The presence of hypertrophic mesothelial cells (HMCs) in peritoneal effluent (PE) has been considered a possible marker for peritoneal sclerosis. We conducted the present study to evaluate if the presence of HMCs in PE or in culture was related to peritoneal function alterations or to the development of sclerosing peritonitis.

We prospectively studied 32 new peritoneal dialysis (PD) patients every 4 months, determining the presence of HMCs in culture (completing 129 studies in total). We isolated mesothelial cells from nocturnal PE and cultured them ex vivo in T-25 flasks. Cell morphology was estimated using the May–Grünwald/Giemsa method. We also examined the histories of a large patient group to determine HMCs directly in PE, and we evaluated 4 of those patients (6%) who showed persistent HMCs.

In 10 of 32 prospectively studied patients, we found HMCs during the culture phase. The cells appeared in the first evaluation in 4 patients and in subsequent cultures in the remaining 6 patients. Ultrafiltration (UF) and solute transport capacity in the 10 patients were similar to those of patients who did not show HMCs. Demographic parameters were not different between the two groups. None of the prospectively studied patients showed any clinical or peritoneal functional abnormality during the study. Cultures performed after the observation of HMCs showed very poor growth capacity.

The evolution of the 4 patients in the historic group occurred as follows:

- 1 patient transferred to hemodialysis 2 years after the observation of HMCs.
- 1 patient died of an unrelated cause after 1 year on PD.

We conclude that the presence of HMCs is not necessarily a marker of sclerosing peritonitis and that such cells may be observed in cultures from new PD patients with no peritoneal function abnormalities.

Key words
Mesothelial cells, mesothelial hypertrophy

Introduction
Early diagnosis of peritoneal abnormalities is necessary to avoid irreversible consequences in patients on peritoneal dialysis (PD). For many physiologic processes, body-fluid cytology is a recognized tool for less invasive or even noninvasive diagnosis. Cytology obtained from PD effluent has demonstrated its usefulness in representing membrane status (1). Furthermore, mesothelial cells from peritoneal effluent (PE) have demonstrated sufficient growth capacity ex vivo to reproduce the cell features of peritoneal tissue (2). Although the presence of hypertrophic mesothelial cells (HMCs) in PE has been considered a marker for peritoneal sclerosis (3,4), other authors have found no remarkable functional abnormalities suggestive of severe peritoneal damage (5).

We conducted the present study to evaluate whether the presence of HMCs in PE or in culture was related to peritoneal function alterations or to the development of sclerosing peritonitis.

Patients and methods

Patients
We prospectively studied 32 new PD patients (17 men, 15 women; mean age 48.1 ± 17.6 years) to determine
the presence of HMCs in culture. All patients were treated with standard PD solutions. Every 4 months, patients were asked to collect nocturnal PE (129 studies completed in total).

Peritoneal function was assessed by calculating mass transfer coefficients (MTCs) for urea and creatinine, using a previously described methodology (6,7). Ultrafiltration (UF) capacity was measured using a 3.86% glucose dialysis solution during a 4-hour dwell.

**Mesothelial cell culture**
Mesothelial cells were isolated from nocturnal PE and cultured *ex vivo*. Bags were drained at the dialysis center and immediately processed for mesothelial cell isolation.

The methodology for mesothelial cell culture has been previously described (8–10). The number of mesothelial cells was estimated in a Neubauer chamber. Afterward, cells were seeded into 25 cm² tissue-culture flasks and counted at confluence. For mesothelial cell subculture, cells were resuspended and seeded into 24-well plates at $2 \times 10^4$ cells per well. Viability of the mesothelial cells was assessed by trypan blue exclusion. Cells were identified as mesothelial cells at isolation from the PE and after trypsinization from confluent 25 cm² flasks. Cell morphology was assessed in cytospin optical microscopy slides [Cytospin (500 rpm): Thermo Electron Corporation, Woburn, MA, U.S.A.] stained with May–Grünwald/Giemsa. The HMCs were defined by size (>100 µm in diameter). Cells were further characterized by immunohistochemical staining. Mesothelial cells were identified by positive staining for cytokeratin and negative staining for CD45, HLA–DR, and von Willebrand factor. All antibodies were supplied by Dako Corporation (Glostrup, Denmark).

