Increasing Peritoneal Contact Area During Dialysis Improves Mass Transfer

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Abstract. Previous studies in mice demonstrated that relatively large volumes in the peritoneal cavity made contact with only 40% of the anatomic peritoneum and that this contact area \((A_{\text{contact}})\) could be increased with use of a surfactant, dioctyl sodium sulfosuccinate (DSS). To investigate the hypothesis that mass transfer rates during peritoneal dialysis are dependent on the area of peritoneum in contact with the dialysis solution, rats were dialyzed for 2 h with a solution that contained \(^{14}\text{C}-\text{mannitol}, \text{with or without 0.02\% DSS. The mass transfer–area coefficients (MTAC) were determined to be (mean \pm SEM, ml/min): no DSS, 0.163 \pm 0.008; with DSS, 0.247 \pm 0.006 (P < 0.002). DSS also caused an increase in total protein loss over 2 h (mean \pm SEM, mg): no DSS, 83.8 \pm 15.8; DSS, 159.5 \pm 6.3 (P < 0.001). In a separate set of animals, the ratio (R) of \(A_{\text{contact}}\) to anatomic area in each plane was measured as in the previous study \(R_{\text{mean}}\) (mean \pm SEM) and equaled 0.466 \pm 0.075, no DSS: 0.837 \pm 0.074, with DSS. The ratio of MTAC (1.52) and protein loss (1.90) approximate the ratio of \(R_{\text{mean}}\) (1.78). Because MTAC = mass transfer coefficient (MTC) \times A_{\text{contact}}, small peritoneal transport chambers were used to determine MTC for \(^{14}\text{C}-\text{mannitol and fluorescein isothiocyanate–albumin. MTC}_{\text{mannitol}}\) did not change significantly with the addition of DSS. MTC}_{\text{albumin}} (cm/min \times 10^4, mean \pm SEM) equaled 1.47 \pm 0.45 without DSS and 1.78 \pm 0.52 with DSS (P < 0.04). It was concluded that DSS increases the mass transfer rates of mannitol and protein by increasing \(A_{\text{contact}}, \text{whereas protein transport is further augmented by an apparent increase in the barrier permeability to protein.}

The rate of mass transfer across the peritoneum can be described by the following equation:

\[
\text{Rate of mass transfer} = \frac{d(C_{\text{plasma}}V_{\text{pc}})}{dt} = \text{MTAC}(C_{\text{plasma}} - C_{\text{pc}}),
\]

where the mass transfer–area coefficient (MTAC) = mass transfer coefficient (MTC) \times peritoneal area in contact with the solution \((A_{\text{contact}})\), where \(C_{\text{plasma}}\) is the plasma concentration, \(C_{\text{pc}}\) is the concentration in peritoneal cavity, and \(V_{\text{pc}}\) is the volume in the peritoneal cavity. An increase in either MTC or \(A_{\text{contact}}\) will result in a rise in the rate of mass transfer. In human studies, \(A_{\text{contact}}\) cannot be easily determined, and the area is typically lumped together with MTC in the term MTAC. We recently developed a technique to determine the area of contact during peritoneal dialysis in anesthetized mice (1). We found that the area of contact between the anatomic peritoneum and a relatively large volume of solution in the peritoneal cavity is a fraction of the area that would be measured after dissection of the tissues that make up the peritoneum. We further showed that extreme agitation of the animal or use of a surfactant in the dialysis solution could significantly increase the area of contact. Because, in equation (1), the rate of mass transfer is directly proportional to \(A_{\text{contact}}, \text{we hypothesized that this rate would change with variation in the contact area. To address this hypothesis, we dialyzed rats with large volumes of solution that contained labeled mannitol to determine the MTAC under conditions of variable \(A_{\text{contact}}, \text{which was increased from control conditions by addition of a surfactant to the dialysis solution. We found that the transfer of both mannitol and protein were increased in approximate proportion to the change in \(A_{\text{contact}}. \text{In control experiments, we also demonstrated that the surfactant did not change the MTC of mannitol across the peritoneum but did have a small effect on the MTC of serum albumin.}

Materials and Methods

Animals and Surgery

Sprague-Dawley rats (purchased from Charles River Labs, Wilmington, Massachusetts) were used in all experiments. In the experiments carried out to measure area or the MTAC, a minimum number of three animals were used per experimental variation; these animals averaged 115 \pm 6 g (mean \pm SEM); the small size made whole- animal sectioning possible. To facilitate chamber experiments that require a significant area of abdominal wall, larger animals (250 to 400 g) were used. There was no apparent difference between the serosal surfaces of the abdominal walls of smaller animals and those of larger animals. All animals were housed in the University of Rochester Vivarium and had ad libitum access to water and standard rat chow. All procedures were reviewed and approved by the University of Rochester Committee on Animal Resources.

