Reactive Changes of the Mesothelium

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Reactive cellular changes of mesothelial cells are still a very embarrassing problem, not only for newcomers to cytology but also for more experienced cytologists. The borderline between reactive changes and the highly differentiated mesothelioma is the best-known example of these difficulties. All new techniques (electron microscopy, tissue culture, morphometry, cytochemistry, and monoclonal antibodies) have been used to try to solve this problem. However, it is still only partly solved.

One of the reasons for the difficulties is our limited knowledge of normal mesothelium in situ. It is limited by the anatomical condition itself since many details of a monolayer such as details of the mesothelium cannot be seen in histological cross sections. We therefore tried to develop techniques allowing a view of the surface of the mesothelium using various technical approaches. The first was a stripping technique which allows larger mesothelial pieces to be studied and we applied it first to the peritoneal mesothelium of fifteen 3- to 4-week-old NMRI mice (Cremerius 1984; Pfitzer 1984). The main steps in the procedure are treatment of unfixed material with 0.3% silver nitrate solution for 30 s, fixation in ethanol for 30 min, treatment with ether, air-drying, and stripping of the mesothelial layer with Tesafilm (hydrated cellulose) which is transferred to slides, cleansed with water and xylene, and finally stained with May-Grünwald-Giemsa stain (MGG).

In a second series of 52 6-month-old guinea pigs used for routine tests of tuberculosis (with negative results), mesothelium from various locations was investigated (Lipke 1987). For this material a second technique that involved freezing with carbon dioxide after silver nitrate had to be applied since the stripping technique worked only with peritoneal mesothelium supported by the abdominal muscles and not with visceral pleura.

The technique resulted in cells having silver-stained borders and demonstrated the surprisingly irregular arrangement of the cells (Fig. 1). They show more or less invaginated margins and not just the theoretically expected regular pavements.

There are reserves of membrane presumably necessary for the changes of shape needed to allow for movement of the underlying organs. The arrangement of the nuclei may be either irregular or surprisingly regular. In the latter case the nuclei are found in a line along their long axes; the direction of this line is independent of the direction of the long axis of the cell and may even be at a right angle to it. What influences such arrangements can only be speculated upon.

Sometimes two nuclei are in positions which seem the consequence of a re-

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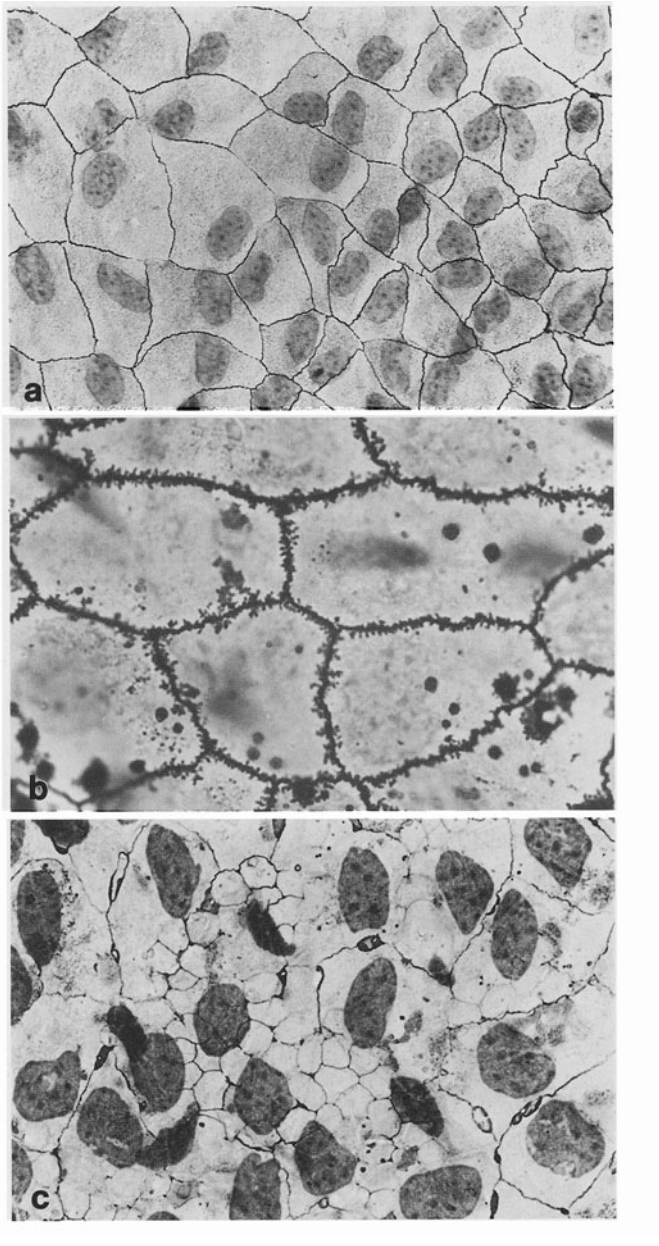

