We performed in vitro experiments with the isolated rabbit parietal peritoneum to evaluate the importance of fluid stirring intensification and of chemical modification of mesothelium and interstitium to the peritoneal transport of glucose and icodextrin. We used a mathematical model of mass transport to calculate the diffusive permeability coefficient, \( P \), in centimeters per second. In control conditions (intact tissue; stirring rate: 11 mL/min), the rate of glucose (2.0 g/dL) transfer remained constant, and no differences were observed for transport from the interstitial to the mesothelial (I \( \rightarrow \) M) side of the membrane or in the opposite direction (M \( \rightarrow \) I). The value of \( P \) (± standard error of the mean) was 2.731 ± 0.472 \( \times 10^{-4} \) cm/s. In contrast, the icodextrin (7.5 g/dL) I \( \rightarrow \) M transport rate was higher than that for M \( \rightarrow \) I (P: 0.319 ± 0.038 \( \times 10^{-4} \) cm/s and 0.194 ± 0.035 \( \times 10^{-4} \) cm/s respectively).

Dynamics of the icodextrin M \( \rightarrow \) I transfer were constant, but I \( \rightarrow \) M increased by 50% over time. The intensification of the stirring rate increased the value of \( P \) at varying rates: the increase was greater for icodextrin than for glucose, and greater for the I \( \rightarrow \) M transport direction than for the M \( \rightarrow \) I direction for both solutes. Chemical modification (by 2.5 mmol/L sodium deoxycholate) increased glucose and icodextrin I \( \rightarrow \) M transfer a mean of 41% and 81% respectively, but increased M \( \rightarrow \) I transfer by 70% and 224% respectively.

The dynamics of glucose and icodextrin peritoneal transfer in vitro are different: glucose diffusion is constant, but I \( \rightarrow \) M icodextrin transfer increases over time and is greater than M \( \rightarrow \) I transfer. Fluid stirring intensification and chemical injury to the peritoneum enhance diffusion of glucose and icodextrin. Glucose and icodextrin M \( \rightarrow \) I transfer, but not I \( \rightarrow \) M transfer, is restricted more by tissue barriers than by stagnant fluid layers.

Key words
Diffusive transport, glucose, icodextrin, unstirred fluid layers, sodium deoxycholate

Introduction
Glucose is usually added to dialysis solution for the development of sufficient ultrafiltration during peritoneal dialysis (1,2). However, with increase in dwell time, ultrafiltration decreases because of rapid glucose absorption from the peritoneal cavity to the vascular bed, with its resulting decrease in the osmotic pressure gradient (3,4). Recently, dialysis fluids with glucose polymer (icodextrin) have been introduced to provide sustained ultrafiltration and to avoid the disadvantageous effects of glucose that are related to long-term application of a hyperosmotic glucose-based fluid during standard peritoneal dialysis (5,6). Icodextrin has been proposed as a carrier solution during intraperitoneal chemotherapy for high doses of drugs in the peritoneal space and for reduction of its concentration in the systemic circulation, and as a prevention measure for adhesion formation caused by intraperitoneal pharmacotherapy (7–9).

Diffusion and convection are the main mechanisms of transperitoneal transport. Transport can occur in two directions—from the peritoneal cavity to the blood, and from the vascular bed to the abdominal space—according to the concentration gradient and the rate of dialysate and blood flow. Diffusion is the main mechanism of glucose (molecular weight: 180 Da) transport, as it is for other small molecules during peritoneal dialysis. Peritoneal absorption of glucose increases during peritonitis as a consequence of peritoneal barrier modifications (10–12). Knowledge of the peritoneal transport of icodextrin, a high molecular weight compound (13,000 – 19,000 Da), is not extensive. Intrapertoneally introduced icodextrin is probably absorbed at a low rate mainly by the lymphatic pathway because of convective fluid movement.
out of the peritoneal cavity; the diffusive component is limited, but not excluded (13,14).

