are obscured by an amorphous layer (A) on the left; entrapped red blood cells (R) are present within this layer. The cells of the muscle layer of the intestinal wall can be seen beneath the serosal cells. Bar, 5 μm.

Fig. 2. (*a*) Scanning electron micrograph of mesentery. The microvilli are short and spaced out. Bar, 1 μm. (*b*) Transmission electron micrograph of a mesothelial cell of the serosa showing microvilli, mitochondria and a nucleus (N). Some of the microvilli have bulbous terminal expansions (arrow). Caveolae are visible near the cell surface close to the right margin of the micrograph. Bar, 1 μm.

Fig. 3. (*a*) Scanning electron micrograph of mesentery. Two stomata can be seen in the micrograph, one of them containing a red blood cell. The microvilli extend on to the margins of the stomata. Bar, 10 μm. (*b*) Transmission electron micrograph of the specimen shown in (*a*) sectioned through a mesenteric stoma. The mesothelial cells on either side of the stoma form the margins of the opening. The mesenteric microvilli are fewer in number (cf. Fig. 2*a*) than in the serosa. Bar, 5 μm.

scription of the mesothelium as consisting of a single layer of flattened cells with thick central regions sloping towards thinner cell margins apply to all species which have been studied to date. The findings in this study are in accord with literature descriptions of murine peritoneum (Baradi & Rao, 1976; Tsilibary & Wissig, 1977, 1987). However, in this study there were two additional features which have not been described in previous studies: the presence, over the serosa, of a surface layer consisting of amorphous material and the presence of mesenteric stomata.

The finding of a surface layer in specimens prepared for scanning electron microscopy but not in those prepared directly for TEM suggests that this layer is dissolved and/or lost at some stage during the process of embedding. The most plausible explanation for the presence of the surface layer is that it represents serous fluid, which fixes onto specimens when these are immersed in fixative, and is dissolved either during osmication or one of the stages in the embedding process. The absence of this surface layer in the mesentery may be explained by the smaller density of microvilli in this part of the peritoneum. Andrews & Porter (1973) have stated that the glycocalyx on mesothelial microvilli helps to trap serous exudate; thus, the greater the density of microvilli, the greater the amount of trapped fluid. The association of the surface layer with red blood cells most likely represents leakage during the process of tissue retrieval. If the assumption that this surface layer represents serous exudate is correct, then the preservation of this fluid layer in this way may be useful in the assessment of changes in the peritoneum and peritoneal fluid following challenge such as dialysis.

Intercellular openings or stomata have been described in the diaphragmatic peritoneum in the mouse and golden hamster (Allen, 1936; Tsibilary & Wissig, 1977, 1987; Fukuo et al. 1990) and in the ovarian bursa in the golden hamster (Shinohara et al. 1985). Whereas the mesothelial stomata in the diaphragmatic peritoneum have been shown to be associated with drainage of peritoneal fluid into subperitoneal lymphatic channels (Tsilibary & Wissig, 1977, 1987; Fukuo et al. 1990; Abu-Hijleh & Scothorne, 1994), the mesenteric stomata appear to be simple, patent portals connecting the peritoneal cavity on either side of the mesentery. On the basis of the morphological evidence obtained in this study, mesenteric stomata do not appear to be related or connected to the lymphatic system. They however appear to be associated with red blood cells. Similar RBC-occupied stomata are apparent in micrographs of at least one previous study (Leak & Rahil, 1978) although no textual reference is made to them. There are two possible explanations for the presence of these RBCs in the peritoneal cavity. These cells could have come from leakage during laparotomy for tissue sampling. The other possibility is that the presence of free RBCs in the peritoneal cavity is normal.

The dimensions of the mesenteric stomata in this study correlate well with those for stomata in the diaphragmatic peritoneum in the mouse (Allen, 1936; Tsilibary & Wissig, 1977). There are no reports in the literature of stomata in other parts of the peritoneum or of mesenteric stomata in the human peritoneum. However, the presence of openings in the mesentery is assumed in calculations used to explain the higher permeability seen in vitro when compared with peritoneum (Gosselin & Berndt, 1962). The significance, if any, of mesenteric stomata in peritoneal dialysis remains to be assessed.

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