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Characteristic alterations of the peritoneum after carbon dioxide pneumoperitoneum

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Abstract
Objective: Any route of entry into the abdomen contributes to alterations of the intraperitoneal organs with different clinical consequences. Characteristic alterations of the peritoneum after CO_2 pneumoperitoneum used in laparoscopic surgery is examined.
Methods: A CO_2 pneumoperitoneum with an intraperitoneal pressure of 6 mmHg was applied for 30 min in 32 nude mice. In the course of 4 days, the animals were killed and the peritoneal surface of the abdominal wall was studied by means of scanning electron microscopy.
Results: Already 2 h after release of the pneumoperitoneum, mesothelial cells were bulging up. The intercellular clefts thereby increased in size, and the underlying basal lamina became visible. This reaction peaked after 12 h. Subsequently, peritoneal macrophages and lymphocytes filled all gaps, thereby recovering the basal lamina.
Conclusion: The morphologic integrity of the peritoneum is temporarily disturbed by a CO_2 pneumoperitoneum.

Key words: Laparoscopy — Mesothelium — Pneumoperitoneum — Scanning electron microscopy

On the basis of clinical observations, animal experiments, and case reports, it was concluded that an increased spread of malignant tumors occurred after surgical laparoscopy, either at the incision site or diffusely in the peritoneum [1–3, 9–11, 13, 14, 16]. As possible reasons for this phenomenon, technical or systemic disadvantages of minimally invasive surgery have been blamed. Examinations of pneumoperitoneum pathophysiology have shown that the CO_2 used during laparoscopic surgery may alter the peritoneal surface [20, 22]. In the animal model, introduction of malignant cells under these circumstances leads to a higher tumor growth rate, a higher tumor load, and a shorter survival rate [19, 21]. At the time of this writing, it is not known whether there is a morphologic correlate substantiating these observations. This study examines the morphologic alterations of the mesothelium after CO_2 application. Because alterations of the peritoneum are reflected on the surface, scanning electron microscopic studies are the method of choice.

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Material and methods

In preliminary experiments, an adequate model was established by varying different strains of mice, intra-abdominal pressure for the induction of pneumoperitoneum, and the type of anesthesia. We used 40 male nude mice (NMRI) 4 months old of about 60-g body weight for the study. These mice were used because of good experiences with them in former animal studies [19, 21]. The specific intraperitoneal immunity of these animals, along with an increased intraperitoneal cellular immune competence as a result of a systemic deficiency in lymphocyte activity, allows a very good evaluation of the regenerating process. To imitate the clinical situation of increased tumor growth in further studies, only this strain of mice would allow the growth of a human cancer cell line.

Thirty-six animals were treated, and four animals served as controls. After a short anesthesia with ether, a CO_2 pneumoperitoneum with an intraperitoneal pressure of 6 mmHg was applied for 30 min. The gas was applied with a microhysteroflator by the insertion of a thin needle (gauge 22) into the right lower abdomen. During and after 1, 2, 6, 12, 24, 48, 72 and 96 h, four randomly chosen animals were killed by an overdose of ketanest and barbiturate.

The peritoneal tissue of the anterior and lateral abdominal wall was fixed in 2.5% glutaraldehyde in 0.05 M (molar) cacodylate buffer (pH 7.2). The fixative was washed out in pure buffer for 30 min. The tissue was then postfixed in 1% osmiumtetroxide in the same buffer for 90 min. For an additional 30 min, the tissue was washed in pure buffer a second time. Thereafter, the preparations were dehydrated stepwise in acetone and dried by means of a critical point apparatus (liquid CO_2, CPD 030 BAL-TEC). The dried specimens were mounted with conductive silver onto specimen holders, sputter coated with gold (SCD 005 BAL-TEC), and studied with a scanning electron microscope (Philips SEM 505). Four animals served as controls to exclude fixation artifact.