Anesthesia was induced with an intramuscular injection of sodium pentobarbital 60 mg/kg, and the animal was subsequently maintained

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at a surgical plane of anesthesia with intravenous injections. Anesthetized rats were maintained at 37°C ± 2°C with an overhead heating lamp and a servo-controlled heating pad (Harvard Apparatus, Holliston, Massachusetts). In all experiments, surgery was carried out after loss of the blink reflex and withdrawal reflex. A trocar was used to make a midline penetration in the lower abdominal wall, and a small catheter was placed into the peritoneal cavity. This was sutured closed with a purse stitch. In longer-term experiments (24 h), no catheter was placed, but an intraperitoneal injection was made directly via a syringe and 22-gauge needle. In all but the 24-h experiments, catheters were placed into a jugular vein for intravenous administration and into the tail artery and carotid artery for continuous monitoring of BP and blood sampling. The mean arterial pressure was observed to be ≈75 mm Hg at all times.

**Isotopic Tracers and Solutions**

In experiments designed to mark the peritoneum which is in contact with the intraperitoneal solution, 125I-IgG (anti-rabbit IgG; IM-134, Amersham Corporation, Arlington Heights, Illinois) was dissolved in all peritoneal solutions 25 μCi/25 to 40 ml. A single batch of labeled IgG was used in the study (specific activity of 12 to 14 μCi/μg) within 2 wk of receipt, with little change in the specific activity (half-life approximately 60 d). As in our previous studies (2), before use of the isotope, the free 125I was removed by dilution with saline and successive ultrafiltrations to limit the free isotope to less than 1%. The approximate loss of label from tracer over the time of use was typically less than 5%. Samples of peritoneal fluid were collected at the end of each experiment, and the free isotope was determined by precipitation with 10% trichloroacetic acid (see Ref. [2]) to be less than 1.5% at 1 h and less than 5% at 24 h. Isotope detection was performed with a Beckman 400 gamma counter (Beckman Instrument, Inc., Fullerton, California).

14C-mannitol was purchased from Moravek Biochemicals (Brea, California) and used directly in both dialysis and chamber experiments. Isotope detection was performed with a Beckman liquid scintillation counter (LS 6000IC, Beckman Instrument, Inc.).

Bovine serum albumin (BSA), labeled with fluorescein isothiocyanate (FITC), was purchased from Sigma-Aldrich Company (St Louis, Missouri). It was detected in plasma and chamber after proper dilution with a Turner TD700 fluorometer (Turner Systems, Sunnyvale, California). Its purity was checked with the same technique as the labeled IgG (2); free FITC was less than 1%.

**Solutions included:** (1) an isotonic Krebs-Ringer-Bicarbonate (KRB, see Ref. [2] for makeup of solution), pH 7.4, 290 mosm/kg, and (2) KRB with a surface active agent, dioctyl sodium sulfosuccinate (DSS, purchased from Sigma-Aldrich Co.), added in concentration of 0.02% by weight.

We did perform some pilot studies with Dexemel (4% Icodextrin solution, a kind gift of ML Laboratories, Sheffield, United Kingdom). The Icodextrin solution was used because human studies (3) had demonstrated that the volume would remain constant for up to 48 h. Because most of the KRB was absorbed in 24 h (<10% remaining), it was assumed that if the peritoneal volume were held constant with Dexemel, the value for peritoneal contact area would be higher than that with an isotonic solution (4). However, the 25- to 35-ml volume of 4% Icodextrin was completely absorbed from the rat peritoneal cavity after 24 h. This is likely due to the level of amylase (the enzyme that metabolizes these starches) present in rodent tissue, which is two orders of magnitude higher than that in humans (5). As in our previous work in mice (1), we collected effluents from six rats dialyzed 24 h with the KRB solution and determined the amylase activity with Sigma Kit 577 (purchased from Sigma-Aldrich Co.). At 24 h, the amylase activity averaged (mean ± SEM units/L) 311 ± 79 for KRB without DSS; the addition of 0.02% DSS increased the activity to 913 ± 80. Although one cannot precisely compare these data with our previous data from 4-h effluents of mice and patients (1), a linear extrapolation to a 4-h level results in values (52 to 152) comparable to an order of magnitude of mice (168 ± 24 units/L) and far above the zero value with this assay for humans. We did not attempt further experiments with Icodextrin solutions because of the species differences between rodents and humans and because results with Icodextrin mirrored those of KRB.