During peritoneal dialysis, transperitoneal transport encounters several resistances: capillary endothelium with basement membrane, interstitium, mesothelium with basement membrane, and stagnant fluid layers on the surfaces of mesothelium and endothelium. Capillary wall is considered the major transport barrier for transperitoneal exchange. Mesothelium is usually expected to be highly permeable. Information about the transport characteristics of the interstitium, which can be a significant transport barrier, especially for small molecules, is scarce. Stagnant fluid layers are not often investigated (10,15).

In the present study, we compared the bidirectional diffusion transfer of glucose and icodextrin across peritoneal membrane in vitro and its change as a result of fluid stirring intensity and chemical modification of the peritoneal membrane.

Material and methods

We studied transfer of glucose and icodextrin (initial concentration gradients, 2.0 g/dL and 7.5 g/dL respectively) from the interstitial to the mesothelial side (I→M) of rabbit peritoneal membrane and in the opposite direction (M→I). The experiments were carried out on fragments of parietal peritoneum taken from the anterior abdominal wall of New Zealand rabbits and mounted in a modified Ussing-type chamber. The active surface of the membrane was 1.1 cm². The chamber was filled with Hanks solution of the following composition (all mmol/L): NaCl, 136.88; KCl, 5.36; NaHCO₃, 4.16; CaCl₂, 1.26; KH₂PO₄, 0.44; Na₂HPO₄×12H₂O, 0.34; MgCl₂×6H₂O, 0.49; MgSO₄×7H₂O, 0.41. A total of 15 mL of solution was circulated at the rate 5.5 mL/min, 11 mL/min, or 22 mL/min. Adequate oxygen content in the medium and a constant pH of 7.4 were maintained by continuous bubbling with a gas mixture consisting of 5% CO₂ and 95% O₂. Polyethylene tubes were used to connect the chamber to the fluid reservoir and a peristaltic pump. The whole system was placed in a thermostat box at 37°C (14).

The fourth procedure used sodium deoxycholate (2.5 mmol/L, 104 mg/dL) added to the medium on the mesothelial side of the peritoneum. After 3 minutes of incubation with the detergent, the chamber was thrice washed with fresh Hanks solution.

Assuming that the transmembrane transport in the Ussing cell is purely diffusive and that mixing of fluid in each compartment of the cell is perfect, we used a mathematical model to estimate the diffusive permeability of the peritoneum (P in centimeters per second), expressed as a mean value ± standard error of the mean (SEM). The changes of P attributable to the experimental modifications were determined as a percentage of the control value before the change, individually for each experiment (that is, separately for each piece of peritoneal membrane), and they are presented as mean ± SEM for the series. In this way, each piece of membrane served, in the initial part of the experiment, as a control for the second part.

For statistical analyses, we used the Student t-test for paired data (14).

Results

In control conditions, the rate of glucose transfer was stable for 120 minutes of the experiment, and no differences were observed for transport directed from the interstitial to the mesothelial side of membrane (I→M) or in the opposite direction (M→I). Values of P were 2.878 ± 0.390 × 10⁻⁴ cm/s and 2.584 ± 0.554 × 10⁻⁴ cm/s, respectively. In contrast, the values for icodextrin were different for transport in both directions:
The dynamics (15 – 60 minutes vs. 75 – 120 minutes) of \( \text{M} \rightarrow \text{I} \) icodextrin transport remained constant, but \( \text{I} \rightarrow \text{M} \) increased by 50% over time \( (p < 0.01; \text{Table I}) \). The increase in the stirring rate to 11 mL/min from 5.5 mL/min enhanced values of \( P \) for glucose by about 74% \( (\text{I} \rightarrow \text{M}) \) and 58% \( (\text{M} \rightarrow \text{I}) \) and for icodextrin by about 95% \( (\text{I} \rightarrow \text{M}) \) and 61% \( (\text{M} \rightarrow \text{I}) \) [Figure 1(A)]. The change in stirring from 11 mL/min to 22 mL/min increased glucose transport by about 42% in both directions, and increased icodextrin transport by about 80% in the \( \text{I} \rightarrow \text{M} \) direction and by about 25% in the \( \text{M} \rightarrow \text{I} \) direction [Figure 1(B)]. Chemical injury to the peritoneum augmented glucose transfer by 41% \( (\text{I} \rightarrow \text{M}) \) and 70% \( (\text{M} \rightarrow \text{I}) \), but augmented icodextrin transfer by 81% \( (\text{I} \rightarrow \text{M}) \) and 224% \( (\text{M} \rightarrow \text{I}) \) [Figure 1(C)].