The preparation of the control animals was performed on examination days 1, 2, 3, and 4. All control animals received a short anesthesia with ether at the first examination day and were killed by an overdose of ketanest and barbiturate. This kind of treatment has no demonstrable effect on the peritoneum [19]. The peritoneal surface was examined in respect to two criteria: (a) changes of the mesothelial cell layer-like visibility of cellular borders, destruction of cells, and changes of microvilli; and (b) the visibility of the basal lamina. A criteria is recognized as such when changes

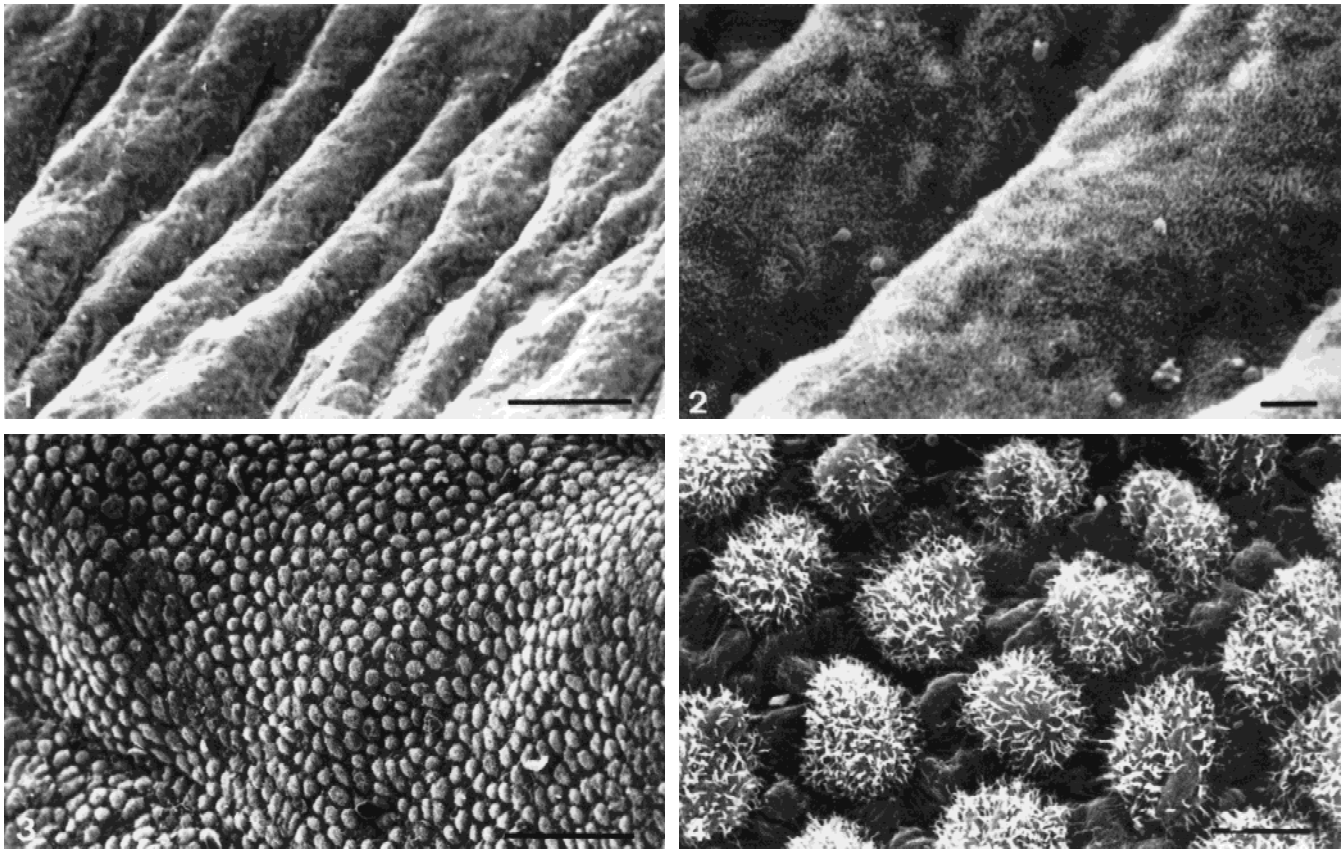


Fig. 1. Untreated animal. The normal peritoneum with intact mesothelial surface and indistinct cell borders. Magnification ×170.