Fig. 1a–c. Various examples of mesothelial tissue of guinea pigs. **a** Parietal pleura, 1 month old, ×400. **b** Peritoneum, 6 months old, ×560. **c** Peritoneum, 3 months old, ×560. Silver impregnation/MGG

cently finished mitotic division and indeed cells in various stages of mitosis can be found. The cells may be isolated or grouped in clusters which seem to represent regions of mesothelial growth.

Some problems arise from the silver staining of cell borders, which may be sharply delineated, flushy, or even missing. Sometimes globular structures such as

pinocytic vesicles appear near the borders or in the cytoplasm. Occasionally pores can be identified.

The appearances of the silver-stained cell borders were independent of the technique used. In some specimens a meshwork of silver-stained structures replaced in parts the solid borderlines, and occasionally nuclei overlapping with cell borders demonstrated that only parts of the plasmalemma were stained. In addition, no cells undergoing mitosis were found in 6-month-old animals. In a younger control (30 days old), however, between 0.2% and 0.9% of cells were undergoing mitotic divisions in the various locations investigated (Fig. 2).

Beneath the mesothelial layer a loose meshwork of fibroblasts and fibrillar structures with some lymphocytes and eosinophils is found, at least in guinea pigs after tuberculosis testing (Fig. 3). In the case of costal pleura there is adipose tissue underneath this mesenchymal net.

Naturally we wanted to extend these investigations to human material. Our

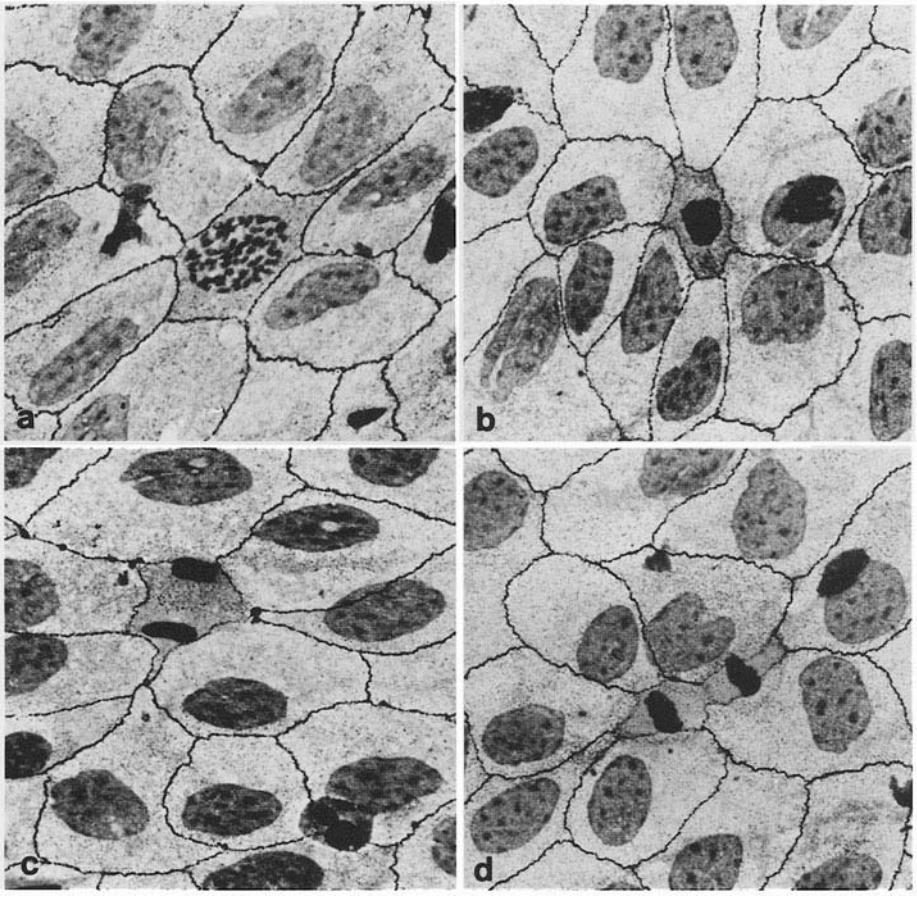

Fig. 2a–d. Various stages of mitosis and mesothelial cell division in peritoneal mesothelium of a 1-month-old guinea pig. Silver/MGG, ×600

