Transperitoneal transport is a complicated process that includes diffusion and convection across the walls of blood microvessels into tissue interstitium, transport through the interstitium, and final passage across the peritoneum to the dialysis solution in the cavity. The purpose of this paper is to briefly review the normal physiology of this process and then to summarize the events that occur in response to inflammation within the cavity. These events begin with stimulation of macrophages, which in turn secrete cytokines. The cytokines stimulate mesothelial cells and fibroblasts in the tissue to synthesize and secrete other mediators. Those mediators initiate the complex events through which leukocytes migrate from blood vessel lumens through the interstitium and into the cavity. Much of the available data is from model in vitro systems, and therefore in vivo events must be deduced or hypothesized.

**KEY WORDS:** Inflammation; hyaluronan; cytokines.

Transport between the blood and a solution in the peritoneal cavity occurs via a complex system which includes: (a) distribution of the solute by the circulation to microvessels that perfuse tissues surrounding the peritoneal cavity; (b) transport across the endothelial barrier of these microvessels; (c) transport through the tissue interstitium (defined as extracellular space that is not contained within blood vessels) that separates the microvessels and the fluid in the peritoneal cavity, and (d) transport across the anatomic peritoneum, which is made up of connective tissue and a single layer of mesothelial cells (1). Transport in this system is passive; it occurs owing to a combination of diffusion and convection (also called solvent drag). The endothelial barrier provides size selectivity to the transport process, while the interstitium prevents direct access of the solution in the cavity to the blood capillary and adds significantly to the total transport resistance between the blood and the cavity (2).

Although each portion of the transport barrier can be defined separately, all of the parts are necessary for the system to operate efficiently. For example, the barrier properties of the mesothelium/peritoneum are equivalent to the underlying interstitium (1,2) and could be grouped with the interstitium with regard to resistance to transport. However, the mesothelial cells lining the peritoneal surface play special roles in surface lubrication and immune response (see below) of the cavity (3). Because an absence of mesothelial cells results in the proliferation of fibroblasts, an intact mesothelium prevents scarring, which would increase transport resistance between the blood vessel and the cavity (4). In addition, the smoothly sliding surface may promote fluid distribution. Thus the mesothelium, although not significant in terms of intrinsic resistance to transport, plays a major role in maintaining the system in a functional state.

The interstitium and the mesothelium present a barrier because interstitial matrix molecules and cells exclude small and large solutes from a large portion of the tissue space (2). However both are considered to have relatively large pores or gaps, with estimated widths on the order of 10 nm – 100 nm (5). This size of channel will severely restrict movement only of the largest molecules, such as hyaluronan (HA; molecular weight > 1 MD) and other interstitial macromolecules. On the other hand, the selectivity of the blood capillaries has been theoretically defined in a three-pore model of the capillary membrane (6): (a) a transcellular pore or aquaporin (7) has a radius of 1.0 nm and permits only water to pass through; (b) small pores have a radius of 4 – 6 nm, make up approximately 90% – 93% of the total pore area, and severely restrict passage of serum proteins; and (c) large pores (radius > 20 nm) make up approximately 5% – 7% of the total pore area (8). Lymphatics remove fluid and solutes from the interstitium without regard to size; however, except for in the diaphragm, their distribution in peritoneal tissue is limited.

The interstitium has been described as a two-phase system that contains colloid-rich, water-poor areas...
MATHEMATICAL MODELS FOR PERITONEAL TRANSPORT CHARACTERISTICS

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Four mathematical models and for the description of peritoneal transport of fluid solutes are reviewed. The membrane model is usually applied for (1) separation of transport components, (2) formulation of the relationship between flow components and their driving forces, and (3) estimation of transport parameters. The three-pore model provides correct relationships between various transport parameters and demonstrates that the peritoneal membrane should be considered heteroporous. The extended three-pore model discriminates between heteroporous capillary wall and tissue layer, which are assumed to be arranged in series; the model improves and modifies the results of the three-pore model. The distributed model includes all parameters involved in peritoneal transport and takes into account the real structure of the tissue with capillaries distributed at various distances from the surface of the tissue.

How the distributed model may be applied for the evaluation of the possible impact of perfusion rate on peritoneal transport, as recently discussed for clinical and experimental studies, is demonstrated. The distributed model should provide theoretical bases for the application of other models as approximate and simplified descriptions of peritoneal transport. However, an unsolved problem is the theoretical description of bi-directional fluid transport, which includes ultrafiltration to the peritoneal cavity owing to the osmotic pressure of dialysis fluid and absorption out of the peritoneal cavity owing to hydrostatic pressure.

KEY WORDS: Fluid transport; solute transport; diffusion; osmosis; convective transport; pore model; perfusion.

Mathematical modeling may help in analyzing peritoneal transport in four respects:

A. Separation of peritoneal transport components: (1) ultrafiltration and fluid absorption in fluid transport, and (2) diffusion, convective transport with ultrafiltrate, and bulk absorption with absorbed fluid for solute transport.

B. Quantitative correlation between the flows and their driving forces: (1) the osmotic pressure for fluid ultrafiltration, (2) the hydrostatic pressure for fluid absorption, (3) the concentration gradient for solute diffusion, (4) the ultrafiltrate flow for convective solute transport, and (5) the absorptive fluid flow for solute absorption. The correlations are described by the so-called “transport coefficients.”

C. Quantitative relationship between the transport coefficients for various solutes and between fluid and solute transport coefficients.

D. Quantitative relationship between the structure and physiological state of peritoneal tissue and its transport characteristics.

According to these four aspects of modeling, the three main models applied for the evaluation of peritoneal transport are: (1) the membrane model for A and B, (2) the three-pore model and extended three-pore model for C, and (3) the distributed model for D.

Mathematical models of peritoneal transport were included into some methods and computer programs for the evaluation of the efficacy and adequacy of peritoneal dialysis. The membrane model was mainly used (1,2), but the three-pore model was used also (3).

THE MEMBRANE MODEL

The membrane model provides a simple relationship between the rates of fluid and solute flows and their respective driving forces (Figure 1). It is derived from linear non equilibrium thermodynamics for the case of two well-mixed compartments, blood and dialysate, separated by a permselective membrane (4).

For crystalloid osmotic agents (as glucose, amino acids, glycerol, etc.) the rate of osmotically driven ultrafiltration, \( Q_u \), is proportional to the difference of osmolality between dialysate and blood, \( \Pi_D - \Pi_B \), and the coefficient of proportionality, \( a_{os} \), is called the osmotic conductance. The net rate of fluid flow between blood and dialysate, \( dV_D/dt \), is equal to the rate of ultrafiltration, \( Q_u \), minus the rate of absorption, \( Q_A \).

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NITRIC OXIDE PRODUCTION IN PERITONEAL MACROPHAGES FROM PERITONEAL DIALYSIS PATIENTS WITH BACTERIAL PERITONITIS

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Nitric oxide (NO) is produced by various cell types, and it is an important mediator in many biological processes, including macrophage-mediated cellular host defense. The relevance and amount of NO production in peritonitis during peritoneal dialysis (PD) treatment is still not clear.

We studied whether human peritoneal macrophages (PMΦ) isolated from healthy PD patients or PD patients with peritonitis showed different spontaneous or lipopolysaccharide (LPS)/interferon gamma (IFN-γ)–induced NO production (LPS, 1 ng/mL – 10 µg/mL; IFN-γ, 10 – 1000 U/mL; incubation between 6 – 48 hours; measured by Griess reagent). Results were compared with human blood monocytes (HBM) isolated from buffy coats. Inducible nitric oxide synthetase (iNOS) mRNA expression was looked for in PMΦ by reverse transcriptase polymerase chain reaction (RT-PCR). Furthermore, plasma (P) and peritoneal dialysate effluent (D) nitrite concentrations were measured in vivo.