Fig. 2. Untreated animal, a closer look at Fig. 1. The normal peritoneum is covered by a sheet of flat mesothelial cells densely strewn with microvilli. No intercellular clefts and no open basallamina can be detected. Magnification ×710.

Fig. 3. Mesothelium 2 h after CO_2 application. The dimensions of the CO_2 application on to the mesothelial lining are clearly visible. It must be noted that the magnification of this figure is the same as in Fig. 1. Magnification ×170.

Fig. 4. Mesothelium 2 h after CO_2 application. The mesothelial cells have partially retracted, strongly bulged up, and appear nearly spherical. Intercellular clefts and the underlying basal lamina are clearly visible. Magnification ×1,310.

are observed in all animals of a group and at a point in time, respectively. The injection site of the peritoneum was not included in the evaluation.

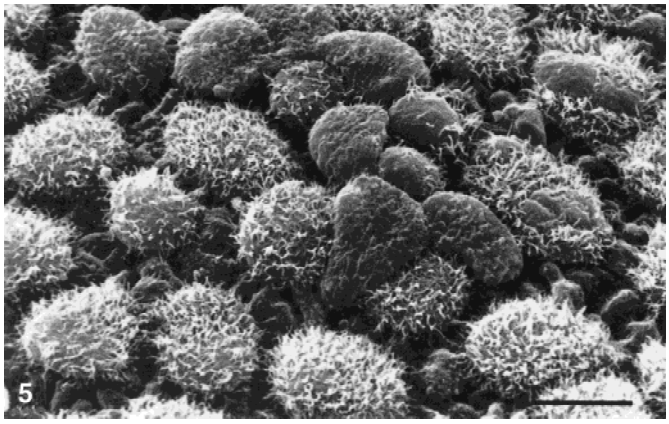
Results

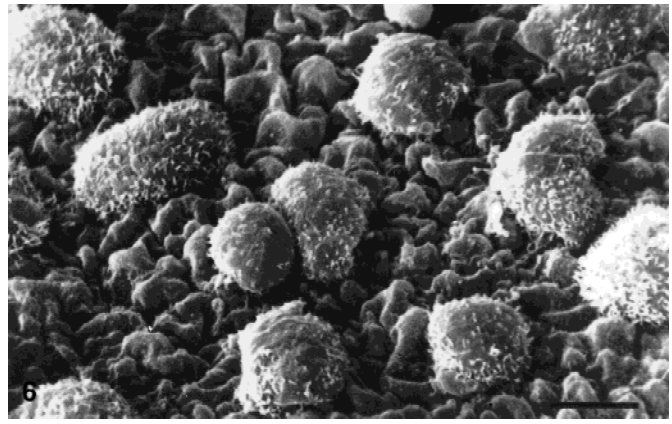
The anesthetic and euthanasia regimen had no demonstrable effect on the morphologic appearance of the peritoneum. The normal morphology of the mesothelium is well known [6, 7, 15]: The peritoneum is covered by a sheet of flat mesothelial cells densely covered with microvilli. No intercellular clefts and no free basal lamina were detected (Figs. 1 and 2). During pneumoperitoneum, no changes from normal morphology were observed in any of the animals. Already 1 to 2 hours after release of the CO_2 pneumoperitoneum, drastic alterations of the surface layer were seen. In all treated animals the mesothelial cells had partially retracted and strongly bulged up so that they appeared nearly spherical. Intercellular clefts become clearly visible and increased in size. The carpet of microvilli was nearly unchanged. By the retraction of the covering cells, the underlying basal lamina was exposed in large areas (Figs. 3 and 4). The continuous uncovering of the basal lamina reached its maximum after 12 h (Fig. 6). Nearly the entire peritoneum was involved.