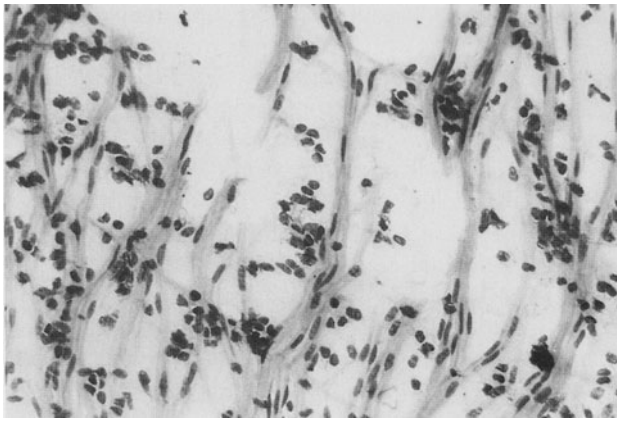

Fig. 3. Submesothelial layer of the peritoneum from a 6-month-old guinea pig. Silver/MGG, ×170

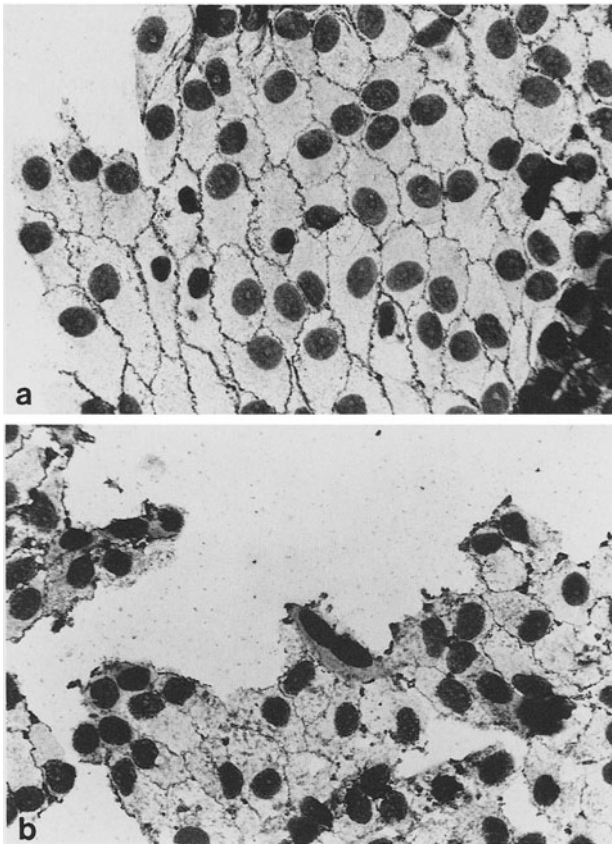

Fig. 4a, b. Human peritoneal mesothelium. **a** 47-year-old male **b** 75-year-old female Silver/MGG, ×500

first series was a complete failure, apparently because of the vulnerability of the mesothelial cells, which had been destroyed by the palpating hands of the surgeon. To check this hypothesis we treated five guinea pigs accordingly and made scanning electron microscopic preparations. They showed a thick irregular fibrinous coat. Knowing this, we tried the freezing technique once more using small untouched strips from four patients who had had abdominal operations and seven who underwent thoracotomies. The patients' ages were between 45 and 95; younger individuals are not included in our series.

The results are consistent with those from guinea pigs as regards the cellular borders and their stainability by silver impregnation (Fig. 4). However, there was some variation in nuclear size, there being some larger nuclei with two or four times the normal DNA content as measured by scanning cytophotometry of Feulgen-stained preparations. This means that some polyploid nuclei are present in situ. Also, some multinucleated cells similar to those found in effusions were also found in situ, and there were indications that local hyperplastic reactions had occurred.