**Experimental Protocol I: Solute Transport in Intact Animals**

The goal of the dialysis protocol was to determine the rate of transport of mannitol (a sugar equal to the molecular size of glucose but not metabolized in mammalian tissue) across the peritoneum during dialysis in intact animals with the different solutions. Animals were anesthetized and surgically prepared as above. Each animal was injected intraperitoneally with solution A or B containing approximately 25 μCi of 14C-mannitol. The initial volume was calculated by scaling to 3-L dwell in 70 kg humans by the following relationship: Vrat = 3000 ml (rat weight in kg/70 kg)5/6 (7). This method of scaling results in larger volumes in small animals (30 ml in a 100-g rat) than would be calculated on purely a “per kg” basis. These volumes however result in intraperitoneal hydrostatic pressures (5 to 6 cm H2O) that closely mimic those in humans (7,8) and help to ensure that the peritoneal contact area is near the maximum attainable (4). The animals tolerated these volumes without respiratory difficulties. Plasma and peritoneal samples for determination of 14C-mannitol concentration were taken during the experiment at 15, 30, 60, and 120 min after injection of the solution. Intraperitoneal pressure was monitored every 30 min. At the end of the designated period of time for the protocol, the animal was killed with an overdose of pentobarbital. The abdomen was then opened, the remaining solution was drained, and its volume was determined by weight.

The concentration in peritoneal fluid (Cp) and the volume (Vp) in the cavity were used to calculate the mass (CpVp) of tracer in the cavity versus time. As in our previous publication (9), equation (1) was fitted to the data, consisting of (CpVp, Cp, and Cplasma versus time), by use of the computer program Scientist (MicroMath, Salt Lake City, Utah) to calculate MTAC for 14C-mannitol.

At the end of the experiment, each rat carcass was rapidly frozen in isopentane, cooled to −70°C. The frozen carcasses were sliced on a cryomicrotome (Hacker-Bright model OTP, Fairfield, New Jersey), and the thin cross-sections of the carcass were dried and placed against film (Kodak Biomax MR, Eastman Kodak, Rochester, New York) to develop into autoradiograms. Unfortunately, after 2 h of dialysis, the surfaces in contact with the fluid could not be differentiated from those in which tracer had not touched because of high background of radioactivity in all tissues. Attempts to use these images to measure Acontact (see Protocol II below) failed because the small-molecular-weight solute, mannitol, diffused rapidly from the cavity into the plasma and redistributed to all tissues surrounding the peritoneal cavity within the dwell period.

**Experimental Protocol II: Contact Area Measurement**

The goal of this protocol was to determine what proportion of the peritoneal surface area is actually in contact with the dialysis fluid during a relatively short dialysis (1 h) and to investigate the effect of a surface active agent (0.02% DSS) in the peritoneal solution on the
contact area. In our previous work (1), we determined that the dwell time of 1 h was long enough for sufficient labeled IgG to interact with the peritoneum to register the autoradiogram. We also determined that 25 to 50 μCi of the <sup>125</sup>I-IgG in 25 to 35 ml (volume was scaled as in Protocol I) of solution provided enough contrast in the staining of the surface to differentiate areas not in contact. As in our previous work, all images above background are weighted equally. Solutions A and B were used in the 1-h experiments. After dialysis for 1 h, each animal was killed, the cavity was drained, and the carcass was frozen. Tissue samples were collected and analyzed as below. The total protein concentration in the 1-h peritoneal effluent was determined with Sigma Diagnostic Kit 690-A.

A second area protocol was designed to answer the question, does the time of dwell change the contact area? Presumably, peristalsis would distribute the fluid to different regions of the peritoneum and broaden the overall exposure. Animals (n = 3 for each solution) were injected under anesthesia with solution A or the Dexemel solution, each of which contained the labeled IgG. The initial volume was scaled as in Protocol I. After injection of the solution via syringe and 22-gauge needle, the rats were allowed to wake up and ambulate freely around the cage and eat and drink ad libitum. At the end of 24 h, each animal was killed, the cavity was opened to check for residual fluid, and the carcass was frozen. Tissue samples were collected and analyzed as below.