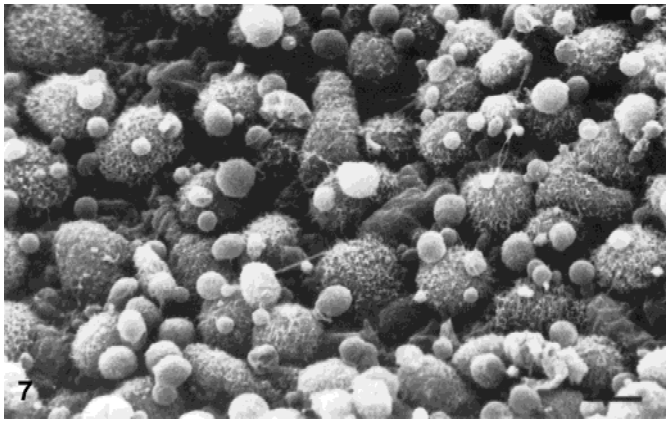
Typical peritoneal macrophages appearing as white, spherical cells of wide diameters and thin folds of the membrane, which is free of micrivilli, were observed in the gaps after 2 h. Also, lymphocytes, which appear smaller in diameter, white, and with stubby microvilli on the surface, were seen [23]. After 24 h they were observed in large numbers on the surface (Fig. 7). After 48 h cells considered to initiate the regeneration of the mesothelium predominated. The basal lamina was covered again (Fig. 8). After 96 h the intercellular gaps had become much smaller, and in some regions had disappeared completely, resulting in a nearly confluent layer of microvilli-covered cells (Fig. 9). In addition, it must be noted that another type of unknown cells, believed also to take part in the regeneration process was seen already after 2 h (Fig. 5). Initially, these cells were characterized by very short stubby microvilli or delicate folds.

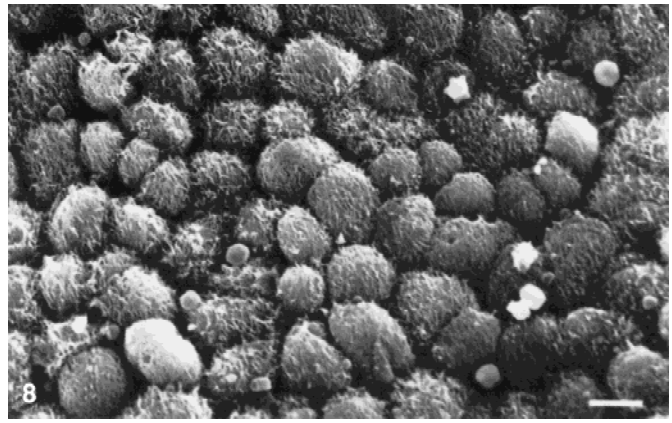
Discussion

The results of this study indicate that the application of a CO_2 pneumoperitoneum induces morphologically recogniz-