The dialysate-to-plasma ratio (D/P) of nitrite concentration was inverse in the case of peritonitis compared to infection-free patients (peritonitis D/P = 1.3, non peritonitis D/P = 0.4; p < 0.01). PMΦ from peritonitis patients produced higher amounts of NO than did those from infection-free patients (0.040 ± 0.044 nmol per microgram cell protein versus 0.018 ± 0.015 nmol per microgram cell protein, p < 0.05). NO release could not be further enhanced by stimulation with LPS plus IFN-γ (1 ng/mL, 250 U/mL, respectively). However, NO production in PMΦ from infection-free patients increased during in vitro stimulation (0.044 ± 0.031 nmol per microgram cell protein versus 0.018 ± 0.015 nmol per microgram cell protein, p < 0.01). An increase of iNOS mRNA expression could be demonstrated by RT-PCR. Blood monocytes from healthy donors also increased NO release during cytokine stimulation (0.032 ± 0.015 nmol per microgram cell protein versus 0.019 ± 0.009 nmol per microgram cell protein, p < 0.05).

Our results indicate that significant amounts of NO are released intraperitoneally in the case of bacterial peritonitis. PMΦ represent a site of NO production, though the absolute amounts released in vitro are only moderate. NO production can be induced in PMΦ and HBM by LPS/IFN-γ stimulation in vitro.

KEY WORDS: Nitric oxide; peritoneal macrophages; peritonitis; lipopolysaccharide; interferon gamma.

Continuous ambulatory peritoneal dialysis (CAPD) has experienced rapid worldwide growth as an alternative renal replacement therapy in addition to hemodialysis. The successful outcome of PD largely depends on the absence of infectious complications, especially peritonitis.

Cellular host defense plays a key role in the early phase of peritonitis. Recent findings indicate that nitric oxide (NO) may act as a cellular mediator and also as a cytotoxic radical in peritoneal host defense. NO, an unstable nitrogen radical, is produced by various cell types. It is a regulator of different processes, including smooth muscle relaxation, neurotransmission, and macrophage-mediated cytotoxicity for microbes and tumor cells, at least in the rat model (1–3). NO is synthesized by the oxidation of L-arginine to L-citrulline by a family of NO synthases (NOS) (4) which can be differentiated into a constitutive, calcium-dependent form and an inducible form, expressed after stimulation with cytokines and endotoxins. NO has a half-life of a few seconds and is oxidized to nitrite (NO3−) and nitrate (NO3). Murine macrophages (including peritoneal macrophages) express high levels of inducible NOS (iNOS) mRNA, iNOS, and NO after stimulation with various agents such as lipopolysaccharide (LPS) and interferon gamma (IFN-γ) (5). Indeed, in human peritonitis, an increase in intraperitoneal levels of IFN-γ could be observed, which coincided with elevated polymorphonuclear cells in the effluent (6).

Although normal human chondrocytes, hepatocytes, and cells of the human colon-tumor cell line DLD-1 can produce high levels of NO after treatment with various cytokines, or with LPS, or both (7–10),
the relevance and amount of intraperitoneal NO production in human peritonitis under peritoneal dialysis is still not clear. Weinberg et al (11) could show a significant, but low NO release by peritoneal macrophages (PM\(\Phi\) obtained from women undergoing laparoscopic investigations) when incubated with LPS and IFN-\(\gamma\) in vitro. In PD patients, Yang et al (12) and Douma et al (13) reported a significant intraperitoneal NO\(_2\) accumulation in peritoneal effluent during bacterial peritonitis.

In this study, we investigated whether elevated amounts of nitrite can be detected in the peritoneal effluent of PD patients with bacterial peritonitis and whether PM\(\Phi\) are a potential site of NO release.

**PATIENTS, MATERIALS, AND METHODS**

**PATIENTS**

Six patients with acute bacterial peritonitis were investigated during the 48 hours after diagnosis of acute peritonitis. The peritoneal effluent was collected on ice after a dwell of 4 hours or more. Seven patients stable on PD served as a control. Clinical data are given in Table 1.

**MATERIALS**

All chemicals, unless otherwise stated, were purchased from Sigma Chemical Company (Poole, Dorset, U.K.). All tissue-culture flasks and multi-well plates were obtained from Falcon (Becton-Dickinson U.K. Ltd., Oxford, U.K.). Recombinant human IFN-\(\gamma\) was obtained from Promega Corp. (Madison, Wisconsin, U.S.A.). The biological activity (expressed as ED\(_{50}\)) was below 1 ng/mL using HLA-DR induction on Colo-25 cells. LPS was purchased from Sigma Chemical Company (Poole, Dorset, U.K.). All cytokine preparations were batched and stored at \(-70^\circ\text{C}\) and were freshly thawed for each experiment.

**PERITONEAL DIALYSIS EFFLUENT AND PLASMA**

The samples of peritoneal dialysis effluent (PDE) were obtained from peritonitis patients and non-infected PD patients (night dwell) by transfer into 50 mL sterile tubes. The effluent was then centrifuged (3000g for 10 minutes to remove any cellular material. Samples of the supernatant from each patient were stored in aliquots at \(-70^\circ\text{C}\) until assayed.

Plasma samples were taken simultaneously, centrifuged, and filtered through a Centricon ultrafiltration membrane with a 10 000 D cut-off (Amicon Pharmaceuticals Inc., Beverly, Massachusetts, U.S.A.) (500g for 45 min).

**ISOLATION AND CULTURE OF HUMAN PERITONEAL MACROPHAGES**

PM\(\Phi\) were obtained from the peritoneal effluent of PD patients with peritonitis (3 \(\times\) 10\(^{-2}\) – 6 \(\times\) 10\(^3\) leukocytes per \(\mu\text{L}\) of dialysate) and from infection-free patients (0.5 \(\times\) 10\(^{-2}\) – 1 \(\times\) 10\(^2\) leukocytes per \(\mu\text{L}\) of dialysate). After centrifugation, cells were washed twice with phosphate-buffered saline (PBS; 4\(^{\circ}\)C) and made adherent to culture flasks for 2 hours at 37\(^{\circ}\)C. Lymphocytes were removed by washing. After detachment, macrophages were identified by peroxidase

| TABLE 1 |
|------------------|------------------|
| **Clinical Data of the Cases Investigated** |
| **Peritonitis** | **Non peritonitis** |
| Patients | 6 | 7 |
| Age (years) | 52±15 (range: 27–69) | 53±16 (range: 31–69) |
| Sex (F/M) | 4/2 | 1/6 |
| Time on PD (years) | 4±3 (range: 2–10) | 4±3 (range: 1–11) |
| PD regime | | |
| CAPD | 3 | 4 |
| CCPD | 3 | 3 |
| Diagnosis of renal disease | | |
| Diabetic nephropathy | 1 | 1 |
| Glomerulonephritis | 3 | 4 |
| Unknown causes | 2 | 2 |
| Microbiologic finding | | |
| Coag. neg. Staphylococcus | 4 | — |
| Streptococcus | 2 | — |
| Leukocytes in dialysate (cells/\(\mu\text{L}\)) | 6.7\(\times\)10\(^2\)–2.5\(\times\)10\(^3\) | <100 |
| Time until resolving from peritonitis (days)\(^a\) | 8±2 | — |

\(^a\) Measured as days until number of leukocytes in dialysate was <200/\(\mu\text{L}\).
staining (>80% positive cells). Cell viability was confirmed by trypan blue exclusion (95% – 98% vital cells). Then, 2 × 10⁶ cells were maintained in 1.5 mL PBS (Dulbecco’s, Gibco BRL: Life Technologies Ltd., Paisley, U.K.) supplemented with 10% fetal calf serum, 1% L-glutamine (Gibco BRL: Life Technologies Ltd., Paisley, U.K.), and 1% glucose (Merck, Darmstadt, Germany). PMΦ cultures were incubated in 24-well plates at 37°C in a humidified 5% CO₂ atmosphere for 6 hours, 12 hours, 24 hours, and 48 hours without stimulation and with the addition of various concentrations of LPS (1 ng/mL – 1000 U/mL) and of IFN-γ (10 – 1000 U/mL). The supernatants were then removed, centrifuged, and stored at –70°C until assayed.