Because the technique of data collection and analysis has been thoroughly documented in our previous publication (1), an abbreviated explanation of the technique will be given. In each area determination, the frozen carcass was sliced in whole cross-sections, 20 μm thick, with a Hacker-Bright Cryotome (Model OTF). After collecting three to four sections, the entire block was sliced 2 mm down to the next sampling point, where several cross-sections were again collected. This was repeated for a total of 10 to 14 sampling points, which spanned the entire peritoneal and pelvic cavities. These sections were then placed against x-ray film (Kodak Biomax MR film, Kodak Corp, Rochester, New York) for 1 to 3 wk, to develop autoradiograms. The autoradiograms of each sampling level represent the linear measurement of tissue in contact with the solution in the cavity in the plane of sampling. The tissue sections corresponding to the autoradiograms were stained with standard hematoxylin and eosin to highlight the anatomic peritoneum. To quantify the area of contact and the area of the anatomic peritoneum, individual autoradiograms and the corresponding histologic slides were imaged (MCID, Imaging Research, St. Catharines, Ontario, Canada), and the linear measurement of each area was determined by manual tracing within the digital image. Where \( I_{\text{contact}} \) and \( I_{\text{anatomic}} \) represent the linear measurement of the contact area and anatomic areas in each section, the ratio (R) for each sampling point is calculated as follows:

\[
R = \frac{I_{\text{contact}}}{I_{\text{anatomic}}}
\]

(2)

The average of R’s at all sampling levels (\( R_{\text{mean}} \)) was calculated and will be reported as \( R_{\text{mean}} \pm \text{SEM} \). Results will be compared with a one-way ANOVA in NCSS (Number Cruncher Statistical Systems, Provo, Utah). A statistic will be considered to be significant if the probability of a type 1 error is \( P < 0.05 \).

**Experimental Protocol III: Effect of DSS on Mass Transfer across the Peritoneum Underlying a Small Chamber**

The goal of these experiments is to answer the question, does DSS increase the “permeability” of the peritoneal barrier to small and large solutes? Because the MTAC determined in Protocol I is a lumped parameter that includes both a permeability term (MTC) and the area (\( A_{\text{contact}} \)), a change in the MTAC could be due to changes in either MTC, \( A_{\text{contact}} \), or both. In the following experiments, we use our chamber technique (9) to isolate a small area of peritoneum to compare rates of solute transfer to or from the chamber with different solutions.

Animals were prepared surgically as in *Animals and Surgery*. The abdominal cavity was opened and small plastic chambers (2 cm high, base area = 1.2 to 1.5 cm², volume approximately 2 ml) were carefully affixed to serosal side of the abdominal wall with cyanoacrylate glue (see Ref. [9]). Care was taken not to stretch the tissue, and KRB was placed in the chamber for 30 min after chamber placement and before the introduction of tracers into the animal, to ensure that the tissue was in a state of normal hydration (10).

To investigate the effects of DSS on the transport of <sup>14</sup>C-mannitol, 2 to 3 μCi were added to a predetermined volume (approximately 2 g by weight) of the KRB solution with or without 0.02% DSS (the order of the solution used in the initial dwell was alternated without significant effect on the results), and the solution was injected carefully into the chamber. The fluid was sampled (sample volume, 10 μl) at time 0 and 15, 30, 45, and 90 min for <sup>14</sup>C-mannitol concentration. At 30, 60, and 90 min, the entire chamber volume was drawn up in the same syringe-needle combination used for the initial injection and its volume determined by weight. After 90 min, the chamber fluid was removed and the chamber was washed with a known amount of KRB without tracer; the residual cpm were measured and divided by the final concentration to determine the residual volume in the chamber (residual volume averaged 18.3 ± 1.9 μl). After the chamber was thoroughly washed with KRB, fresh KRB was allowed to dwell in the chamber for 30 min. After the 30-min period, a premeasured volume of the alternate test solution was added to the chamber, and the experiment was repeated. At the end of the second period, the final concentration and volume of the chamber were determined. The animal was killed, and the area under the chamber was measured. To calculate MTC, the data (chamber concentration and volume, both functions of time; chamber area) were fitted to the following equation with the use of the computer program Student (MicroMath) (9):

\[
\text{Rate of disappearance} = \frac{d(C_{\text{cham}}V_{\text{cham}})}{dt} = -\text{MTC}(A_{\text{cham}})(C_{\text{cham}} - C_{\text{plasma}}).
\]

(3)

where \( C_{\text{cham}} \) is the concentration in the chamber, \( V_{\text{cham}} \) is the chamber volume, \( t \) is time, \( A_{\text{cham}} \) is the area of contact (area of the base of the chamber), and \( C_{\text{plasma}} \) is where plasma concentration = 0 (because the transfer area is so small, there is no significant transfer to the blood).

