The results thus suggest that disturbance of the adrenergic innervation in the terminal zones of the circulation modifies their adaptive and trophic properties and contributes to the development of changes in the microcirculation detectable in the preclinical period of atherosclerosis.

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RESPONSES OF PERITONEAL MESOTHELIAL CELLS IN RATS WITH ASEPTIC AND BACTERIAL PERITONITIS

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KEY WORDS: peritoneal mesothelial cells; contractions of mesotheliocytes; peritonitis; scanning electron microscopy; transmission electron microscopy.

Contractions of nonmuscular cells (endotheliocytes, fibroblasts, leukocytes) play an important role in physiological and pathological processes [1, 11, 13, 14]. There is evidence in the literature to suggest that active contractions of the peritoneal mesothelial cells are possible, for example in inflammation [3, 9, 12], although this problem requires further study in order to elucidate the regulatory and structural mechanisms of the contractile responses of mesotheliocytes (MC). In the investigation described below this problem was studied by means of transmission and scanning electron microscopy (TEM and SEM respectively), in a step by step analysis of responses of MC in different forms of experimental peritonitis.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 280-320 g were used. There were four series of experiments. In series I aseptic peritonitis was induced by intraperitoneal injection of 3% aqueous solution of amylodextrin (15 animals). In series II aseptic peritonitis was induced by intraperitoneal injection of a suspension of edible starch [11]. In series II (18 rats) and IV (10 rats) the ascending part of the large intestine was constricted [4] and an incision

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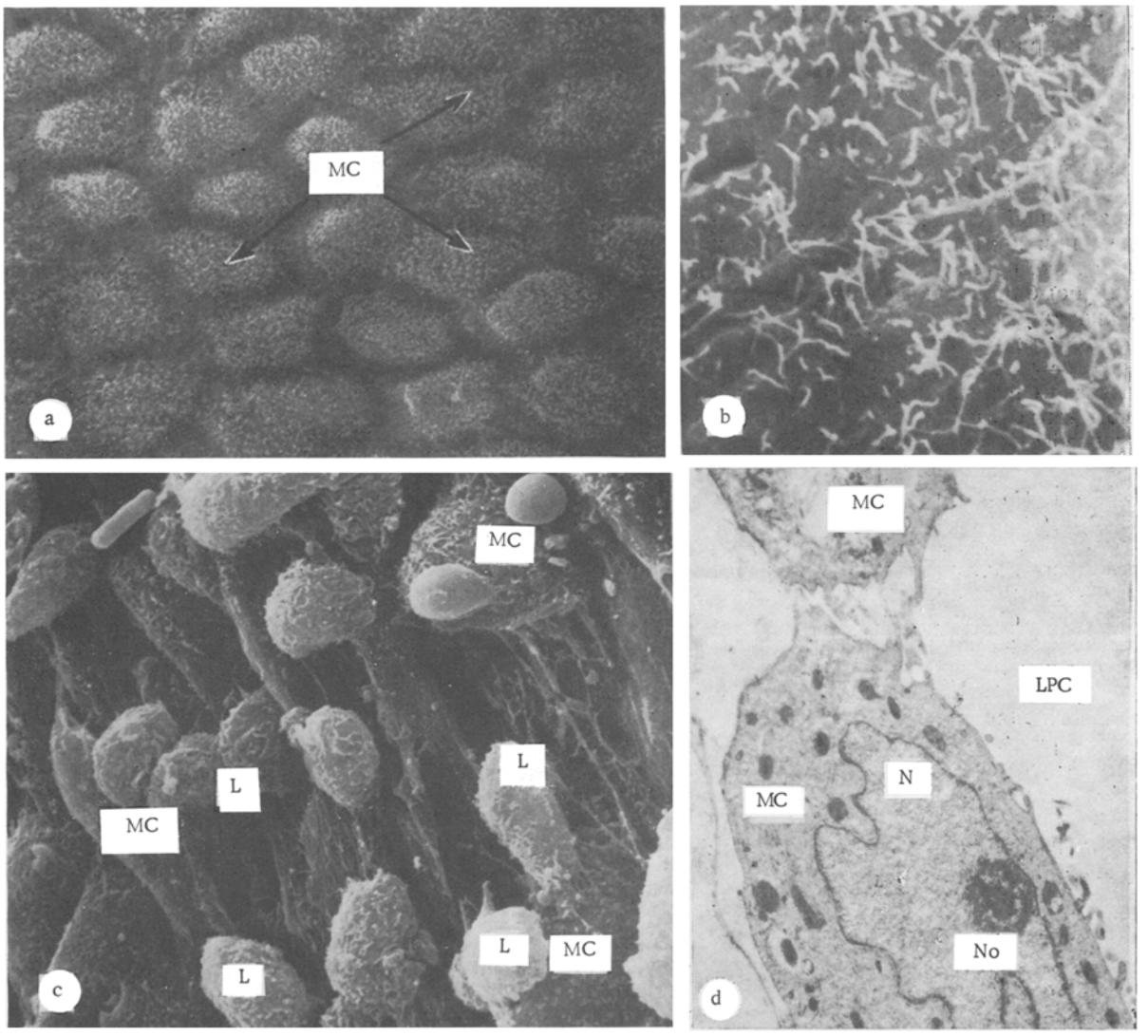