ISOLATION AND CULTURE OF HUMAN BLOOD MONOCYTES

Human blood monocytes (HBM) were isolated fromuffy coats of healthy donors (Institute of Transfusion Medicine, Heinrich-Heine-University, Düsseldorf, Germany). The cells were isolated by a Ficoll gradient from the interphase (Pharmacia Biotech, Uppsala, Sweden), washed with PBS and made adherent on tissue flasks. Detached cells (5 × 10⁶) were incubated and stimulated by the same procedure described above for PMΦ.

NO₂ MEASUREMENT, CELL PROTEIN CONCENTRATION

The nitrite level as an end-product of NO was measured colorimetrically in the supernatants by the Griess reagent as described elsewhere in detail (14,15). Briefly, aliquots of supernatant were mixed with Griess reagent (1% Sulfanilamide, 0.1% naphthylethenediamine dihydrochloride, 2.5% H₃PO₄) and formed a purple azo dye. The absorbance was measured at 550 nm using an ELISA-reader, and NO₂ concentration was determined using sodium nitrite as a standard (sensitivity: 0.5 µmol/L). Total cellular protein was analyzed by the modified Bradford method using bovine serum albumin (Sigma Chemical Company, Poole, Dorset, U.K.) as a standard. Repeated cell counts (Neubauer chamber) showed that 1 µg of cell protein was equivalent to 1.16 × 10⁶ cells (n = 6) for PMΦ and 1.69 × 10⁶ cells (n = 6) for HBM. NO₂ concentrations are expressed as nmol/µg of cellular protein in the supernatants of PMΦ and HBM.

iNOS REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION

Total RNA was extracted from 5 × 10⁶ cultured cells by the Trizol reagent (Gibco BRL: Life Technologies Ltd., Paisley, U.K.). For the reverse transcriptase polymerase chain reaction (RT-PCR) assay, 1 µg of total RNA was reverse transcribed into cDNA. The total reverse transcribed RNA was amplified by PCR, using 25 pmol of each primer in PCR buffer. Sense primer for iNOS was 5'-CCATGTCTGGCAGGACGA GAAGCG and antisense primer, 3'-GAATTGTGTT GAGCTCTTCAGC, which yielded a 366 bp product. The amplification conditions were: after 4 minutes predenaturation at 94°C, 40 cycles were performed at 94°C for 1 minute, 65°C for 1 minute, 72°C for 1 minute, followed by 72°C for 10 minutes (Trio-Thermoblock: Biometra, Gottingen, Germany). A glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) fragment of 246 bp length was coamplified using primers sense 5'-TGATGACATCAAGAAGGTGAA and antisense 5'-TCCTTGAGGCGCATGTAGCCAT. Aliquots of PCR products were run on a 1% agarose gel.

STATISTICAL ANALYSIS

All statistical analyses were performed using t-test for unpaired samples or Student's t-test with an alpha error of less than 0.05 being considered significant. All data are presented as mean ± standard deviation.

RESULTS

DIALYSATE-TO-PLASMA RATIO OF NITRITE IN PERITONITIS AND INFECTION-FREE PATIENTS

The nitrite concentration in infection-free patients was 5.95 ± 1.85 µmol/L in the plasma and 2.59 ± 0.89 µmol/L in the dialysate (Figure 1) representing a dialysate-to-plasma ratio of <1. In cases of peritonitis, the NO concentration in the dialysate (3.97 ± 1.33 µmol/L) surpassed the plasma value (3.08 ± 1.05 µmol/L) in each of the studied patients. This finding corresponded to a markedly higher D/P ratio of nitrite in peritonitis episodes compared to the infection-free state (peritonitis D/P = 1.3; non peritonitis D/P = 0.4; p < 0.01).

NITRITE RELEASE FROM PMΦ OF PERITONITIS PATIENTS AND INFECTION-FREE PATIENTS

Constitutive NO Release The non stimulated, constitutive NO release from PMΦ in cases of peritonitis was about 2.2-fold increased compared to the infection-free controls after 24 hours of incubation in vitro (peritonitis: 0.040 ± 0.044 nmol/µg cell protein; non peritonitis: 0.018 ± 0.015 nmol/µg cell protein; p < 0.05) (Figure 2). LPS/IFN-γ Stimulation: PMΦ from peritonitis patients produced only small additional amounts of nitrite when stimulated with LPS (1 ng/mL) and IFN-γ (250 U/mL) for up to 48 hours (0.063 ± 0.041 nmol/µg cell protein versus 0.040 ± 0.044 nmol/µg cell protein;
NO PRODUCTION IN HUMAN MONOCYTES UNDER DIFFERENT PATTERNS OF STIMULATION AND TIME-DEPENDENT RELEASE

HBM were stimulated with different concentrations of LPS (1 ng/mL - 10 µg/mL), IFN-γ (10 - 1000 U/mL), and a combination of both, for between 6 hours and 48 hours.

In the case of LPS stimulation, a 1 ng/mL concentration was already enough to induce a significant NO accumulation after 6 hours of incubation (0.024 ± 0.008 nmol/µg cell protein, p < 0.05, n = 6) and 24 hours of incubation (0.032 ± 0.015 nmol/µg cell protein, p < 0.05). The values clearly surpassed the constitutive release of control cells (Figure 3). Concentrations above 1 µg/mL LPS did not further enhance NO release, but even suppressed it.

Stimulation with different concentrations of IFN-γ alone showed only a small, non significant increase of NO. With IFN-γ stimulation only, maximum NO levels versus that of control cells were registered using 250 U/mL for 6 hours (0.019 ± 0.014 nmol/µg cell protein versus 0.010 ± 0.002 nmol/µg cell protein, n = 6, non significant).

Different combinations of LPS and IFN-γ were tested. The stimulation with 1 ng/mL LPS plus 250 U/mL IFN-γ turned out to give the strongest signal at all time intervals and was used as the standard stimulation pattern. A clear increase of NO release was already noticed after 6 hours of stimulation with this regime. The maximum level was reached after 24 hours (0.032 ± 0.015 nmol/µg cell protein, p < 0.01). No further rise could be observed in the further incubation period up to 48 hours.

Besides supplemented PBS buffer, Krebs buffer and RPMI 1640 (by Roswell Park Memorial Institute) were used. Non stimulated, constitutive release is opposed to the additional stimulation with lipopolysaccharide/interferon gamma (LPS/IFN-γ) (1 ng/mL, 250 U/mL, respectively).

Figure 3 — Stimulation of isolated human blood monocytes (5 × 10⁶) with lipopolysaccharide (LPS) and interferon gamma (IFN-γ). Effect of dose and incubation time on NO release (measured by the Griess reagent) into the supernatant. Some elective data from these experiments are shown. Data represent mean ± standard deviation of six experiments performed with cells from separate donors. * p < 0.05 versus control.
also tested. No superiority concerning NO release for the latter buffers could be found. As the background of nitrite contamination was lowest in PBS buffer, this one was chosen as the standard medium.

**iNOS EXPRESSION IN PMΦ**

A positive signal for iNOS mRNA expression could be observed in PMΦ from peritonitis patients. It was increased compared to infection-free controls (Figure 4). However, a high amplification rate with 40 cycles was necessary to get an adequate signal. The additional stimulation with LPS/IFN-γ clearly increased iNOS expression in PMΦ. But an equivalent increase of the product NO could not be observed as already described above.

**DISCUSSION**

The NO system is regarded as an important component of antimicrobial or antineoplastic activity of cytokine-activated cells including macrophages (16). In this study, an increased nitric oxide production was found in the peritoneal dialysate effluent of PD patients with bacterial peritonitis compared to infection-free patients. A similar finding was reported by Yang et al (12). High levels of dialysate nitrite concentration coincided with elevated counts of dialysate leukocytes in acute peritonitis. An increased D/P ratio of nitrite (> 1) was indicative for local peritoneal pro-inflammatory cytokines such as IL-1, IL-8, IL-6, IFN-γ, and TNF are increased in the dialysate of PD patients. Macrophages as well as resident peritoneal cells have been shown to contribute to the local production (1,17–20). Bacterial lipopolysaccharide and cytokines (especially IL-1 and IFN-γ) are also known as potential activators of iNOS. Whether peritoneal macrophages are a relevant site of NO production in humans is still controversial.