To investigate the effect of DSS on protein transfer from the tissue into the cavity, labeled protein (20 mg FITC-BSA in 0.5 ml of KRB) was injected intravenously, and the rate of appearance of the protein into the chamber was determined. Because protein transports more slowly than small solutes, the experimental period had to be doubled to 3 h to allow sufficient accumulation of tracer. To facilitate this lengthy experiment, two chambers (one for KRB solution and one for KRB + DSS) were affixed side by side to the abdominal wall. The experiment paralleled the one described for mannitol, except that the chamber volume and concentration were determined every 60 min. Because of the slow appearance of albumin in the chamber and experimental restriction of the sample size from the chamber, the data from early time points were quite variable. Fitting the data to an
equation analogous to equation (3) was not practical. In this case, MTC was estimated with the following equation:

\[
MTC = \frac{\Delta(C_{\text{chamber}}V_{\text{chamber}})(A_{\text{chamber}})}{\Delta t} (4)
\]

The calculation was performed with the 180-min chamber data. The plasma concentration decreased less than 5% over 3 h; the mean plasma concentration, \(C_{\text{plasma}}\), was typically two orders of magnitude greater than the chamber concentration; therefore, the \(C_{\text{chamber}}\) of equation (3) was set to zero in equation (4).

**Statistical Analyses**

Rates of mannitol transport, total protein appearance, and mannitol MTAC and values for \(R_{\text{mean}}\) were compared with a one-way ANOVA. Values for \(MTC_{\text{mannitol}}\) and \(MTC_{\text{BSA}}\) for solutions A and B were compared with paired \(t\) tests. The program NCSS 6.0 (Ogden, Utah) was used for all calculations. An effect was considered significant if the chance of a type I error was less than 0.05 (\(P < 0.05\)). All results are reported as mean ± SEM.

**Results**

**Protocol I**

The experiments in Protocol I demonstrated a significant effect of DSS on \(^{14}\text{C}\)-mannitol transport after 2 h of dialysis with the KRB solution. Figure 1 shows the normalized mass of tracer in the cavity (mean ± SEM, \(n = 3\) in each set of data) versus time for the two solutions. DSS causes a marked increase in the rate of decline for the KRB solution. The mean (± SEM, ml/min) values for MTAC are KRB, 0.163 ± 0.007 and KRB + DSS, 0.247 ± 0.006. A one-way ANOVA of the MTAC versus the solution type was significant at \(P < 0.002\). From a series of one-way ANOVA, there was no significant effect on these results of the following variables: weight of the animal (range, 80 to 122 g), volume of fluid injected (range, 26 to 34 ml) or recovered (range, 16 to 28 ml), or intraperitoneal pressure (4.2 to 6.1 cm H₂O).

**Protocol II**

In Protocol II, DSS had significant effects on the contact area of the peritoneal fluid. Animals were dialyzed for 1 h with either KRB or KRB with 0.02% DSS that contained a macro-molecular surface marker (\(^{125}\text{I}\)-IgG) to determine the contact area of the fluid. The \(R\) values (contact area/anatomic peritoneal area, mean ± SEM, \(n = 3\) each) were 0.466 ± 0.075 for KRB alone and 0.837 ± 0.074 for KRB with DSS (\(P < 0.001\)). The protein concentrations in the effluent at 1 h were (mean ± SEM mg/ml) 2.16 ± 0.25 for KRB and 4.83 ± 0.19 for KRB with DSS (\(P < 0.001\)), whereas the mean total protein in the cavity at 1 h was (mean ± SEM, mg) 83.8 ± 15.8 for KRB and 159.5 ± 6.3 for KRB with DSS.

As in our previous work in mice (1), extension of the dwell time to 24 h resulted in an increase of the area marked in the cavity. At 24 h, \(R\) was (mean ± SEM, \(n = 3\) each) 0.90 ± 0.01 for KRB and 0.94 ± 0.01 for Dexemel (NS, \(P > 0.5\)). At 24 h, less than 2% of the KRB solution was recovered and virtually none of the Dexemel. As we noted in the Materials and Methods section, the fact that Dexemel does not have the same properties in rats as in humans is likely due to its rapid metabolism in rodents that have high levels of amylase in subperitoneal tissues (5).