**Discussion**

Earlier investigations showed that, in contrast to glucose, intraperitoneally introduced icodextrin is transported slowly to blood. About 40% of this macromolecule (7.5 g/dL) is absorbed during a 12-hour dwell (17). By comparison, glucose absorption is much higher; about 85% is transferred during 2 hours of peritoneal dialysis (4). In *in vitro* conditions, the transperitoneal transport of icodextrin (average weight: 14,556 Da; initial concentration gradient: 7.5 g/dL) was about ten times smaller than that of glucose (molecular weight: 180 Daltons; initial concentration gradient: 2.0 g/dL). The peritoneal transport of glucose was stable during the 120 minutes of the experiment, and differences between the two transfer directions were not observed. Icodextrin transport across the peritoneal membrane from the interstitial to the mesothelial side of the tissue increased over time and showed asymmetry: \( \text{I} \rightarrow \text{M} \) transfer was greater than \( \text{M} \rightarrow \text{I} \) transfer. The reasons for the described *in vitro* icodextrin transport phenomena are not known. They may be connected with partial degradation of the glucose polymer, an increase in fluid osmolality, and modifications in peritoneal transport function (18,19).

Glucose polymer degradation in the rat is related to high amylase activity, but no data are available for the rabbit. In contrast, in humans, intraperitoneal metabolism of icodextrin is small, and amylase activity in peritoneal effluents from peritoneal dialysis patients is undetectable or very low (13,17,18). Previous *in vitro* results showed that glucose polymer modifies the diffusive permeability of the peritoneum and induces asymmetry of uric acid and albumin transport (20). Frajewicki and co-authors detected a significant increase in the peritoneal excretion of protein after a single use of glucose polymer (7.5 g/dL) in rats and, more importantly, after long-term administration. Moreover, the latest investigations underline the influence of icodextrin on the peritoneal membrane: chemical peritonitis, reduction in mesothelial cell volume, and membrane lipid peroxidation (21,22).

Unstirred fluid layers are a barrier to the peritoneal transport of molecules, but the significance of the phenomenon is not clear. A variety of methods have been used *in vivo* and *in vitro* to reduce the stagnant fluid resistance on the peritoneal surface: for example, vibration of the abdominal wall, rhythmic shaking of experimental animals, or changes in fluid flow rate (14,23,24).

In the present *in vitro* study, we examined the importance of stagnant fluid layers by changing the fluid flow speed on both the mesothelial and interstitial sides of the peritoneal membrane. An increase of fluid mixing to 11 mL/min from 5.5 mL/min augmented glucose and icodextrin peritoneal transport bidirectionally by about 42% – 95% (higher percentages for glucose polymer than for glucose). Further increasing the fluid flow

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**Table I**  
Transperitoneal glucose and icodextrin transport *in vitro* expressed as diffusive permeability \( (P) \) in control conditions (15 – 60 minutes and 75 – 120 minutes)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Initial concentration</th>
<th>Experiments (n)</th>
<th>Transport direction</th>
<th>Diffusive permeability ( (P) ) ( \times 10^{-4} \text{ cm/s} )</th>
<th>( 15–60 \text{ Min} )</th>
<th>( p ) Value</th>
<th>( 75–120 \text{ Min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2 g/dL</td>
<td>10</td>
<td>( \text{I} \rightarrow \text{M} )</td>
<td>2.797±0.388</td>
<td>NS</td>
<td>2.959±0.393</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>( \text{M} \rightarrow \text{I} )</td>
<td>2.733±0.679</td>
<td>NS</td>
<td>2.436±0.429</td>
<td></td>
</tr>
<tr>
<td>Icodextrin</td>
<td>7.5 g/dL</td>
<td>11</td>
<td>( \text{I} \rightarrow \text{M} )</td>
<td>0.280±0.038</td>
<td>0.01</td>
<td>0.394±0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>( \text{M} \rightarrow \text{I} )</td>
<td>0.195±0.039</td>
<td>NS</td>
<td>0.207±0.035</td>
<td></td>
</tr>
</tbody>
</table>