Fig. 1. Ultrastructure of peritoneal MC of intact rats (a, c) and of rats with aseptic peritonitis (b, d). a) SEM of peritoneal surface of intact rat: cell boundaries packed together, MC rhomboidal, microvilli on surface; b) SEM of peritoneal surface 4 h after injection of starch; spaces formed between MC, leukocytes visible on surface of MC and between them; c) details of villi in MC of intact rat; d) TEM of MC after aseptic peritonitis for 4 h: space visible between cells with cytoplasmic processes, cytoplasm and nucleoplasm of MC well structured, nucleus round and loosely packed. L) Leukocyte, N) nucleus, No) nucleolus, NL) neutrophilic leukocyte, LPC) lumen of peritoneal cavity. Magnification: a) 1200, b) 2200, c) 5000, d) 7000.

made in the cupola of the cecum (defect about 2 mm). In the experiments of series IV the saltatory group of muscles of the hind limbs was injured 2 days before the intestinal operation [5, 8]. Material for cytological and electron-microscopic study was taken 4 h and 1, 2, 4, and 6 days after the beginning of the experiment (on the 4th day in series IV).

Films of the peritoneal contents were studied after staining with Fast green and azure A [6] and the structure of the peritoneum was studied by TEM and SEM. In both cases glutaraldehyde fixative [12], injected intraperitoneally while the animals were alive, was used for prefixation. Pieces of parietal peritoneum were taken from the lateral zones of the abdominal wall. Postfixation was carried out in 1% OsO_4 solution and the material was embedded in a mixture of Epon and Araldite or in Spur (for TEM) and studied in the Tesla-500 microscope.

Material for SEM was dehydrated with acetones of increasing concentration, dried by taking through the critical point in liquid CO_2, and sprayed with gold by ionic bombardment [7]. The specimens were examined and photographed in the Hitachi S-500 scanning electron microscope.