**Protocol III**

The experiments in Protocol III are designed to answer the question, does DSS alter the peritoneal barrier such that the

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*Figure 1. Normalized mass of \(^{14}\text{C}\)-mannitol [Mass(t)/Mass(\(t = 0\))] in the peritoneal cavity of rats versus time. ○, data for Krebs-Ringer-Bicarbonate (KRB) solution without dioctyl sodium sulfo-succinate (DSS); ●, data for KRB solution with 0.02% DSS. The data demonstrate a striking effect of DSS on the rate of disappearance of mannitol from the dialysis solution. All normalized masses are mean ± SEM.*

*Figure 2. DSS effects on mannitol transport. The mass transfer–area coefficients (MTAC) from experiments in intact animals, the mass transfer coefficient (MTC) from chamber experiments, and the average of ratios at all sampling levels (\(R_{\text{mean}}\)) from the area measurements are displayed for KRB and KRB with 0.02% DSS. There are significant differences between the solutions for MTAC and \(R\), which demonstrates that the DSS enhancement of the MTAC is due to increase of peritoneal surface area. All quantities are mean ± SEM. \(^*P < 0.05\) compared with KRB.*
barrier permeability is changed to mannitol or to protein? Small chambers fixed to the abdominal wall serosa restrict transfer to and from the chamber to the tissue area directly under the chamber. Thus, area of contact becomes a measurable quantity, and the MTC or permeability factor can be calculated from equation (3).

In the case of $^{14}$C-mannitol, there is no apparent change in the MTC of the peritoneum. The MTC for KRB (cm/min, mean ± SEM, $n = 5$ each) is 0.0019 ± 0.0003, whereas that for KRB + DSS is 0.0021 ± 0.0003. There is no significant difference between these quantities when they are compared with the paired $t$ test ($P > 0.1$).

In the case of FITC-BSA, injected intravenously, DSS causes an increase in the rate of mass transfer. MTC for the KRB solution (cm/min, mean ± SEM, $n = 5$ each) is 1.47 ± 0.45 × 10$^{-4}$, whereas that for the DSS solution is 1.78 ± 0.52 × 10$^{-4}$ ($P < 0.04$).

Discussion

**DSS Effects on $A_{contact}$**

Previous studies on rats (9) in our laboratory indirectly showed that the area of peritoneum that made contact with dialysis fluid was significantly less than the total area found on dissection and measurement of peritoneal tissues. In those experiments, individual MTC of different surfaces of the peritoneum were multiplied times the area of each of the surfaces measured after the animals were killed and their abdominal cavities dissected. By summing the MTC × $A$ products of all the tissues surrounding the cavity, we estimated the MTAC for the whole cavity. In a separate set of animals, the MTAC for the entire cavity was measured in intact rats with a volume of dialysis solution scaled to 2000 ml in a 70-kg human (a smaller volume than that used in this study) and was found to be 25% to 30% of the sum of all MTC × $A$ values. This discrepancy between calculated MTAC and measured MTAC implied that much of the area of the anatomic peritoneum was not available for transport during a large-volume dialysis. A separate set of animals was then dialyzed with an intensely staining dye for 1 h to detect those surfaces that came in contact with the solution. Large parts of the peritoneal surface had no staining: i.e., one side of the cecum, colon, and stomach and large parts of the abdominal wall and diaphragm. Although qualitative, these observations demonstrated that large portions of the peritoneum were untouched by solution while the animal was in an anesthetized, quiescent state. The observations also imply that the surfaces of the visceral and parietal peritoneums are in contact and can exclude intraperitoneally fluid from entering the space between them.

In this study and in our previous study (1), we have confirmed our earlier observations that even with larger volumes (scaled to 3 L in human instead of 2 L as it was in Ref. [9]) in the peritoneal cavity, only a fraction of the anatomic area is in contact with the dialysis solution. In mice (1), $R_{mean}$ after a 1-h dwell of KRB was 0.43 ± 0.03. With the use of 0.05% DSS, the entire area of the peritoneum was brought in contact with the intraperitoneal solution. In this study in rats, a similar experimental protocol to our study in mice produced $R_{mean}$ of 0.47 ± 0.08 with the same KRB solution. The use of DSS resulted in a marked enhancement of $A_{contact}$. Together, these observations demonstrate that a surface active agent in the dialysate fluid results in fluid contact with surfaces that might otherwise be unavailable to the solution. The results also imply that the surfaces of the peritoneum are coated with a substance that results in surface tension when placed in contact.