I = interstitial side of the peritoneal membrane; M = mesothelial side of the peritoneal membrane; NS = statistically nonsignificant.
rate (to 22 mL/min from 11 mL/min) evoked less-evident changes (25% – 80%).

When animals were shaken, in vivo diffusion of glucose was more than four times enhanced, but transfer of macromolecules (protein) increased only by about 50% (24). During continuous flow-through peritoneal dialysis in dogs, glucose clearance was 11 ± 5 mL/min for a flow rate of 3.6 L/h and 16.5 ± 6 mL/min for a flow rate of 6 L/h (25,26). These in vitro and in vivo findings are probably all connected with an increase of fluid contact with the peritoneal membrane and the resulting reduction in stagnant fluid layers. In contrast, in transport experiments with a plastic chamber affixed to rat parietal peritoneum, fluid mixing did not affect the transfer of mannitol, just as in a previous in vitro investigation, an increase in fluid flow did not change the transport of urea, but enhanced transfer of albumin (14,27). The reasons for the described diversity are unknown, but complexity of the transperitoneal transport mechanisms, adaptation of the peritoneal membrane, and differences in the experimental models cannot be excluded.

In our in vitro study, chemically modified peritoneum was used as a surrogate for change in the peritoneal membrane during long-term dialysis with recurrent peritonitis. Within the peritoneum, peritoneal inflammation induces structural and functional disturbances that influence peritoneal transfer dynamics (13,28,29). To injure the experimental peritoneal membrane, we used 2.5 mmol/L (104 mg/dL) sodium deoxycholate, which is known to denude the peritoneal and pleural mesothelium and the epithelium of renal tubules, but not to damage the associated basement membranes (28,30). Other data from our study showed that the detergent induces apoptosis and necrosis of mesothelial cells, lipid peroxidation, and generation of free radicals. Morphologic and morphometric examinations showed that sodium deoxycholate removed mesothelial cells and induced loosening of adjacent connective tissue. The detergent increased the thickness of the rabbit parietal peritoneum by about 40% (p < 0.001, unpublished data). This chemical injury to the peritoneum increased diffusion transport of glucose and icodextrin in both transport directions, but to a higher degree for glucose polymer than for glucose. Similar effects were obtained for albumin and urea after sodium deoxycholate was administered in peritoneal dialysis in animals (28).
In humans using 1.5% glucose dialysis solution, peritoneal transport of glucose from the peritoneal cavity to the blood increased during peritonitis by 74%. In *in vivo* investigations with animal models, peritonitis increased peritoneal absorption of glucose by 155% during a 2-hour dwell with 4.25% glucose solution (14,29). No data are available about the effect of peritonitis on the absorption of icodextrin during peritoneal dialysis in humans. An increase of glucose polymer degradation and dialysate osmolality were observed in rats during peritoneal inflammation (13).

**Conclusions**

Our study results indicate that the *in vitro* peritoneal transfer dynamics of glucose and icodextrin are different: glucose diffusion is constant, but I → M icodextrin transfer increases over time and is greater than M → I transfer. Intensification of dialysate stirring (a decrease in stagnant fluid resistance) and chemical injury to the peritoneum can both induce an increase in peritoneal diffusive transport of glucose and icodextrin. Glucose and icodextrin M → I transfer, but not I → M transfer, is restricted more by tissue barriers than by stagnant fluid layers. Changes in diffusive transport may be unfavorable for peritoneal dialysis efficiency.

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**References**


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