**Studies with HMCs obtained from PE (in vivo)**
We also evaluated 4 other patients who persistently showed HMCs *in vivo* at long term in a prior study. Those patients repeatedly showed large, flat mesothelial cells with a diameter greater than 100 µm (5).

**Statistical analysis**
Results are presented as mean ± standard deviation. The Mann–Whitney test for nonparametric data was used. Probabilities less than 0.05 were considered statistically significant.

**Results**

**Studies with cultures of mesothelial cells obtained *ex vivo***
In 10 of 32 patients (31.3%), HMCs were found during the culture phase. All were observed in T25 flask cultures [Figure 1]. In 4 patients, the cells appeared only in the first evaluation; in the remaining 6 patients, the cells appeared in subsequent cultures. In 1 patient, HMCs were detected twice. Cultures performed after observation of HMCs showed very poor growth capacity (Figure 2).

We found no significant differences in age, sex, or cause of renal failure between the patients with and without HMCs. All of the patients showed no clinical or peritoneal functional abnormalities during the study.

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**FIGURE 1** Morphology of hypertrophic mesothelial cells in culture (T25 flask cultures).

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**FIGURE 2** Cultures performed after the observation of hypertrophic mesothelial cells.
Urea and creatinine MTCs were not different in either group (with HMCs: 22.1 ± 4.5 mL/min and 10.8 ± 3.1 mL/min respectively; without HMCs: 21.8 ± 4.2 mL/min and 10.5 ± 3.8 mL/min respectively). Ultrafiltration capacity was similar in both groups of patients (with HMCs: 875 ± 357 mL; without HMCs: 900 ± 393 mL). No patient showed clinical or functional data compatible with peritoneal sclerosis.

Studies with HMCs obtained in vivo

Table I shows the evolution of patients who had persistent HMCs in vivo. Two years after the observation of HCMs, 1 patient was transferred to hemodialysis because of peritonitis. One patient died on PD from an unrelated cause after 1 year. Another patient received a successful kidney graft 5 years later, and the final patient was transferred to hemodialysis because of a type I UF failure.

Figure 3 shows representative HMCs in PE.

Discussion

Mesothelial transformation as a consequence of PD has previously been documented by our group (2). In the course of practicing ex vivo cultures of mesothelial cells, we have occasionally observed hypertrophy in the flasks, a phenomenon that has unknown significance. Some years ago, during our first studies assessing PE cytology, we detected and described HMCs in 4 patients (5). Although both observations represent a similar transformation by mesothelial cells, they happened under different conditions. We have therefore attempted to determine possible relationships.

Some authors have mentioned the presence of giant mesothelial cells with the development of peritoneal sclerosing syndromes (4). Peritoneal sclerosis has been reported as a frequent complication in patients on long-term PD (11–13). The condition may progress to scle-roaing encapsulating peritonitis, which is a serious complication of the PD modality (12,13). Because we have had the opportunity to observe some patients who showed HMCs in vivo at long-term, we tried in the present study to reply to the questions posed by authors who have associated HMCs with peritoneal sclerosis (3,4).

Our results fail to demonstrate that ex vivo HMCs—that is, in HMCs culture—are related to demography or peritoneal function. However, our most interesting finding is related to the loss of subsequent growth capacity after the first detection of HMCs in culture. Cell hypertrophy could be the consequence of an arrest of the cell cycle.