We isolated PMΦ from patients in the early phase of bacterial peritonitis and studied PMΦ from infection-free PD patients as a control. Furthermore, peripheral blood monocytes were used to investigate different stimulation patterns in vitro with regard to NO release. PMΦ isolated from peritonitis patients demonstrated a significantly higher NO release when cultured over 6–48 hours in a supplemented PBS buffer, as compared to infection-free controls. In our study, we also found a moderate, but clearly inducible increase of NO release in non peritonitis macrophages and also in human blood monocytes when incubated under stimulation with LPS and IFN-γ. NO release was presumably dependent on the activation of iNOS. We could demonstrate an enhanced iNOS mRNA expression in PMΦ from peritonitis by means of RT-PCR. Moreover, different calcium concentrations in the incubation buffer did not change NO concentrations. But, compared to the high levels of NO release described for mouse or rat macrophages, the levels of NO measured in our experiments were comparatively low (5). Though some investigators have been unable to show NO production by human blood monocytes or peritoneal macrophages (21–23), others have noticed some induction of NO release or iNOS expression (11,24,25).

According to our results, PMΦ represent one site of NO release in peritonitis. However, it can not be excluded that other resident cell systems may also contribute in an important manner (for example, endothelial cells or fibroblasts).

From the technical point of view, measurement of nitrite as a rather stable oxidation product of NO provides a simple method of detecting peritoneal inflammation. Owing to its small molecular size, nitrite readily diffuses from the peritoneum into the blood. On the other hand, nitrite concentrations in plasma and dialysate also depend on the nutritional uptake of nitrite salts. For this reason, not the absolute amounts of nitrite, but the dialysate-to-plasma ratio

**Figure 4** — iNOS mRNA expression in peritoneal macrophages (PMΦ) from a patient with bacterial peritonitis. Cells were harvested from a 4-hour dwell effluent (lane 1), and mRNA expression was measured by reverse transcriptase polymerase chain reaction. Another aliquot of the cells (5 x 10⁶) was incubated and further stimulated over 24 hours with lipopolysaccharide (LPS) (1 ng/mL) and interferon gamma (IFN-γ) (250 U/mL) (lane 2). Peripheral blood monocytes (5 x 10⁶) were submitted to the same procedure (untreated cells, lane 3; stimulation with LPS/IFN-γ for 24 hours, lane 4).
appears to be the adequate marker for intraperitoneal NO release.

It now remains a further question to elucidate whether the peritoneal release of NO and related radicals [for example, peroxynitrite formed by the reaction with superoxide (26)] must be regarded as relevant agents in peritoneal host defense or, on the other hand, bear the additional risk of toxic peritoneal membrane damage.

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Therefore, the change of dialysate volume is given by the following formula (5):

\[
d\overline{V}_D/dt = a_{OS}(\overline{\Pi}_D - \overline{\Pi}_B) - Q_A. \tag{1}
\]

Equation (1) provides a simplified but useful description of fluid flow. For the general thermodynamic description of fluid flow and alternative approximations, see Refs. 4–6.

The rate of solute flow between blood and dialysate may be described as the sum of three terms: (1) the rate of diffusive flow, which is proportional to the difference of solute concentration in blood and dialysate, \( C_B - C_D \), with the coefficient of proportionality called the diffusive mass transport coefficient, \( K_{BD} \), and also denoted as mass transfer area coefficient (MTAC) or permeability–surface area coefficient (PS); (2) the rate of convective solute flow owing to ultrafiltration, which is proportional to the rate of ultrafiltration, \( Q_U \), the solute concentration in blood, \( C_B \) (or some mean value of blood and dialysate concentrations), with the coefficient of proportionality \( S \), called the sieving coefficient; and (3) the rate of bulk absorption together with absorbed fluid, which is equal to the rate of fluid absorption, \( Q_A \), times solute concentration in dialysate, \( C_D \). Thus, the rate of net change of solute amount in dialysate, \( d(\overline{V}_D C_D)/dt \) is described as (7):

\[
d(V_D C_D)/dt = K_{BD}(C_B - C_D) + S Q_U C_B - Q_A C_D. \tag{2}
\]

Alternative versions of the model, which differ mainly in the description of the convective transport component (4,6,7), also exist.

Equation (1) and Eq. (2) may also be written for the amount of fluid and solute at time \( t \) as follows (5,7):

\[
V_D(t) = V_D(t_0) + a_{OS} (\overline{\Pi}_D - \overline{\Pi}_B) \Delta t - Q_A \Delta t \tag{3}
\]

\[
(V_D C_D)(t) = (V_D C_D)(t_0) + K_{BD}(C_B - C_D) \Delta t + S Q_U C_B \Delta t - Q_A C_D \Delta t \tag{4}
\]

where \( \Delta t = t - t_0 \), with \( t_0 \) being an earlier moment (for example, the beginning of fluid dwell in the peritoneal cavity) than \( t \). A bar over a variable denotes its mean value over the time period \( (t_0,t) \). Here, we assume that the transport coefficients \( a_{OS}, K_{BD}, \) and \( S \), as well as the flow rate \( Q_A \), are constant. Note however, that according to some recent studies, the transport coefficients depend on dwell time for the standard glucose-based dialysis fluids, but are relatively stable for some alternative dialysis fluids (6–9).

Equation (3) and Eq. (4) are applied for the estimation of the transport parameters for fluid and for each solute separately, using the linear regression method and the values of dialysate volume and solute concentration measured versus dwell time (5,7).

The transport parameters derived from the membrane model are used to describe the transport characteristics of the peritoneal membrane for peritoneal dialysis patients and in animal experiments. The importance of the evaluation of the transport parameters may be shown for two groups of patients with permanent loss of ultrafiltration capacity (UFC), Table 1 (10,11). The reason for the UFC loss was obviously quite different in each of the two groups of patients. The patients from Group I had decreased osmotic conductance and increased diffusive mass transfer coefficients for small solutes, which means that their peritoneal membrane was hyperpermeable. In contrast, the patients from Group II seemed to have a normal peritoneal membrane, but substantially increased peritoneal absorption. The mechanisms for these divergent types of change in peritoneal transport are not known.

The membrane model allows for the estimation of the transport parameters for fluid transport and for each of the investigated solutes separately. However, the structure of the membrane, the size of solute, and the transport parameters are related in passive membrane transport. To describe a possible relationship for peritoneal transport, we need further assumptions about the peritoneal membrane.

THE THREE-PORE MODEL

The three-pore model is based on a concept of transport through a cylindrical uniform pore across the membrane. Solute and fluid transport through the pore is evaluated using the hydrodynamic theory of fluid flow along a cylindrical pipe and diffusion and convective drag of spherical molecules along the pore (12). The theory provides so-called “restriction factors” for diffusive and convective solute transport; these factors describe how much the solute transport is re-
tarded owing to the presence of the pore wall, compared to free transport in unbound medium (6,12).

