In patients with end-stage renal disease on peritoneal dialysis, the peritoneum is effectively utilized as an endogenous semipermeable membrane. Chemical agents may alter the structure of the peritoneum and induce changes in its transfer properties. The purpose of our in vitro study was to compare the influence of sodium hyaluronan (a cytoprotective glycosaminoglycan, 2000 kDa, 40 mg/dL) and sodium deoxycholate (an intense oxidative stress factor, 414 Da, 104 mg/dL) on the transfer of insulin (5.8 kDa, 100 mg/dL) and albumin (68 kDa, 1000 mg/dL) through isolated rabbit parietal peritoneum. 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) in three separate series of experiments:

- Control conditions without chemical agents
- Before (15 – 60 minutes) and after (75 – 120 minutes) introduction of sodium hyaluronan into the experimental system
- Before (15 – 75 minutes) and after (90 – 150 minutes) introduction of sodium deoxycholate into the experimental system

The results are expressed as a diffusive permeability coefficient $P$ in centimeters per second. During the 120 minutes of the control stage, the rate of insulin and albumin bidirectional passage remained constant. Values of $P \pm$ standard error of the mean did not correlate with the molecular weight of the compounds and measured 0.145 $\pm$ 0.033 $\times$ 0.0001 cm/s and 0.146 $\pm$ 0.022 $\times$ 0.0001 cm/s for insulin, and 0.271 $\pm$ 0.056 $\times$ 0.0001 cm/s and 0.315 $\pm$ 0.057 $\times$ 0.0001 cm/s for albumin in the I→M and M→I transport directions respectively. Application of sodium hyaluronan did not change the bidirectional transport of either solute. However sodium deoxycholate caused the values of $P$ to increase by 142% ($p < 0.05$) for insulin I→M transfer and by 102% ($p < 0.02$) for insulin M→I transport. Albumin passage increased by 193% ($p < 0.01$) for both transfer directions.

We conclude that, in vitro, sodium deoxycholate but not sodium hyaluronan increases the bidirectional peritoneal transport of insulin and albumin. In the case of larger molecular weight solutes, the modification is greater.

Key words
Peritoneal transport, hyaluronan, deoxycholate, macromolecules

Introduction
High molecular weight fractions of hyaluronan have found application in fluids for peritoneal dialysis (1). The hyaluronan molecule is an essential component of the peritoneal extracellular matrix. It shows anti-adhesive properties and participates in restoring peritoneal integrality and remodeling the peritoneum (2-4).

In a model of the chemically modified peritoneal membrane, deoxycholate sodium is used as a surrogate for the changes induced by prolonged peritoneal dialysis with peritonitis episodes (5-12). This detergent can damage mesothelial cells and generate free radicals (12).

The influence of sodium hyaluronan and deoxycholate on transperitoneal transport of large molecules is currently little understood (1,3,12).

Material and methods
Our experimental model used fragments of the parietal peritoneum of New Zealand male rabbit placed into a modified Ussing-type chamber. The active surface area of the membrane was 1.1 cm². The chamber was connected by peristaltic pump to a fluid reservoir.
containing Hanks solution, circulating at a rate of 11 mL/min. Adequate oxygen in the medium and a constant pH of 7.4 were both maintained by continuous bubbling with a gas mixture of 5% CO₂ and 95% O₂. The whole system was maintained in a thermostatic box at 37°C (8).

The transfer rates of insulin (ICN Biomedicals, Aurora, OH, U.S.A.; initial concentration gradient: 100 mg/dL; molecular weight: 5.8 kDa) and albumin (Sigma Chemical, St. Louis, MO, U.S.A.; 1000 g/dL; 68 kDa) from the interstitial to the mesothelial side (Sigma Chemical, St. Louis, MO, U.S.A.; initial concentration gradient: 100 mg/dL; molecular weight: 5.8 kDa) and albumin (Sigma Chemical, St. Louis, MO, U.S.A.; 1000 g/dL; 68 kDa) from the interstitial to the mesothelial side of the peritoneal membrane and in the opposite direction (M→I), were studied in three separate series:

- Control conditions (120 minutes)
- Before (15 – 60 minutes) and after (75 – 120 minutes) introduction of sodium hyaluronan (SERVA Electrophoresis, Heidelberg, Germany; 40 mg/dL; 2×10⁵ Da) on the mesothelial side of the peritoneum
- Before (15 – 75 minutes) and after (90 – 150 minutes) application of sodium deoxycholate (POCH, Gliwice, Poland; 104 mg/dL; 414 Da) on the mesothelial side of the peritoneal membrane

Sampling of the medium was carried out at regular 15-minute intervals. The insulin and albumin concentrations were determined using the Peterson modification of the micro-Lowry method (Sigma Chemical). All procedures were validated for accuracy, specificity, linearity, and sample stability.

We used a mathematical model of mass transport to estimate a diffusive permeability coefficient \( P \) for the examined specimens. The changes of \( P \) attributable to experimental modifications were determined as a percentage of the control value before the change individually in each experiment and are presented as mean value ± standard error of the mean (SEM) for the whole series. In this way, for each piece of membrane, the initial portion of the experiment served as a control for the second portion (8). A SEM was applied instead of standard deviation because of diversity of series number. For statistical analysis, we used the Wilcoxon test for paired data (Statistica version 6.0: StatSoft, Tulsa, OK, U.S.A.). The Shapiro–Wilks test was applied to the evaluation of data distribution. Values of \( p < 0.05 \) were considered statistically significant.