Mesothelial cells usually grow in culture by duplicating themselves until they reach confluence (8–10). When the growth capacity of the cells is limited or exhausted, confluence is delayed or fails to occur. That is the situation in which HMCs are more

<table>
<thead>
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<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
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<td>CGN</td>
<td>Diabetes</td>
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<tr>
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<td>2</td>
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<td>Renal allograft</td>
<td>Death</td>
<td>Peritonitis</td>
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PD = peritoneal dialysis; ESRD = end-stage renal disease; CGN = chronic glomerulonephritis.
frequently observed. The coincidence of that phenomenon with the lack of mesothelial cell growth capacity in later assays and the reproducibility of peritoneal tissue findings by effluent cytology (2) are all very suggestive of the loss of mesothelial cell growth capacity in vivo.

Regarding HMCs found in vivo in PE, our findings do not support the presence of coexisting sclerosing syndromes. The fact that the patients continued on uneventful PD (up to 10 years after the first detection) demonstrates that sclerosing peritoneal syndrome was definitely not present at the time of culture. We therefore believe that the proposal of other authors (4) to suspend PD when HMCs are observed so as to prevent the development of sclerosing syndromes is inappropriate. The change of mesothelial cells into a hypertrophic morphology is a complex phenomenon that requires more study. We think that the detection of HMCs, either in vivo or ex vivo, represents cell-cycle arrest—probably as a consequence of a negative relationship with dialysate components. Two different approaches based on mesothelial cell biocompatibility and cellular rest, which combine several weeks of peritoneal rest (14) with restarting PD using more biocompatible fluids, would likely be a more appropriate treatment. The possibility of continuous monitoring for the presence of HMCs in effluent makes the latter approach possible.

Conclusion

The presence of HMCs is not necessarily a marker of sclerosing peritonitis. Such cells may be observed in culture from new PD patients with no peritoneal functional abnormalities.

References


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In the present study, we compared the influence of icodextrin (2 g/dL) on uric acid (20 mg/dL) and albumin (1 g/dL) transfer through isolated rabbit parietal peritoneum. In separate series of experiments, we used a mathematical model to calculate the transport rate of solutes from the interstitial to the mesothelial side of peritoneal membrane (I→M) and in the opposite direction (M→I) before and after introduction of glucose polymer.

In control conditions, the rates of uric acid and albumin passage across the rabbit peritoneum remained constant. Mean values (x ± standard error of the mean (SEM)) of the diffusive permeability coefficient $P$ were 1.936 ± 0.324 (I→M) and 2.078 ± 0.186 (M→I) for uric acid and 0.341 ± 0.066 (I→M) and 0.389 ± 0.084 (M→I) for albumin (all ×10 $^{-4}$ cm/s), respectively. The introduction of glucose polymer into the experimental system did not alter the I→M transport of either solute; but, in the opposite direction, it caused a 17% decline in the $P$ for uric acid ($p < 0.05$), and an increase of 77% in the same parameter for albumin ($p < 0.03$). Hence, I→M transfer dominated M→I transfer of uric acid ($p < 0.04$). In contrast, in albumin transport, M→I was higher than I→M ($p < 0.02$). We conclude that, in vitro, icodextrin modifies the diffusive permeability of the peritoneum and induces transport asymmetry for some small and large solutes.

Key words
Peritoneal transport, icodextrin, uric acid, albumin

Introduction
Glucose polymer (called icodextrin) has been used in dialysis fluid to respond to the disadvantages of long-term glucose use during peritoneal dialysis (PD), especially in patients with a highly permeable peritoneum (1–3). Glucose polymer has been also proposed as a carrier solution in intraperitoneal chemotherapy, keeping higher doses of drugs in the peritoneal space and reducing the concentration of those drugs in the systemic circulation (4). Recent analyses also demonstrate the possibility that icodextrin can assist in preventing adhesion formation caused by intraperitoneal pharmacotherapy (5).