The parameters used for the description of the transport through such a pore are: pore radius, \( r_P \); pore length, \( \Delta x \); Stokes radius of the solute, \( r_S \) (calculated from the solute molecular weight); and fluid viscosity, \( \eta \). The restriction factor for diffusion is presented as the ratio of the effective surface area of the pore cross-section, \( a_{eff} \), over its nominal surface area, \( a_0 = \pi r_P^2 \) (6):

\[
\frac{a_{eff}}{a_0} = \frac{(1 - \alpha)^{3/2}}{1 - 0.3956 + 1.0616 \alpha^2}
\]

where \( \alpha = r_S/r_P \). Thus, the pore diffusive permeability is given by the following formula (6):

\[
P = \left(\frac{a_0}{\Delta x}\right)\left(\frac{a_{eff}}{a_0}\right)D_W
\]

where \( D_W \) is the diffusion coefficient for the solute in water, and \( a_{eff}/a_0 \) is given by Eq. (5). The retardation factor for convective solute transport is called in thermodynamics the sieving coefficient, \( S \) (with \( S = 1 - \sigma \), \( \sigma \) being the Staverman reflection coefficient), and may be calculated as (6):

\[
S = \frac{(1 - \alpha)^2[2 - (1 - \alpha)^2](1 - \alpha/3)}{1 - \alpha/3 + 2\alpha^2/3}
\]

The pore hydraulic conductivity, \( l_P \), of the pore is calculated as (6):

\[
l_P = \left(\frac{a_0}{\Delta x}\right)(r_S^2/8\eta)
\]

Note that, in the formulas for \( P \) [Eq. (6)] and for \( l_P \) [Eq. (8)], the same factor, \( a_0/\Delta x \), appears. By multiplying \( P \) and \( l_P \) by the number of pores in the whole membrane, the global transport coefficients are obtained for the whole membrane: diffusive mass-transport coefficient, \( K_{BD} \), often denoted \( PS \) (permeability – surface area); and hydraulic permeability, \( L_P S \).

According to the three-pore model, the peritoneal membrane is heteroporous with three sizes of equivalent pores: large pores (L), of radius about 250 Å – 300 Å; small pores (SM), of radius about 40 Å – 50 Å; and ultrasmall pores (USM), of radius about 2 Å – 4 Å (6). (See Figure 2.) Diffusive permeability (PS), hydraulic conductivity (\( L_P S \)), sieving coefficient (S), and reflection coefficient (\( \sigma = 1 - S \)) may be calculated for small and large pores by adjusting formulas (5) – (8), multiplied by the respective number of pores, if appropriate, to the available data about peritoneal transport (6,13).

Ultrasmall pores are not permeable for solutes, so \( PS_{USM} = 0 \), \( S_{USM} = 0 \), and \( \sigma_{USM} = 1 \). Hydraulic conductivity of the ultrasmall pores, \( L_P S_{USM} \), is about 1% – 2% of the total hydraulic conductivity (6,13). Large pores play important role in the transport of macromolecules (of the size of albumin and larger) mainly by convective flow. Small pores are the main routes for the exchange of small and middle molecules. Osmotically-driven water flow passes small and ultrasmall pores. The number of large pores is about 12 500 times lower than the number of small pores.

### Table 1

<table>
<thead>
<tr>
<th>Transport parameter</th>
<th>Control group CAPD patients</th>
<th>Group I UFC loss patients</th>
<th>Group II UFC loss patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a_{OS} ) (mL/min)/(mOsm/kg)</td>
<td>0.133±0.033</td>
<td>0.094±0.041\textsuperscript{a}</td>
<td>0.127±0.025</td>
</tr>
<tr>
<td>( Q_A ) (mL/min)</td>
<td>1.6±0.6</td>
<td>2.0±0.4</td>
<td>4.9±0.3\textsuperscript{a}</td>
</tr>
<tr>
<td>Urea ( K_{BD} ) (mL/min)</td>
<td>29.5±11.9</td>
<td>59.3±42.8\textsuperscript{a}</td>
<td>25.4±1.3</td>
</tr>
<tr>
<td>Creatinine ( K_{BD} ) (mL/min)</td>
<td>10.6±5.7</td>
<td>43.1±13.0\textsuperscript{a}</td>
<td>14.2±4.9</td>
</tr>
<tr>
<td>Glucose ( K_{BD} ) (mL/min)</td>
<td>10.7±2.7</td>
<td>20.4±4.1\textsuperscript{a}</td>
<td>15.4±2.6</td>
</tr>
</tbody>
</table>

\( a_{OS} = \) Osmotic conductance; \( Q_A = \) rate of peritoneal absorption; \( K_{BD} = \) diffusive mass transport coefficient.

\( a p < 0.05 \) versus the control group.

---

**Figure 2** — Schematic presentation of the three-pore model for the membrane separating blood and dialysate compartments with pores of various cross-sectional surface area.
The data about equivalent pores were obtained by analysis of fluid and solute transport in peritoneal dialysis, as well as of the previous models of the capillary wall. In the analysis of small-solute transport, a lumped parameter appeared: \( \frac{A_0}{\Delta x} \), where \( A_0 \) is the total nominal surface area of (mainly) small pores. From fitting to the data about diffusive transport, the value of \( \frac{A_0}{\Delta x} \) was found to be about 27 000 – 45 000 cm on average (6). This “area parameter” was estimated for individual patients on peritoneal dialysis and proposed to be used for discrimination between different patient groups as in the peritoneal equilibration test (PET) (13). An important result yielded by the three-pore model was the explanation of the apparently contradictory values of the net sieving coefficient for small solutes (which is about 0.5) and a very low Staverman reflection coefficient (less than 0.1) (6). Note, that \( S = 1 - \sigma \) only for a homoporous membrane; in the case of a heteroporous membrane, the mean values of \( S \) and \( \sigma \) depend on the size and number of different pores. With appropriate description of the peritoneal membrane pores, the theoretical mean values of \( S \) and \( \sigma \) may be fitted to the measured ones.

THE EXTENDED THREE-PORE MODEL

A modified version of the three-pore model was also formulated with the aim of taking into account two transport barriers arranged in series within the tissue for the transport between blood and peritoneal dialysate: capillary wall with three different sizes of pores, and the tissue itself (6). (See Figure 3.) The transport of most solutes in the tissue is mainly through interstitium, but lipophilic gases may pass through cells, too. Thus, in the case of diffusion, the model describes the total transport resistance, \( 1/PS \), as the sum of the resistances of the capillary wall, \( 1/PS_{\text{cap}} \), and of the tissue (interstitium), \( 1/PS_{\text{int}} \). Diffusive permeability of the capillary wall was modeled using the three-pore model with essentially the same radii for the pores, but the value of the parameter \( A_0/\Delta x \) was increased to 270 000 cm (6). The diffusive permeability of the interstitial layer, \( PS_{\text{int}} \), was adjusted to get the fit to the measured values of \( PS \) (6).

An important result yielded by this extended version of the model was the agreement in the theoretical values of \( PS \) and \( L_pS \) — which are linked by the parameter \( A_0/\Delta x \) [see Eqs. (6) and (8)] — with the respective measured values. However, the model does not separately include any direct information about the available pore surface area, \( A_0 \), and about the thickness of this layer (they are included in the lumped parameter \( A_0/\Delta x \)), nor about the effective surface area available for diffusion in the tissue layer, \( A_{\text{int}} \), and about the thickness of the tissue layer \( \Delta x_{\text{int}} \).

THE DISTRIBUTED MODEL OF PERITONEAL DIFFUSIVE TRANSPORT

The distributed model describes the capillary bed with capillaries distributed within the tissue at various distance from the mesothelial surface of the tissue (14,15). (See Figure 4.) The transport of solute between each of the capillaries and the tissue is governed by the transport characteristics of the capillary wall (for example, the diffusive mass transport coefficient for the capillary wall per unit tissue volume, \( k_{BT} \)) and the local solute concentration at the capillary site. The transport of the solute across the tissue is governed by the transport characteristics of the tissue (for example, tissue diffusivity, \( D_T \)), but depends also on the supply (or washout) of the solute by blood in the local capillaries (blood perfusion). A characteristic profile of solute concentration within the tissue arises as a result of these interactions (15–17). For the case of diffusive transport, this distribution of the solute within the tissue may be described by the following equation (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation):

\[
d^2C_x/dz^2 = -(1/\Lambda^2)(C_B - C_x)
\]

where \( \Lambda = (D_T/k_{BT})^{1/2} \), and \( z \) describes the distance from the mesothelial surface of the tissue. In the fol-
following discussion, we assume that the coefficients in Eq. (9), Λ and C_B, are independent of z. Solute concentration in blood, C_B, is the concentration in systemic blood (that is, in blood at the entrance to the capillary bed) and therefore does not depend on the position of the capillary. Coefficient Λ has the unit of length and is called the depth of penetration. To solve Eq. (9), the total thickness of the tissue, L, must be known. The concentration profiles are described as (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation; 18):

$$C_T(z) = C_B + (C_D - C_B) \frac{\cosh(\Phi(1 - z/L))}{\cosh(\Phi)}$$  (10)

where Φ = L/Λ is a non dimensional parameter called the relative tissue width. This parameter describes the tissue width as measured in units of the penetration depth, Λ. For a large Φ — that is, small penetration depth compared to the tissue width — the equation is (14):

$$C_T(z) = C_B + (C_D - C_B) \exp(-z/\Lambda).$$  (11)

To calculate K_BD, it is necessary to know the surface area, A_M, for the contact between dialysate and tissue. Then, using the formula for the total solute flow at z = 0 (that is, at the mesothelial surface of the tissue), one gets (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation; 18):

$$K_{BD} = A_M(D_T k_{BT})^{1/2} \tanh(\Phi).$$  (12)

For high values of Φ, tanh(Φ) = 1.