There is little known of the precise makeup of the substance that makes up the layer covering the mesothelial cells. Effluent from patients undergoing peritoneal dialysis has been shown to contain phospholipids and phosphatidylcholine on thin layer chromatography (11). These peritoneal “washings” are similar to the results from pleural washings of dogs, which demonstrated the presence of phosphatidylethanolamine, sphingomyelin, and predominantly phosphatidylcholines (12). In fact, the layer is only seen in electron microscopy when osmication is not used to prepare the specimens (13). Its thickness has been observed in mice to be 10 to 15 μm (13), although it was reported to be thicker in rat tissues (14). Mesothelial cells are known to be covered with microvilli, which are connected with many strands that are polyanionic and arise from a surface glycocalyx (15). These are hypothesized to support the “serous exudate,” which presumably contains the surface active agents and provides the lubrication between surfaces (15). We presume that it is this layer with which DSS interacts.

**DSS Effects on Mass Transfer Correlate with $A_{contact}$**

The combined results from Protocols I, II, and III uphold our hypothesis that an increase in the contact area results in an increase in mass transfer. Figure 2 illustrates the results for mannitol. Because the MTAC = MTC × $A_{contact}$, the ratio of the MTAC should be proportional to the ratio of the products of MTC and $A_{contact}$. In the case of mannitol, the MTC were not different for each solution. Therefore, the ratio of $MTAC_{DSS}/MTAC_{KRB} = 0.247/0.163 = 1.51$ should approxi-

![Figure 3. DSS effects on protein transport. Total protein at 1 h of dialysis, $R_{mean}$, and MTC$_{BSA}$ are plotted for KRB and KRB with 0.02% DSS. The results demonstrate a significant effect of DSS on all three quantities. All quantities are mean ± SEM. *$P < 0.05$ compared with KRB.](image-url)
mate the ratio of the relative contact areas (R), which equals $(0.84/0.47) = 1.78$. The lack of one-to-one correspondence between the MTAC and the area ratios is likely due to the fact that different sets of animals were used for each set of data and to the technique used to measure $A_{\text{contact}}$, which may overestimate this quantity because of peristalsis during the 1-h dwell (see discussion in reference 1). From these experiments, we conclude that the increase in R or $A_{\text{contact}}$ accounts for the increase in MTAC for small solutes such as monosaccharides.

The results for protein loss are more complicated and may involve both the increase in surface area and a change in barrier characteristics. Figure 3 illustrates the total protein lost to the solution in the cavity over a single hour as well as the R values and the MTAC. Under the assumption that MTAC is representative of all the protein lost, the ratio of protein lost is $159.5/83.8 = 1.90$. The ratio of the R values (1.78) is nearly equal to this number. In this case, the protein loss was measured in the same animals in which the contact areas were determined, and, therefore, we would anticipate a closer correlation of the total protein lost. Given that the total protein transport should be proportional to the MTAC (under the assumption of equivalent concentration gradients for all sets of experiments), the ratio of the values for $MTC_{\text{BSA}} \times A_{\text{contact}} = [(1.78 \times 10^{-4})/(1.47 \times 10^{-4})] \times (0.84/0.47) = 2.15$. The area change makes up most of the change, but there is an apparent change in MTAC as well. The apparent changes in the MTC and $A_{\text{contact}}$ are more than sufficient to account for the observed change in the protein transport.

**Correlation with Other Studies**

Although no other group has determined $A_{\text{contact}}$, MTC, and MTAC in a single study as we have, there have been several sets of data that have demonstrated that maneuvers to increase surface area have resulted in an increase in the clearance of small solutes and protein. Vigorous shaking of anesthetized animals (16–18) and the use of surfactants (19–22) in the dialysis solution have resulted in marked increases in mass transfer. Although all of these studies have used methods similar to our Protocol I, some have observed larger enhancements of the MTAC or clearance primarily because the initial volume was much lower than our scaling criteria would have called for. By starting at a lower volume and therefore a smaller $A_{\text{contact}}$, they obtained a much larger enhancement in their experiments. We previously discussed these issues in the mouse study (1).