Proliferations in effusions were our main object of study in a second series of investigations on reactive mesothelial changes, concentrating on a sort of "experimental" ascites: continuous ambulatory peritoneal dialysis (CAPD). This method was introduced by Popovich and co-workers in 1976–1978 and consists of lavage of the peritoneal cavity with 2000 ml glucose solution over 4–5 h. It can be performed by the patients themselves. This independence of the patient from machinery naturally demands that the bags be changed carefully. Infections are continuous danger. We first received the material in the cytology laboratory when it was

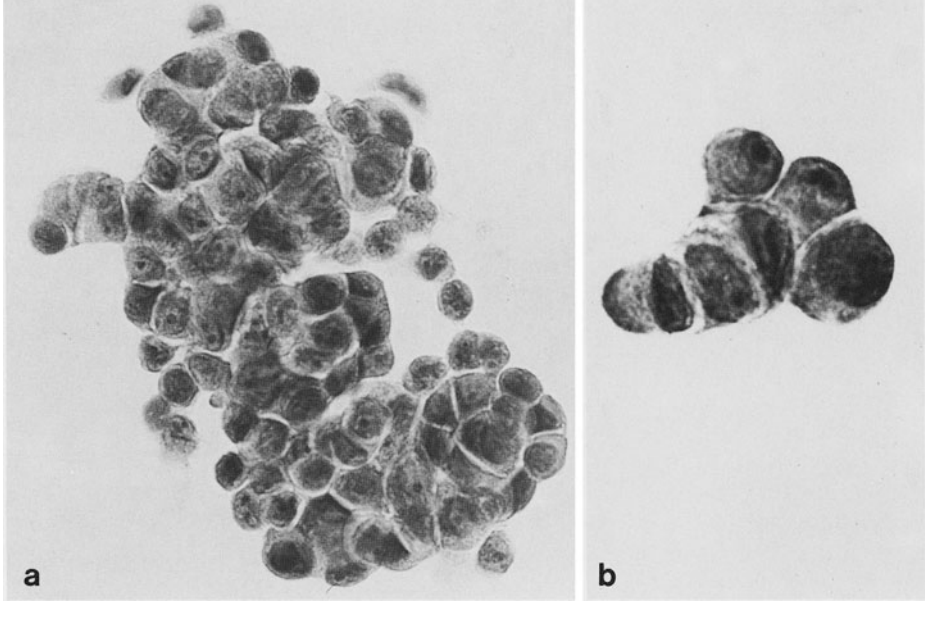

Fig. 5 a, b. Mesothelial proliferations after CAPD. **a** Papanicolaou, ×690. **b** MGG, ×1000

sent to us for confirmation of suspected eosinophilic inflammations; we found various degrees of general inflammation in 85% of the 362 specimens. The inflammations did not occur continuously during the study period but there was a certain increase in chronic form with time. Eosinophilic reactions were observed sporadically in 27 of the 32 patients (84%).

The mesothelial reactions which occurred in 39% of the specimens are especially interesting from the cytologic point of view (Fig. 5). Some of them appear highly suggestive of malignancy because of the variability of the clusters and some of the single cells. However, if their numbers are considered in relation to the 2000 ml of fluid they are rather rare. However, the number of atypical, often polyploid mitoses found is rather high (7.5% of the CAPD specimens; Fig. 6). Such mitoses are occasionally found in routine smears with high mesothelial cell populations, but in CAPD fluid they appear isolated because of the low cellularity.