The diffusive transport across the capillary wall depends on three factors: (1) the diffusive permeability of the capillary wall, P_C; (2) the density of the capillary surface area, a_C (that is, a_C is the capillary surface area per unit volume of the tissue); and (3) the rate of blood perfusion, q_B (q_B is blood flow expressed per unit volume of the tissue). The relationship is given by the Kety–Renkin formula (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation; 19,20):

$$k_{BT} = \frac{q_B}{1 - \exp(-P_C a_C/q_B)}.$$  (13)

The values of P_C may be calculated using the three-pore model, or experimentally measured P_C values may be applied (6,14,15). The capillary surface area density, a_C, is usually assumed on the basis of histological measurements and physiological hypotheses; however, the values of a_C for the organs involved in peritoneal transport usually have not been directly measured. Perfusion rate, q_B, may be measured using some physiological methods, but such measurements in the context of peritoneal dialysis can be made only in animal models (21,22).

Perfusion rate is very variable and depends on the physiological state of the organ as well as the structure of its capillary bed. The parameters a_C and q_B are usually linked, but the relationship may be quite different and depends on the physiological state of the organ (23–25). The manipulation of perfusion during peritoneal dialysis — using, for example, vasodilatory or vasoconstrictive drugs or other means — was the aim of many studies (26). In general, such manipulation may influence all three parameters: P_C, a_C, and q_B.

Equation (13) was applied in most of the physiological studies of capillary transport (27). In contrast, approximate formulae for k_{BT} were often used for the consideration of peritoneal transport (14,28). For solutes with a very high diffusive permeability, P_C — such as lipid-soluble gases CO_2 and O_2 — k_{BT} equals q_B; for slowly diffusing solutes and high perfusion rates, k_{BT} equals P_C a_C. These approximations are usually correct; but, for low perfusion rates and small solutes such as urea and creatinine, the general equation (13) should be used (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation).

Detailed knowledge of the parameter values for the distributed model is usually not available. The parameters may vary substantially, depending on the clinical conditions of the study, applied dialysis fluid, time of the peritoneal dwell, animal species used for the experimental model, preparations for the experimental study (for example, anesthesia, surgery, and other manipulations), investigated organs, and so on. Often, only a net measure of transport, such as solute...
clearance or $K_{BD}$, is obtained. Data about concentration profiles within the tissue are scarce (17). The detailed structure and physiological state of the studied tissue is, as a rule, not known. Therefore, the parameter values must be to some extent “guessed” and adjusted to the results of the study, using general physiological knowledge and the results of other studies. To illustrate the problems met in this approach, the results of the search for possible explanations of some clinical and experimental data are presented in the next section.

APPLICATIONS OF THE DISTRIBUTED MODEL FOR THE EVALUATION OF BLOOD PERFUSION IN PERITONEAL DIALYSIS

The diffusive mass transport parameter, $K_{BD}$, for small solutes was found to be high at the beginning of a peritoneal dwell of a standard glucose-based dialysis solution. Later, it decreased exponentially to a steady-state value after about 2 hours of the dwell (6,8,9,13). The initial $K_{BD}$ values were higher by 60% than the final ones (8,9). This phenomenon was attributed to the impact of the increased perfusion of the tissue owing to the vasodilatory effect of the dialysate.

We applied the distributed model to try to explain this finding (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation). The values of the parameters were selected according to the previous estimates for the model and the available results of physiological studies. The values of $D_T$ and $P_C$ were dependent on the molecular weight of the solute as described by Dedrick et al (14). The capillary surface area density, $a_C$, was linked to the perfusion rate, $q_B$, as described by Haraldsson and Rippe (23). The peritoneal surface area was assumed to be 1 m$^2$, and the typical width of the tissue involved in peritoneal transport was selected to be 5 mm. The initial and final values of the perfusion rate were adjusted to yield the measured initial and final values of $K_{BD}$ for creatinine: 14.8 mL/min and 8.8 mL/min, respectively (8,9). The results are shown in Table 2. According to our estimation, the perfusion rate decreased during the dwell by a factor of six: from 0.30 mL/min/g to 0.05 mL/min/g. The final perfusion rate was close to the value reported for the resting muscle (15).

Granger et al (21) reported a several-fold increase in perfusion rate in thin tissue layers that were in contact with peritoneal dialysate compared to the control values. Note that, in spite of such a high increase in perfusion rate, the net transport coefficient, $K_{BD}$, increased only by 60%. This effect is partly due to the decrease in penetration depth with increased perfusion (that is, less tissue is effectively involved in the transport — see Table 3 and Figure 5), and partly due to the “square root” rule for the values of $K_{BD}$ [Eq. (12): a fourfold increase in $k_{BT}$ results in only a twofold increase in $K_{BD}$]. Note also, that $K_{BD}$ for lipophilic gases is much more sensitive to changes in blood perfusion than is $K_{BD}$ for small solutes (Table 3). Investigations of gas transport may therefore be helpful in the assessment of changes in perfusion rates, even if $K_{BD}$ values for gases fail to provide any measure for “peritoneal blood flow” (Waniewski J. Effect of perfusion on diffusive transport in peritoneal dialysis: Distributed modeling. In preparation; 28). The penetration depth for the solute is lower for the high perfusion rate than for the low perfusion rate (Table 2 and Figure 5). This effect is due to the fact that the solute that diffuses from peritoneal dialysate into the tissue is more effectively washed out from the tissue if the perfusion and the capillary surface area (which is linked to the perfusion rate) are high.

The change in total blood flow need not necessarily have any substantial impact on net diffusive transport. This was demonstrated by Flessner, and by Kim et al, in studies of mannitol and urea transport using diffusion chambers affixed with glue to the tissue of several rat organs (29–31). The blood flow to the organ was mechanically reduced by 60% - 70% without any significant change in the measured $k_{BD}$ values ($K_{BD}$ is the surface density of $K_{BD}$; that is, $K_{BD}$ expressed per unit surface area of the contact surface between dialysate and tissue). (See Table 4.) The blood flow, however, was not measured.