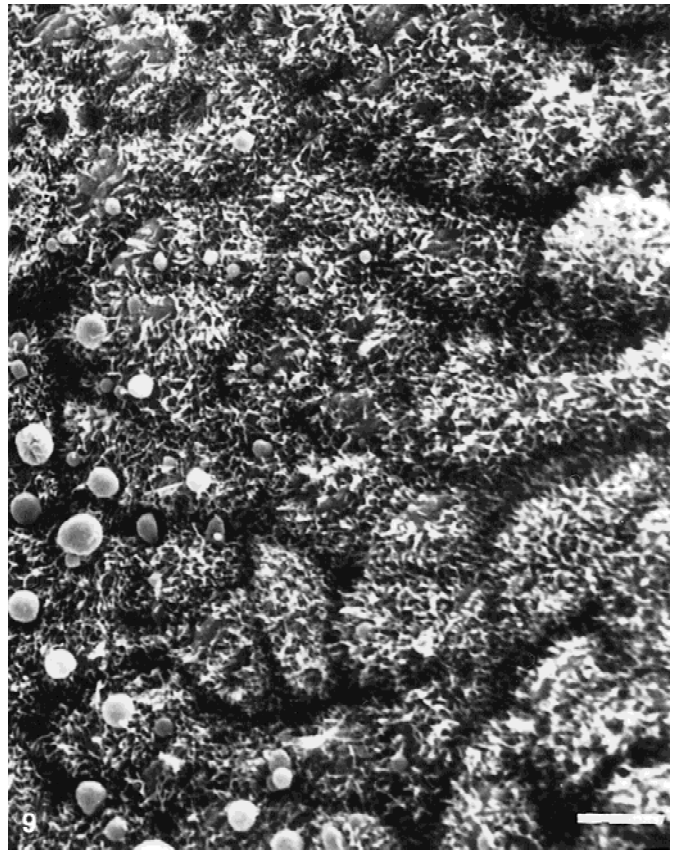


Fig. 5. Mesothelium 2 h after CO_2 application. Other type of cells believed to take part in the regeneration process are seen lying between mesothelial cells on the basallamina. Magnification ×1,250.

Fig. 6. Mesothelium 12 h after CO_2 application. Large parts of the basal lamina are denuded. Magnification ×1,200.

Fig. 7. Mesothelium 24 h after CO_2 application. Numerous peritoneal macrophages and lymphocytes fill the gaps between the rounded mesothelial cells. Magnification ×810.

Fig. 8. Mesothelium 48 h after CO_2 application. Regeneration cells and mesothelial cells form a cobblestone-like sheet. The basal lamina is hidden underneath. Magnification ×710.

Fig. 9. Mesothelium 96 h after CO_2 application. A nearly confluent layer of microvilli strewn cells is now visible. The intercellular gaps have become much smaller and have disappeared into other regions. Magnification ×710.

able marked alterations in the superficial layer of the entire peritoneum. The mesothelial integrity is temporarily disturbed. It appears important that during these changes large parts of the basal lamina were laid bare. It must be assumed that this phenomenon may have fatal consequences. The disturbances of the superficial layer were shown to be reversible, which means that the mesothelium was repaired after a limited time. The denuded basement membrane was subsequently covered by regenerating cells.

Importantly, no immediate changes of the peritoneal surface are seen during the pneumoperitoneum. It was seen earlier that applying aggressive substances such as phytohemagglutin antigen (PHA) [5] or distilled water [8] drastically altered the peritoneal surface. The question therefore arises whether the expansion of the abdominal cavity by CO_2 or the acidity of the medium in the cavity induced by CO_2 causes damages of the mesothelium. From our observations on using different gases for expanding the abdominal cavity [14], we presume that the chemical stimulus is the more probable agent than the physical irritation. Buck [4] and Rovensky [18] showed that tumor cells attach better to a bare basal lamina and to the wound site, and that the basal lamina itself supports tumor growth.

We have shown in this study that large portions of the peritoneum basal lamina are uncovered in the course of damage and regeneration after CO_2 pneumonperitoneum. Therefore, it must be assumed that the uncovered basal lamina is the best environment for the attachment of straying tumor cells. Regarding the regeneration process, our results are strongly supported by Raftery's findings [17]. He wounded different parts of the peritoneum and found macrophages to help in the regeneration. When the basal lamina is denuded [23], peritoneal macrophages and lymphocytes also are involved in the regeneration of the mesothelial layer. Watter's and Buck [23] also found cells that differed from the spherical cells by their flattened appearance.

We also have demonstrated that cells of a different size with a relatively smooth surface take part in the regeneration process. Our findings give plausible explanations for clinical and experimental results about specific tumor growth in the abdominal cavity [12, 19] after laparoscopic surgery. Earlier, CO_2 was considered a physiologic and innocuous gas. Now, increasing evidence suggests that CO_2 may be harmful in the specific circumstances of laparoscopic surgery. To create a physiologic pneumoperitoneum should be the aim of further studies.

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