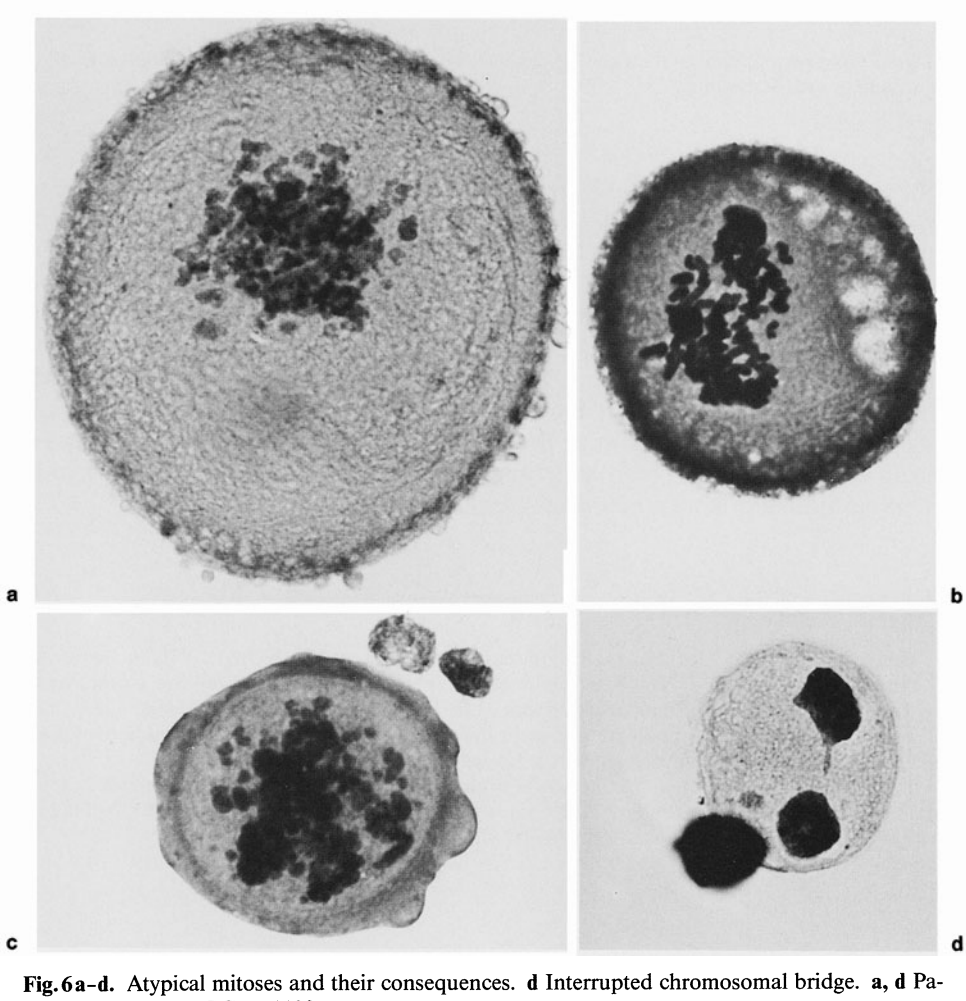

Fig. 6a–d. Atypical mitoses and their consequences. **d** Interrupted chromosomal bridge. **a, d** Papanicolaou **b, c** MGG. ×1100

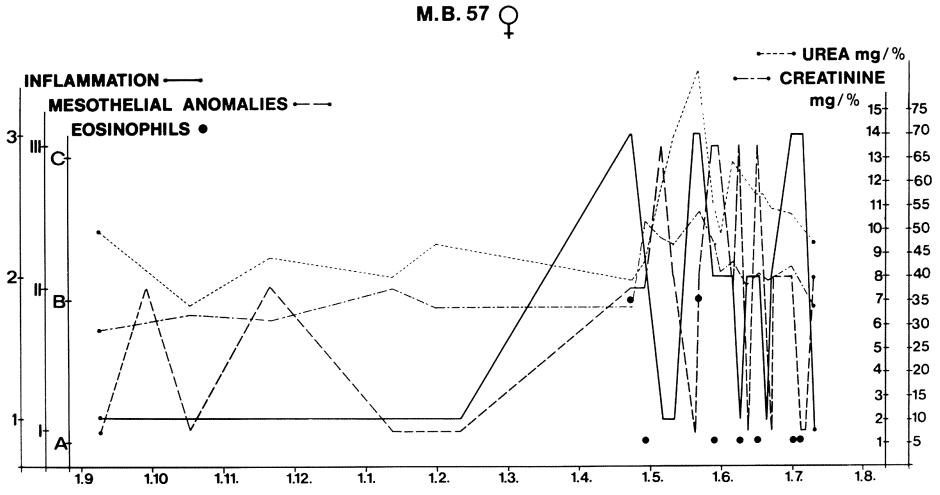

Fig. 7. Follow-up of clinical data and cytological changes in a semiquantitave evaluation show no correlation over 10 months

No relevant correlation of cytological results with the clinical data or the histories of the patients was found. Graphs (Fig. 7) demonstrate only the irregularity of the changes in all 362 samples.

The most interesting results of the cytological examination of the CAPD fluid are certainly the quality of the mesothelial changes and the relatively high number of atypical mitoses found after a period of only 4–6 h. This is certainly a very short time in comparison with the time for which a spontaneous peritoneal effusion will have existed before a puncture biopsy is taken by the clinician for cytologic analysis. However, it does demonstrate the extent of the reactive mesothelial changes in man, controlled under nearly experimental conditions, and it is a reminder to be cautious in interpreting mesothelial proliferation.

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