The $K'_{BD}$ values found in the chamber experiments were high. If scaled to clinical conditions — that is, to the surface of the peritoneum 1 m$^2$ — they yield a $K'_{BD}$ for urea of more than 40 mL/min and, for glucose/mannitol, of more than 20 mL/min. These values are much higher than usually observed. To explain those results, it is therefore necessary to increase the local transport parameters, comparing them to

---

**TABLE 2**

Theoretically Estimated Diffusive Mass Transport Coefficient ($K_{BD}$) and Tissue Penetration Depth ($\Lambda$) at the Beginning ($q_B = 0.30 \text{ mL/min/g}$) and at the End ($q_B = 0.05 \text{ mL/min/g}$) of Clinical Six-Hour Peritoneal Dwell Studies (18)

<table>
<thead>
<tr>
<th>Solute</th>
<th>$K_{BD}$</th>
<th>$\Lambda$</th>
<th>$K_{BD}$</th>
<th>$\Lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mL/min)</td>
<td>(mm)</td>
<td>(mL/min)</td>
<td>(mm)</td>
</tr>
<tr>
<td>$H_2$</td>
<td>0.68</td>
<td>263.9</td>
<td>1.66</td>
<td>105.7</td>
</tr>
<tr>
<td>$CO_2$</td>
<td>0.39</td>
<td>136.1</td>
<td>0.95</td>
<td>55.6</td>
</tr>
<tr>
<td>Urea</td>
<td>0.18</td>
<td>19.8</td>
<td>0.31</td>
<td>11.3</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.18</td>
<td>14.7</td>
<td>0.30</td>
<td>8.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.19</td>
<td>11.6</td>
<td>0.30</td>
<td>7.2</td>
</tr>
</tbody>
</table>
those used for the evaluation of the clinical studies presented in Table 2. Selecting a $P_C$ of the same value as in the evaluation of the clinical data in Table 2, and a $D_T$ three times higher than that used for the clinical study provided good estimations of the measured $k'_B$ values. For high perfusion rates, $q_B$ equals 1 mL/min/g; and, the capillary surface area density, $a_C$, being increased twice, the values of $k'_B$ calculated for urea and mannitol using Eq. (12) for $A = 1 \text{cm}^2$ were essentially the same as those found experimentally (Table 3 and Table 4). Furthermore, the reduction of perfusion rate to 0.3 mL/min/g — that is, by the same ratio as in the experiments with diffusion chambers — yielded only a small decrease in the values of $k'_B$ (Table 4). Further reduction of perfusion to 0.15 mL/min/g, concomitant with the reduction of the effective capillary area, may substantially decrease the value of $k'_B$ (Table 4). Note again that lipophilic gases are much more sensitive to change of perfusion rate than are small solutes, and therefore these gases might be used for assessing the changes in the local state of the capillary bed.

**CONCLUSIONS**

The distributed model for diffusive transport seems to be the most general one. It includes all parameters that may be of interest for the evaluation of peritoneal transport: capillary surface area, diffusive permeability of the capillary wall, perfusion rate, tissue diffusivity, peritoneal surface area, and tissue-layer width. Diffusive permeability of the capillary wall may be modeled using the three-pore model. However, the values of the parameters are usually not
known; the modeling needs many assumptions and the application of the "trial-and-error" method. Nevertheless, the model can provide better understanding for many approximate models in use and their limitations. It may also help to test various hypotheses and to serve as a guide in future investigations of peritoneal transport.

Distributed modeling of fluid transport and convective solute transport is more difficult than modeling pure diffusion as presented in the current study. Physiological explanation of bi-directional water flow during peritoneal dialysis within the tissue (osmotic pressure-driven flow to the peritoneal cavity and hydrostatic pressure-driven flow out of the peritoneal cavity) is still an unsolved problem. Some results for combined diffusive and convective solute transport with one of these fluid-flow components were, however, obtained (16,18,32,33).

The three-pore model in its extended version (capillary wall plus interstitial tissue layer) seems to be an approximation for the distributed model, but the relationship between the models has not been investigated yet. The three-pore model has clearly demonstrated that various transport routes must be considered for various flow components and various driving forces, as well as for solutes of various transport characteristics. Furthermore, the model has managed to explain the quantitative relationships between various transport parameters, such as sieving and reflection coefficients, diffusive mass transport coefficients and hydraulic conductivity, and so on. However, the model includes at least two lumped parameters: \( A_c/\Delta x \) for the capillary wall, and \( A_{int}/\Delta x_{int} \) for the tissue layer. The further understanding of the role of the geometrical characteristics of peritoneal tissue in peritoneal transport needs the application of the distributed model.

The membrane model is the basic model for the evaluation of clinical and experimental data from dwell studies. The transport parameters obtained may be further analyzed using the three-pore model, or the distributed model, or both.

ACKNOWLEDGMENT

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REFERENCES

20. Renkin EM. Effects of blood flow on diffusion kinetics
alternating with water-rich, colloid-poor areas (9). The matrix of molecules that makes up the interstitial "ground substance" is no longer considered an amorphous mixture of macromolecules, but is now known to be a highly ordered structure made up of collagen fibers that are anchored to surrounding cells by adhesion molecules called integrins (10).

Reed and colleagues (11) have shown that the interruption of β₁ integrin linkages results in expansion of the interstitial space and a significant lowering of the interstitial hydrostatic pressure. These observations imply that, under normal physiologic conditions, the collagen fibers hold cells together in active tension. Wrapped around the collagen molecules are very large hyaluronan molecules (1 MD – 40 MD), which imbibe large amounts of water and to which are bound glycoproteins such as chondroitin sulfate, keratin sulfate, and heparin sulfate. The distribution of these molecules surrounding the cells and the blood vessels is quite heterogeneous (12).

Because of the cells and the colloid spaces filled with macromolecules, solutes transporting from the blood capillaries are restricted to the water-rich areas of the interstitium and must take a tortuous path toward the cavity. In animal studies, we have determined that under control conditions less than 20% of the abdominal wall muscle volume is available to a small solute such as mannitol, while a large protein such as IgG is restricted to 5% of the total tissue volume (13). The solute may be absorbed into a local lymphatic capillary or, if it is a protein or a charged molecule, the solute may interact with the charged molecules of the colloid-rich space and be retarded in its transport. The result of the tortuous path and the exclusion is that solute diffusion through tissue interstitium is 30 times to 100 times slower than the corresponding rate of diffusion through water (1). The larger the distance between the capillary and the peritoneal surface, the more significant the resistance of the interstitium becomes. The portion of resistance attributable to the interstitium in the diffusive transport of a small solute such as sucrose has been estimated to be 29% if the capillary is located 50 µm from the peritoneum, but it increases to 83% of the total peritoneal resistance if the capillary is located 600 µm from the peritoneum (2). Thus the interstitium has the overall effect of increasing the resistance to diffusion between the lumen of blood vessels and the dialysis solution in the cavity.

Convection, or fluid movement, through the interstitium is a complex process that is under active investigation by our laboratory. It plays a minor role in the overall transport of small solutes (MW ≤ 6000 D), but it may be the dominant mechanism of large-solute transport. The hydrostatic pressure-driven fluid movement from the cavity into the tissue results in hydration of the tissue, and the volume of the tissue interstitium of the abdominal wall doubles with the increase in intraperitoneal (IP) pressure from 2 cm to 4 cm H₂O (14). We have recently demonstrated that isotonic fluid with an IP pressure of 6 mmHg in the cavity expands the abdominal wall interstitium by 100%, causes an apparent movement of HA from the abdominal muscle outward to the subcutaneous space (14), and results in a fourfold increase in hydraulic conductivity of the muscle (15). Studies in the synovial interstitium have also demonstrated that HA depletion increases the hydraulic conductivity to a much greater extent than predicted from the concentration change alone (16).

Thus, increase in the proportion of space that is available to solutes and water, coupled to changes in the interstitial structure, result in a decrease in the resistance to fluid flow and solvent drag (17) and an increase in the rate of diffusion (13). Because IP pressures during peritoneal dialysis are typically 4 – 18 mmHg (18), the tissue of the peritoneal transport system expands with two to three liters in the cavity. Because of the expansion, solute transport from the blood vessels toward the cavity will accelerate through the interstitium, but the fluid loss from the cavity into the tissue will increase as well.

Dextrose (or another solute used in the peritoneal solution as the osmotic agent) in sufficient concentration (≥ 15 g/L) causes fluid movement from the tissue into the cavity. The solute does not make contact with blood capillaries in concentrations equal to those in the cavity. Rather, it diffuses from the cavity into the interstitium and, from there, exerts its osmotic force across blood capillaries. Research in animals has shown that the dextrose concentration profile in the interstitium will decrease from the level in the tissue at the peritoneum to the concentration in equilibrium with the blood at a point 500 – 700 µm from the peritoneum (19). The preservation of this concentration profile of the osmotic agent in the tissue is important to the function of the peritoneal system and to the removal of fluid from the patient during dialysis.