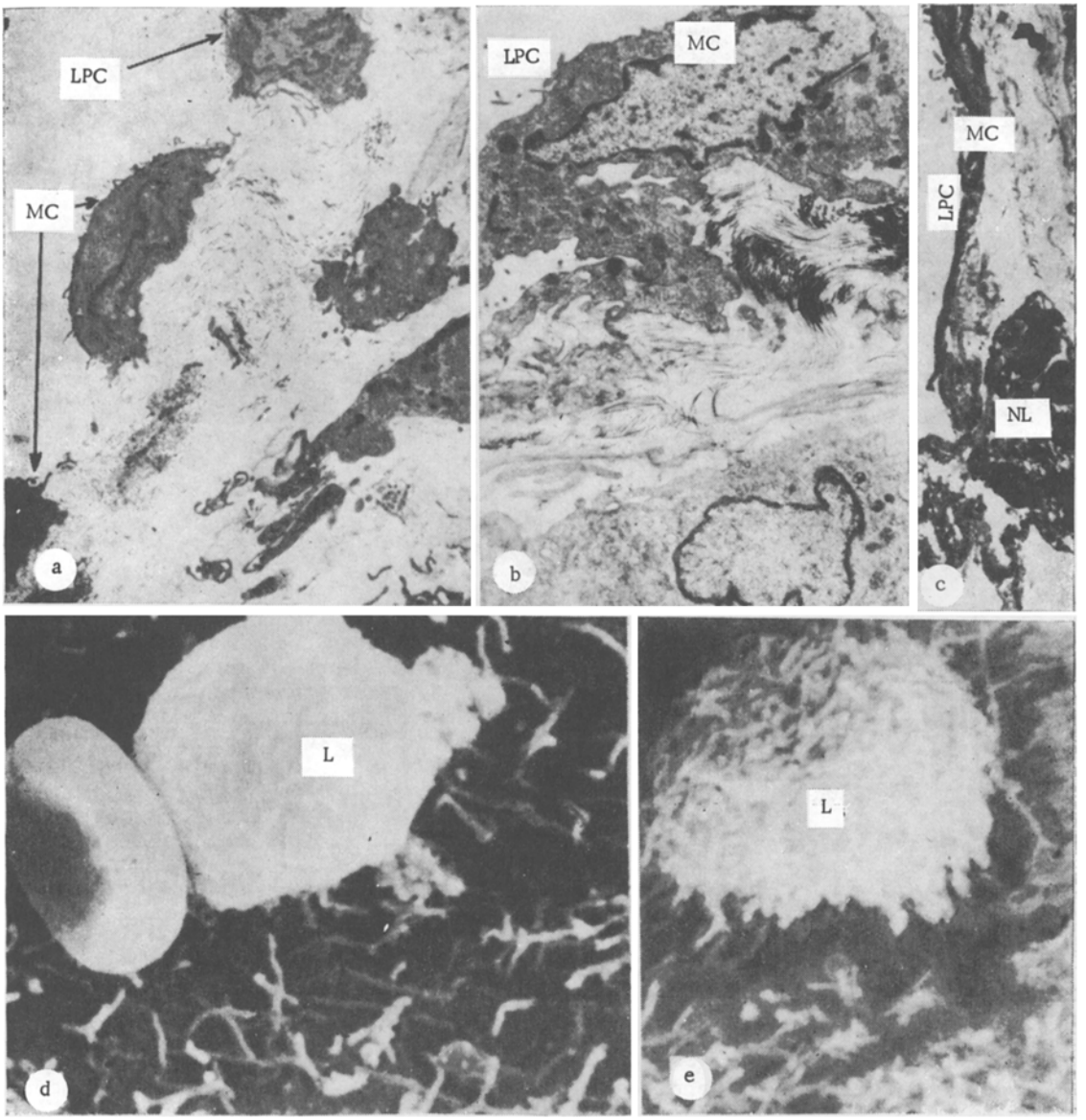

Fig. 2. Ultrastructure of peritoneal MC of rat with bacterial peritonitis (a, b) and on 2nd-4th day of aseptic peritonitis (c, d, e). a, b) TEM: MC greatly contracted, wide gaps between cells (a), no sign of degenerative changes in MC, basement membrane follows folds of contracted MC (b); c) TEM, 4th day of aseptic peritonitis. MC flattened, polymorph emerges at junction between two MC; d, e) SEM, 4th day of aseptic peritonitis: no gaps between MC, well-marked villi, leukocytes (lymphocytes) with surface of different structure lie on surface of MC. Magnification: a) 1800, b) 4000, c) 7000, d) 8000, e) 8000. Remainder of legend as to Fig. 1.

EXPERIMENTAL RESULTS

In aseptic peritonitis the predominant cells found in the peritoneal contents 4 h after injection of soluble or edible starch were polymorphonuclear leukocytes (polymorphs), mainly neutrophils: they accounted for 80-90% of all cells found in films from the peritoneal cavity. By the end of the first day the relative number of these cells was reduced on account of an increase in the proportion of mononuclear phagocytes; on the 2nd-3rd day mainly mononuclear phagocytes and lymphocytes were found, in agreement with data in the literature [13].

In bacterial peritonitis without preliminary injury to the saltatory group of muscles a loop of small intestine or the omentum was adherent to the cecum after 2-4 h, thus closing the defect. However, the relative proportion of polymorphs in films taken 4 h after the operation was sharply increased and it remained high on the 2nd and 4th days (30-40%). Under

these circumstances the peritoneum was smooth and shining, but covered with a film of thick sticky liquid.

After preliminary injury to the saltatory group of muscles in the hind limbs (series IV) more than 50% of polymorphs were found in the peritoneal contents on the 4th day after injury to the cecum and in this case the peritoneum was hyperemic and dull, with focal purulent deposits.

Proteus vulgaris was cultured from the peritoneal exudate in both series of "fecal" peritonitis (the bacteriological analysis was carried out in the Laboratory of the No. 7 Moscow City Hospital), but in series IV cocci as well as bacilli were found in the films.

Examination of the peritoneum of the intact rats by SEM revealed a continuous layer of MC which were rhomboidal in shape. The cell boundaries were closely packed together (Fig. 1a). Thin (0.10-0.14 μ wide and 1.5-2 μ long) villi, branched at their ends in some places, were present on the inner surface of MC (Fig. 1a, c). The presence of such structures in MC has been reported previously [3, 13]. Villi are evidently analogs of the reactive structures of the peritoneum found in large mammals [2]. The MC became round in shape 4 h after injection of soluble starch, their nuclear part projected into the lumen of the peritoneal cavity (Fig. 1d), and spaces appeared between the cytoplasmic regions of the cells (Fig. 1b). These spaces could be seen mainly between the lateral zones of MC. In the region of the poles (the "angles") of the cells cytoplasmic bridges still remained (Fig. 1b).