Application of glucose polymer during PD causes both positive and negative effects. It is observed to produce effective and sustained ultrafiltration, with minor absorption to the systemic circulation and lower osmotic stress. It also lessens the patient’s caloric load, so that hyperglycemia and hyperinsulinemia are absent, with the concomitant achievement of a more favorable lipid and lipoprotein profile than that produced by glucose-based solutions. Disadvantageous changes include accumulation of breakdown products, a small decline in serum sodium and chloride, cytotoxic effects on monocytes and cultured mesothelium, chemical peritonitis, and DNA injury through a mechanism of lipid peroxidation. Icodextrin also modifies the transport functions of the peritoneum (1–3,6,7).

Peritoneal transfer characteristics with the use of icodextrin-based fluid have previously been investigated in both humans and animals, with conflicting results. Also, the studies looked at solute transport in vivo (1,2,7). Our aim in the present study was therefore to examine, in vitro, the direct effect of glucose polymer on the bidirectional transport rate for small (uric acid) and large (albumin) molecules across the peritoneal membrane.

Material and methods
Fragments of parietal peritoneum from New Zealand male rabbits were placed into a modified Ussing-chamber system as an experimental model. The tissue (with an active membrane surface area amounting to
1.1 cm²) was connected through a peristaltic pump to a fluid reservoir containing Hanks solution, which circulated at the rate of 11 mL/min. Adequate oxygen content in the medium and a constant pH (7.4) were both maintained by continuous bubbling of a gas mixture consisting of 5% CO₂ and 95% O₂. The entire system was maintained at 37°C during the experiments (8).

The transfer rates of uric acid (initial concentration gradient: 20 mg/dL; Ubichem, Eastleigh, England) and of albumin (initial concentration gradient: 1 g/dL; Sigma Chemical, St. Louis, MO, U.S.A.) from the interstitial to the mesothelial side (I→M) of the peritoneal membrane, and in the opposite direction (M→I), were studied in two separate series:

- Control conditions without icodextrin (120 minutes; n = 22)
- Before (15 – 60 min) application of icodextrin, and after (75 – 130 min for uric acid; 75 – 120 min for albumin) application of icodextrin [2 g/dL (average molecular weight 14.6 kDa); ML Laboratories, Liverpool, England] on the mesothelial side of the peritoneum

Sampling of the medium was carried out at regular intervals (10 minutes or 15 minutes). The uric acid concentration was determined using an enzymatic test with uricase (Cormay, Lublin, Poland). For albumin, the Peterson modification of the micro Lowry method (Sigma Chemical, St. Louis, MO, U.S.A.) was applied. A mathematical model of mass transport was used to estimate a diffusive permeability coefficient $P$ (scaled to the surface area of the membrane) for the examined specimens.

Changes of $P$ attributable to experimental modifications were determined as percentages of the control value for each individual experiment (that is, separately for each sampler of peritoneal membrane) and are presented as mean value ± standard error of the mean (SEM) for the whole series. In this way, the initial part of an experiment with a particular membrane sample served as a control for the second part.

For statistical analysis, the Student $t$-test for paired and unpaired data was used (8).

Results
In control conditions, the rates of uric acid and albumin transport from the interstitial to the mesothelial side of membrane and in the opposite direction remained constant. For the cases of I→M and M→I, respectively, the values of the diffusive permeability constant $P$ were 1.936 ± 0.324 and 2.078 ± 0.186 for uric acid and 0.341 ± 0.066 and 0.389 ± 0.084 for albumin (all $\times 10^{-4}$ cm/s).

Icodextrin introduction at 60 minutes of the experiment did not change I→M transport of the analyzed solutes. However, in the opposite direction, glucose polymer caused a decrease of about 17% in uric acid diffusion and an increase of about 77% in albumin transfer. Hence, uric acid and albumin transport showed asymmetry (Figure 1):

- I→M passage dominated M→I passage of uric acid.
- In contrast, in albumin transfer, M→I was higher than I→M.