EFFECTS OF PERITONEAL INFLAMMATION

Acute inflammation is a relatively common event, occurring approximately once per year in each peritoneal dialysis patient (20). Inflammation often results in marked changes in the transport of solutes and water; small solutes such as urea, creatinine, and glucose increase their rates of transport to or from the cavity by 50% – 80%, while the rates of net water transport from the blood to the cavity decrease by 30% – 40% (21).

Subsequent biopsies of the parietal peritoneum often reveal scarring of the tissue, with a loss of
microvessels next to the peritoneum and a thickening of the layer of tissue between the vessels and the edge of the cavity (22). This situation typically results in loss of transport function and transfer of the patient from peritoneal dialysis to hemodialysis. An even larger problem may be the possibility that the peritoneum is in a state of chronic inflammation, owing to solutions that are non compatible with biologic tissue (23). This constant state of inflammation may be responsible for the nearly 40% of patients who experience technique failure and must transfer to hemodialysis after 6 years of continuous therapy (24). Others in this forum will provide details of the pathologic changes and the alterations in water and solute transport with time on PD. This review will focus on the mechanisms that result in the pathologic alterations seen in chronic or acute inflammation.

Studies of the dialysis solution drained from the cavity of patients with acute peritonitis have suggested marked increases in the local synthesis of a number of inflammatory mediators: tumor necrosis factor (TNFα), interleukins (IL-1, IL-6, IL-8), prostaglandins (PGE₂, 6-keto-PGF₁α, TxB₂), macrophage chemotactic peptide (MCP-1), and growth factors (huGROα, TGFβ) (25–27).

The cascade of cytokines that is induced during peritoneal inflammation has recently been reviewed (28,29), and a chain of inflammatory events has been hypothesized from in vitro cell-culture studies and apparent concentrations of mediators in the cavity. Peritoneal macrophages are the first line of defense and, when stimulated, release IL-1, IL-6, IL-8, and TNFα. Mesothelial cells respond to IL-1 and TNFα by generating prostaglandin (PGE₂, 6-keto-PGF₁α) and various cytokines (MCP-1, IL-1β, IL-6, and IL-8). IL-1 and TNFα act synergistically to induce infiltration of polymorphonuclear cells into the cavity (30) and likely induce fibroblasts to secrete IL-6, IL-8, PGE₂, and PGI₂ (31).

Very few in vivo studies of inflammatory effects on the interstitium have been performed, and therefore the mere presence of high levels of inflammatory cytokines in the peritoneal cavity may have little to do with the changes in the tissue that result in alteration in transport. The controlling factors of leukocyte migration from the blood through the interstitium into the cavity are therefore poorly defined, but the chemoattractants leukotriene B₄ (secreted by macrophages), IL-8 and MCP-1 (both secreted by stimulated mesothelial cells), and the expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) may be involved in this process (32,33). During the inflammatory process, plasma proteins — including fibrinogen and fibronectin — and fluid likely accumulate in the interstitium (34). Leukocytes, which roll and adhere to the endothelium owing to the action of P-selectins and L-selectins (35), may exit the blood space and bind to the fibronectin/fibrin in the tissue via β₂-integrins (CD11b/c, CD18), which are upregulated in inflammation (35). It has been proposed that white cells attached to the interstitial fibrin and fibronectin molecules form “extracellular compartments” that are protected from protease inhibition in the tissue. In this compartment, it is further hypothesized that the leukocyte's protease will digest the extracellular matrix and allow the leukocyte to pass toward the source of inflammation (the peritoneum) (36). This digestive process will likely increase local edema formation.

Specific effects of the various cytokines on blood vessels and components of model interstitia have been reported in the literature. TNFα is known to induce increases in leakage in postcapillary venules in cremaster and abdominal muscles of rats (37–39). In addition, TNFα has been reported to cause the release of nitric oxide (40), which likely explains its vasodilatory effect on vascular smooth muscle (41). IL-1 is synergistic with TNFα and, if infused in sufficient dose, also results in a clinical “capillary leak syndrome” and hypotension when the endothelium is exposed to activated neutrophils (42,38). IL-1 increases expression of cellular adhesion molecules and induces fibroblast proliferation (43) and may therefore have effects on the interstitial matrix as well as on neutrophil transmigration. Of the prostaglandin class of inducible substances, levels of 6-keto-PGF₁α and PGE₂ in the peritoneal dialysis solution are significantly elevated during acute peritoneal inflammation and correlate with rates of transfer into the cavity of the following proteins: total protein, albumin, β2-microglobulin, IgG, α2-macroglobulin (44). Recent studies in the skin have demonstrated that analogs of PGE₁ and PGF₂α resulted in a lowering of the interstitial pressure and produced edema, presumably by their action on fibroblast contraction of collagen fibers (45).

EVIDENCE OF INTERSTITIAL INVOLVEMENT IN PERITONEAL INFLAMMATORY CHANGES

Because of the difficulties of separating transport changes owing to microvessel alterations from those owing to interstitial changes, specific effects of inflammation on peritoneal interstitial transport have not been reported. Recent findings, however, support the hypothesized chain of events in the inflammatory process as presented above. There is a marked decrease in inflammation-induced protein loss to the rabbit peritoneal cavity when either the plasma is depleted of leukocytes or an antibody to CD18 (β₂-integrin) is administered (46). Presumably, leuko-
cyte invasion of the tissue space is necessary for transfer conditions, and the antibody blocks one of the first steps in leukocyte migration through the interstitium. It is known that stimulated mesothelial and fibroblasts significantly increase their production and release of HA into the dialysis solution during peritonitis (47). There is also evidence of increased synthesis by peritoneal tissue of procollagen I upon exposure of the peritoneum to dialysis solutions (48).

Besides the effects of HA on the structure and function of tissue, HA is known to moderate the laying down of collagen after tissue injury, to act as a free-radical scavenger, to foster tissue repair, and to prevent scar formation (49). The passage of leukocytes, recruited from the circulation, through the interstitium must require some alteration of the collagen and glycosaminoglycan matrix that occupies the space between cells. While this specific process has not been studied in vivo, indirect effects of HA on transperitoneal transport have been observed. Infusion of a saline solution supplemented with 10 mg/dL of HA reduced the dialysis of the rat peritoneum (50); and chronic infusion of a dialysis solution containing N-acetylglucosamine (monomer that forms HA) over an eight-week period resulted in a more compact tissue space with an enhanced glycosaminoglycan content and improved transport characteristics (51). In contrast, infusion of solutions containing hyaluronidase, to deplete the peritoneal tissue of HA, resulted in significantly higher rates of small-solute transfer and decreased net ultrafiltration (52).

SUMMARY

The peritoneal transport barrier is a complex system of blood vessels surrounded by interstitial space and bordered by the anatomic peritoneum. The interstitium, or the portion of the tissue that is both extracellular and extravascular, is an orderly structure of collagen fibers that are attached to cells by integrin molecules. Under normal conditions, the interstitium is expanded by fluid flowing under the influence of hydrostatic pressure from the cavity during dialysis. Inflammation in the peritoneal cavity stimulates a cascade of mediators produced by macrophages, mesothelial cells, and fibroblasts in the tissue surrounding the peritoneal cavity. It is hypothesized that these mediators result in changes in blood vessels and in the interstitium, leading to leukocyte migration from the blood into the peritoneal cavity. This migration of white cells results in alterations in endothelial cell junctions, disruption of the collagen-cell linkages, and changes in the glycosaminoglycan content of the interstitium. These changes acutely lead to the increased rates of diffusion and convection across the blood microvessels and through the interstitium summarized by the phenotypic changes of acute peritonitis. Specific long-term effects to the transport system are more difficult to predict and will require further research to elucidate.

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REFERENCES

Flessner Interstitial Transport Changes