Results

The rate of insulin and albumin passage remained constant for both I→M and M→I transfer during the control stage (120 minutes). Values of the diffusive permeability coefficient \( P \) were 0.145 ± 0.033 × 0.0001 cm/s and 0.146 ± 0.022 × 0.0001 cm/s for insulin \((n = 16)\) and 0.271 ± 0.056 × 0.0001 cm/s and 0.315 ± 0.057 × 0.0001 cm/s for albumin \((n = 26)\) in the I→M and M→I transport directions respectively. The introduction of sodium hyaluronan into the experimental system did not change the bidirectional transport of either solute [see Figure 1(A)]. However, after application of sodium deoxycholate, we observed an increase in \( P \) of 142% \((I→M, p < 0.05)\) and 102% \((M→I, p < 0.02)\) for insulin transfer [see Figure 1(B)]. Moreover, this oxidative stress factor augmented albumin passage by 193% \((p < 0.01)\) across the membrane in both directions [see Figure 1(B)].

Discussion

In vivo and in vitro studies point to complexities in the transport of macromolecules across the peritoneal membrane. The process is limited by the anatomic barriers of the peritoneum: endothelium, mesothelial cells, and interstitium (13–15). Large solutes are probably transferred through the pores in the peritoneal structure (diffusion and convection) and through transcellular pathways (pinocytosis). The percentage participation of these mechanisms and the importance of individual peritoneal barriers in macromolecule transperitoneal transport are not well known (15,16). Clinical studies show that insulin is transported slowly from the peritoneal cavity to the blood after its intra-peritoneal introduction; however, albumin is transferred through the peritoneum in the opposite direction, mainly through lymphatic channels (13,15).

Stability of albumin and insulin peritoneal transport was observed in both transfer directions \((I→M, M→I)\) in the present study when we compared the first and the second hour of the experiments. In in vivo conditions, peritoneal clearances of albumin, \(\beta_2^-\)microglobulin, \(\alpha_2^-\)macroglobulin, and immunoglobulin G are highest in the first 60 minutes of a dwell; after that, reduction and stabilization of the process have been observed (16). Furthermore, in the present in vitro study, the diffusive permeability coefficients for insulin and albumin were not correlated with the molecular weight of those solutes. We noted relative
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low values of $P$ for insulin. Those results are probably connected with specific ability of molecules of insulin to create (through hydrogen bonds) new compounds with large and complicated structures (17). In macromolecule transport, we cannot exclude the possibility that shape and size are more important than molecular weight.

Application of sodium hyaluronan (0.04 g/dL; $2\times10^6$ Da) on the mesothelial side of the rabbit peritoneum did not change the value of the diffusive permeability coefficient $P$ for bidirectional transfer of albumin and insulin in vitro. The results of in vivo studies show that the effect of hyaluronan on peritoneal transport of macromolecules varies. As in the in vitro studies, some of the large-solute molecules show no change of peritoneal permeability after hyaluronan use; others show a reduction in transfer. For example, intraperitoneal introduction of hyaluronan (0.01 g/dL) to phosphate-buffered saline during a 4-hour dwell in rats did not modify bidirectional albumin transport (18). Similarly, application of a glucose solution (0.025 g/dL; $1.6\times10^6$ Da) with hyaluronan during 7 days of dwells did not influence the $K_{BB}$ coefficient (measured after 4 hours’ equilibration) for protein and, as compared with a control non dialyzed group, did not affect albumin absorption from the peritoneal cavity to border tissue (3). In contrast, a single intraperitoneal dose of hyaluronan (0.05 g/dL) reduced albumin clearance in experimental animals (18).

It has been suggested that the ability of hyaluronan to inhibit peritoneal permeability for macromolecules is connected with a reduction of hydroconductivity in the peritoneum. The three-dimensional structure of high molecular fractions of hyaluronan (molecular weight above $1.6\times10^6$ Da) make water binding and a concomitant change in water control possible (2,4). Notably, we observed no such effects in the present study.

In our in vitro analyses, sodium deoxycholate intensified peritoneal transport of insulin (by a mean of 122%) and of albumin (by 193%). These modifications probably result from exfoliation of the mesothelium and disruption of the interstitium because of oxidative stress. As a solute, sodium deoxycholate can denude the peritoneal mesothelium (5,12). Studies in vivo show that it can induce apoptosis and necrosis of mesothelial cells and accelerate free-radical reactions. Furthermore, Gotloib et al. showed a considerable increase in the concentration of malondialdehyde in plasma after 5 minutes of sodium deoxycholate application; this finding was the result of intensive peroxidation of membrane lipids (12).

In our previous preliminary studies in vitro, we observed an increase in the thickness of the parietal peritoneum of about 40% after sodium deoxycholate introduction (9,11). Furthermore, a concomitant
intensification of transperitoneal creatinine transfer of 33% was noted (9). Sodium deoxycholate also intensified transport of other small molecules (glucose, urea) through the peritoneum, but at a lower level than was the case for large solutes (6–11). Comparison of our in vitro results (7,9–11) indicate that the mesothelium and interstitium restrict the transfer of high molecular weight compounds (including albumin, icodextrin, and insulin) more than they do small molecules (urea, creatinine, glucose). Similar data have been obtained in in vivo conditions: Exposure of dialyzed animals to sodium deoxycholate for 10 minutes caused albumin concentration in abdominal cavity to increase by 65%, but glucose concentration to increase by only 20% (12).

Conclusions
Sodium deoxycholate, but not hyaluronan, intensifies peritoneal transport of insulin and albumin in vitro. These observations may be clinically important in addressing the problems of malnutrition and efficiency of hormonal therapy in dialyzed patients.

References

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