Discussion
The results of previous in vivo studies concerning the effects of icodextrin on transperitoneal transport are ambiguous (1,2,7). Application of 7.5 g/dL glucose polymer instead 3.86 g/dL glucose in stable continu-
ous ambulatory peritoneal dialysis (CAPD) patients during a 4-hour dwell did not affect the mass transfer area coefficients (MTACs) of such low molecular weight solutes as urea, creatinine, and urate (1). Similarly, in studies with an animal model in vivo, the urea dialysate-to-plasma (D/P) concentration ratio was not significantly different between two groups of rats injected daily for 30 days with 7.5 g/dL icodextrin or 3.86 g/dL glucose (7). However, urea Kt/V and creatinine clearance values were both higher for icodextrin solution (7.5 g/dL) than for glucose solution (2.27 g/dL) in automated peritoneal dialysis patients (2). Additionally, 7.5 g/dL icodextrin–based fluid did not change clearances of albumin and other serum proteins (immunoglobulin, macroglobulin) in CAPD patients, but did increase $\beta_2$-microglobulin clearance in comparison with 1.36 g/dL and 3.86 g/dL glucose solutions [about 26% and 44%, respectively (1)].

In contrast to those studies, Frajewicki and co-authors detected a significant increase in the excretion of protein (including albumin), unrelated to molecular weight, with a single use of glucose polymer (7.5 g/dL) in rats. The effect was more prominently after long-term administration of icodextrin. Notably, the simultaneous use in the animals of a mixture of 3.86 g/dL glucose and 7.5 g/dL icodextrin induced the highest levels of protein in the peritoneal effluent (7). These contrasting results are probably related to interspecies heterogeneity in regard to both the structure and the function of peritoneal membrane and the models used (1,7,9–11).

In the present in vitro study, application of icodextrin (2 g/dL) modified diffusive peritoneal permeability and caused asymmetry in the transperitoneal transfer of uric acid and albumin, which, in the control conditions, had been similar in both directions and dependent on molecular weight. The modifications varied for examined compounds. In the case of uric acid, $I \rightarrow M$ diffusion dominated $M \rightarrow I$ diffusion. In contrast, albumin $I \rightarrow M$ transport was lower than that in the opposite direction. The reasons for the observed changes are not known, but can probably be attributed to the separate mechanisms of uric acid and albumin peritoneal transport.

Uric acid is a low molecular weight compound, and diffusion is the most important process of its passage across the peritoneal membrane. Among other factors, it depends on the transperitoneal concentration gradient, the molecular weight of molecule, and the properties of the peritoneum (9,11).

The mechanism of large-solute peritoneal transport is not clear. Controversy exists concerning whether in vivo transfer of macromolecules (for example, albumin) through the large-pore system occurs mainly by diffusion or by convection induced by a hydrostatic and osmotic pressure gradient. The size, shape, and electric charge of high molecular weight solutes are factors with a potential influence on transport from the plasma to the peritoneal cavity. However, the disappearance of large molecules from the intraperitoneal space seems to be independent of the molecular size (9). In vitro, the role of transmesothelial pinocytotic transport cannot be excluded (11). Furthermore, modifications of transport processes after introduction of glucose polymer need also to consider the physicochemical nature of the icodextrin molecule. That is, the molecule’s oncotic properties, high viscosity, and possible degradation to lower molecular weight fragments may result in an increase of fluid osmolality and metabolic activity with regard to peritoneal transport functions (3,6,10).

Notably, the asymmetry of uric acid and albumin transperitoneal transport induced by icodextrin (2 g/dL) in vitro may be also observed in vivo during PD. That asymmetry seems to be important and beneficial from the clinical viewpoint. The phenomenon indicates better excretion of certain small uremic toxins from the blood to the peritoneal fluid and lower losses of serum proteins.

**Conclusion**

The results of the present experiment show that, in vitro, glucose polymer modifies the diffusive permeability of the peritoneum and induces asymmetry of uric acid and albumin transport—an asymmetry that could be favorable for the efficiency of PD. The causes of these effects are not yet recognized. Further studies are needed on how the application of icodextrin solution affects the mechanisms that underlie transport through the peritoneum.

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