TEM showed the presence of gap junctions between MC in intact animals, similar to intercellular junctions of endothelial cells of microvessels, and that the villi of MC are outgrowths of the cytoplasm (Fig. 1d; Fig. 2a, c). Separation of the junctions between MC with preservation of cytoplasmic bridges could be clearly seen 4 h after injection of soluble starch (Fig. 1d). The state of the nucleus and nucleolus (Fig. 1d) showed that the functions of the cell were preserved and it was not undergoing degeneration. Changes in the shape of MC were thus not attributable to death or "desquamation" of these cells. Many leukocytes appeared on the peritoneal surface at this period, and on the basis of data in the literature and cell counts in the films, they were classed mainly as polymorphs. They were round or slightly oval in shape and about 7 μ in diameter. The surface of the leukocytes was covered with indistinct folds (Fig. 1b).

Similar changes, and with a similar time course, also were observed in aseptic peritonitis caused by insoluble (edible) starch.

In the later stages of aseptic peritonitis (2nd-4th day) the gaps between MC closed, as shown both by SEM and by TEM (Fig. 2c-e). Comparatively small (under 5 μ in diameter) round cells with three different types of surface structure were revealed on the surface of MC by SEM: 1) the presence of many long, thin villi (Fig. 2e); 2) the formation of short projections of cytoplasm with club-shaped thickenings; 3) the presence of small pectinate projections. These cells were identified as lymphocytes. Typical macrophages 10-12 μ in diameter also were found.

In "fecal" peritonitis in both series of experiments more marked changes in the shape of MC were observed in the first 4 h than in aseptic peritonitis (Fig. 2b), with marked separation of the cell edges (Fig. 2a). One very characteristic feature of bacterial peritonitis was the appearance of a fibrin network on the surface of the peritoneum.

In the experiments of series IV (fecal peritonitis accompanied by injury to the saltatory group of muscles) the structure of the peritoneal MC had not yet returned to normal on the 4th day. TEM revealed intensive infiltration of the peritoneum by polymorphs and macrophages. Among the fibrinous exudate on the surface of the peritoneum a zone of productive inflammation formed, in which the predominant cells were mononuclear phagocytes and fibroblasts. During this period no normally organized MC could be seen, only a few degeneratively changed cells with wide projections of cytoplasm and many lysosomes — evidently dead MC.

In aseptic peritonitis and in the initial stages of bacterial peritonitis changes in the shape (contraction) of MC thus take place, with separation of the intercellular junctions and the formation of intercellular spaces in the parietal peritoneum. A good state of preservation of MC and signs of their functional activity are evidence that the change in shape of MC under these circumstances is an active contractile response. The cytological mechanisms of this response and its functional significance require further clarification. A stereotyped cellular response evidently takes place to the mediators of inflammation, to histamine, for

example, which as has now been shown is secreted from the peritoneal mast cells in rats during the development of peritonitis [11]. Contractions of MC, in turn, may evidently affect processes of cellular migration, exudation, and resorption of fluid and colloids. In bacterial peritonitis contraction of MC is followed by destruction and death.

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ELECTRON-MICROSCOPIC STUDY OF PARCHMENT

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KEY WORDS: parchment; collagen fibrils; destruction of collagen fibrils.

If optimal conditions for the preservation of ancient manuscripts on parchment are to be ensured, it is important to know the causes of destruction of parchment during long-term storage. The present investigation was undertaken in order to study the ultrastructure of parchment and to identify the submicroscopic changes which could explain the changes in its physical properties.

EXPERIMENTAL METHOD

Nine parchments from the 11th-19th centuries, which had been well, satisfactorily, or badly kept, and a sample of modern parchment, prepared from calf skin at the "Moskozhob"edinenie" Factory for restoring old manuscripts, were studied.

Pieces of the parchments measuring less than 1 mm^2 were fixed in 1% OsO_4 solution in 0.1 M cacodylate buffer, pH 7.3, with or without the addition of ruthenium red in a concentration of 0.0005% to the fixative. Some specimens also were fixed consecutively with 3% glutaraldehyde in 0.1 M phosphate buffer and 1% OsO_4 by Caulfield's method. After dehydration in alcohols of increasing concentration the fragments were embedded in Araldite. In the course of dehydration (at the 70% alcohol stage, 16 h) they were treated with 3% uranyl acetate. Ultrathin sections were stained successively with 3% phosphotungstic acid solution and with lead

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