In several studies in both rats and rabbits, DSS has been shown to enhance mass transfer of small solutes across the peritoneum. Penzotti and Mattocks (19) used DSS (0.05% to 0.5%) in rabbits and were able to demonstrate a three- to fourfold increase in the mass transfer of small solutes. These early studies with DSS (19) in rabbits used volumes of 60 ml/kg (compared with 400 ml/kg in our study). Further studies in rabbits dialyzed with a range of DSS concentrations (0.005% to 0.04%) in the solution demonstrated dose-dependent increases of 75% to 240% in the peritoneal clearances of urea and creatinine (20). Leypoldt et al. (21) carried out experiments with 0.005% DSS in rabbits and calculated a 50% increase in mass transfer of creatinine. In another study in rats (22), 25 ml of 3.86% dextrose with 0.005% DSS caused significant increases in fluid gain over 4 h (to 37 ml with DSS versus 33 ml without DSS) and 20% to 30% increases in urea and sodium clearance to the cavity. The variation in the degree of DSS enhancement of mass transfer seen in the various studies is presumably due to the different species and experimental designs used by the different groups of investigators.

The use of DSS in the dialysate solution increases the protein loss into the dialysate. Leypoldt et al. (21) determined that the transport of protein into the dialysate is significantly enhanced with the use of 0.005% DSS in the dialysis solution. In our previous study in mice (1), we demonstrated a clear correlation of DSS concentration, the peritoneal contact area, and protein transport to the dialysate. In this study, we showed that the use of 0.02% DSS in rats caused a 90% increase in the loss of total protein. Although the increase in protein transport was chiefly accounted for by the increase in contact area, we have also demonstrated that the MTC for labeled albumin is increased significantly. From all of these observations, we conclude that protein loss would be accelerated in mammals undergoing peritoneal dialysis with DSS in the solution.

**Clinical Use of Surfactants in Patients Undergoing Peritoneal Dialysis**

As far as we know, there has not been a study in human patients undergoing dialysis with DSS in the solution. Addition of phosphatidylcholine (PPC) (50 mg/L) to standard glucose dialysis solutions, which were instilled into the peritoneal cavities of patients with low ultrafiltration, tripled the net fluid removal over 24 h (23). Creatinine and urea clearances increased by 20% to 25% in these same patients. The effects of the addition of PPC to the solutions lasted the length of the experiment, 72 h (23). In patients with normal ultrafiltration, addition of the substance did not change the ultrafiltration or clearances (23). Even intravenous and oral doses of PPC in patients with low ultrafiltration characteristics were shown to enhance the ultrafiltration, and the effect was sustained over 4 mo. PPC levels in the peritoneal effluent were shown to increase significantly after administration of the substance via any route. If we assume that the layer over the mesothelial cells of the peritoneum in these patients with low ultrafiltration was altered by the PPC, we can hypothesize that perhaps one effect might be that their $A_{\text{contact}}$ may have been increased.

$A_{\text{contact}}$ in human dialysis patients with a 2-L volume in the cavity has been estimated to be 0.55 m$^2$ (24), which is 30% to 60% of the anatomic area in patients who weigh 70 to 80 kg (25–28). The area of peritoneum that is not in contact with the dialysis solution is a potential site for dialysis enhancement, given that the MTAC = MTC $\times A_{\text{contact}}$. In patients who are apparently inadequately dialyzed (weekly KT/V$\text{area}$ $< 2.1$), a 20% increase in the MTAC may be enough to produce adequate dialysis and allow them to continue peritoneal dialysis. Surface active agents are particularly attractive with patients who have adhesions and may have pockets of peritoneal surface that might not be readily available to the typical dialysis solution.
Issues of toxicity must be carefully considered with these substances. We observed that 0.5% DSS in the peritoneal cavity of mice resulted in 100% lethality within 2 h (1). We have also observed a direct correlation between DSS concentration, contact surface area, and the protein concentration in the dialysate fluid after 1 h of dwell; protein loss must be investigated before implementation of this in long-term dialysis in patients. Another item that must be investigated before human implementation is the long-term effect on the peritoneum and the subperitoneal tissue space that forms the barrier between the dialysis fluid and the blood. Other investigators (20) observed fibrinoid material in the dialysis effluents of two of three rabbits in which a 0.04% DSS solution had been used; this finding implies that DSS may set up or enhance conditions of inflammation within the peritoneal cavity. Use of any additive to peritoneal dialysis fluid will require further study for potential complications during long-term use in humans.

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**References**