In patients on long-term continuous ambulatory peritoneal dialysis (CAPD), peritoneal dysfunction is considered to be due to the loss of peritoneal mesothelial cells and to subsequent peritoneal fibrosis and neovascularization. Our aim in the present study was to clarify the role of various components of peritoneal dialysis fluid in the occurrence of peritoneal dysfunction in CAPD patients. We used a cell counting assay and ELISA to study the viability of human peritoneal mesothelial cells and their secretion of basic fibroblast growth factor (bFGF)—which induces peritoneal fibrosis and neovascularization—by cells cultured with various components of peritoneal dialysis fluid.

The viability of cultured cells, ranked from highest to lowest by solution type, was bicarbonate (40 mEq/L) > lactate (15 mEq/L) + bicarbonate (25 mEq/L) > lactate (40 mEq/L). Viability also showed a concentration-dependent decrease in the presence of advanced glycation end-products of bovine serum albumin. The bFGF level in the supernatant showed a concentration-dependent increase in the presence of glucose and glycated albumin; bFGF level decreased as the bicarbonate concentration increased. Low levels of glucose, lactate, and glycated albumin, and a high concentration of bicarbonate may preserve the viability of peritoneal mesothelial cells and prevent bFGF secretion.

Key words
Fibrosis, vascular reactivity, growth factor

Introduction
Continuous ambulatory peritoneal dialysis (CAPD) has been used for more than two decades as a treatment for end-stage renal failure, but peritoneal dysfunction is still an important problem. In patients on long-term CAPD, peritoneal dysfunction is considered to be attributable to the loss of peritoneal mesothelial cells and to subsequent peritoneal fibrosis and neovascularization (1,2). We previously reported that basic fibroblast growth factor (bFGF), a cytokine that participates in fibrosis and neovascularization (3,4), can cause peritoneal fibrosis in CAPD patients (5).

Our aim in the present study was to clarify the role of peritoneal dialysis (PD) fluid in the occurrence of peritoneal dysfunction in CAPD patients by investigating the viability and bFGF secretion of human peritoneal mesothelial cells (HPMCs) after incubation with various components of PD fluid.

Material and methods
From pieces of human omentum (3 – 5 cm²) obtained at laparotomy, HPMCs were isolated according to the method of Stylianou et al. (6). The cells were cultured in M199 medium containing 10% fetal calf serum (FCS: Mitsubishi Kasei Corp., Tokyo, Japan). Cells from the first or second passage were used for the experiments.

Advanced glycation end-products of bovine serum albumin (AGE-BSA) were prepared by incubating
fatty-acid-free BSA (Sigma, St. Louis, MO, U.S.A.) in phosphate-buffered saline (PBS: Nissui Pharmaceutical, Tokyo, Japan; pH 7.3) with 250 mmol/L glucose 6-phosphate (Sigma) at 37°C for 8 weeks in the presence of 1.5 mmol/L phenylmethylsulfonyl fluoride and 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA: Life Technologies, Grand Island, NY, U.S.A.), as described elsewhere (7). An anti-carboxymethyllysine monoclonal antibody was used to detect the AGE-BSA.

Various culture media were tested in the experiments. Sodium bicarbonate–free M199 medium was supplemented with 10% FCS (volume/volume), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine before use. That medium was further supplemented with d-glucose (Kanto Chemical, Tokyo, Japan) and sodium lactate (Katayama Chemical, Osaka, Japan) or sodium bicarbonate (Katayama Chemical), or both, and with AGE-BSA. Lactate or bicarbonate alone was added at a concentration of 40 mmol/L. The lactate–bicarbonate combination contained lactate at 15 mmol/L and bicarbonate at 25 mmol/L. All media were sterilized by filtration (0.22 μm Millex-GV: Millipore, Bedford, MA, U.S.A.) and buffered to pH 7.4 with 1 N sodium hydroxide (Katayama Chemical) or 1 N hydrochloric acid (Katayama Chemical) just before use.

The viability of HPMCs was assessed after incubation in the various media for 24 hours. The cell counting kit (Dojindo, Kumamoto, Japan) employed a colorimetric assay based on cleavage of the watersoluble tetrazolium salt WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] to a formazan dye by mitochondrial dehydrogenase in viable cells. The WST-1 solution (5 mmol/L WST-1, 20 mmol/L HEPES, and 0.2 mmol/L 1-methoxy-5-methylphenazinium methylsulfate) was added at 10 μL per well for the last 4 hours of each incubation. An ELISA reader (SLT Lab Instruments, Salzburg, Austria) was used to determine absorbance at a reading wavelength of 420 nm and a reference wavelength of 630 nm.

A sandwich enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, U.S.A.) was used to measure the level of bFGF in the culture supernatants. Supernatants were harvested after mesothelial cells (1×10^4 per well) had been incubated for 24 hours with the various media (1.0 mL per well) in 24-well dishes coated with rat type I collagen (Becton–Dickinson, Mountain View, CA, U.S.A.), and were stored at –30°C until assay.

Statistical analysis was performed using the unpaired Student t-test as appropriate. Results are expressed as the mean ± standard error of the mean (SEM), with each experiment being performed in triplicate on cells from each of three donors. Values of p less than 0.05 were considered statistically significant.

**Results**

The viability of HPMCs was lowest in the presence of 40 mEq/L lactate, higher in 15 mEq/L lactate + 25 mEq/L bicarbonate, and highest in 40 mEq/L bicarbonate. In addition, we noted an AGE-BSA concentration–dependent decrease in cell viability (Figure 1).

The bFGF level in the supernatant showed a concentration-dependent increase when cells were incubated with glucose or with AGE-BSA (Figure 2). In addition, the bFGF level showed the following order after incubation of HPMCs with lactate or bicarbonate or a combination: 40 mEq/L lactate > 15 mEq/L lactate + 25 mEq/L bicarbonate > 40 mEq/L bicarbonate (Figure 2).

**Discussion**

Our study demonstrates that lower levels of glucose, AGE-BSA, and lactate, and a higher level of bicarbonate in the culture medium could improve the viability of HPMCs and prevent secretion of bFGF by the cells.
In patients on CAPD, the peritoneal mesothelium acts as a protective barrier. It is also involved in the transport of water and solutes, in tissue remodeling after injury, and in the production of many biologically active agents including cytokines, growth factors, and adhesion molecules. Mesothelial injury has been suggested as the primary cause of peritoneal hyperpermeability and peritoneal fibrosis (or both) in patients on CAPD. Dobbie (8) reported that mesothelial cells develop abnormalities and then eventually disappear during the course of peritoneal membrane sclerosis in CAPD patients. A decrease in the viability of mesothelial cells may cause changes in the peritoneal tissues, and progressive sclerosis may eventually lead to peritoneal dysfunction. In fact, degeneration of the peritoneum in patients on CAPD seems to start from submesothelial layer, and its progression depends on the duration on CAPD (2,9).

In 1974, Gospodarowicz described the presence of a potent mitogen for mouse 3T3 fibroblasts in extracts of the brain and the pituitary gland (10). This activity was named “fibroblast growth factor,” and bFGF was subsequently purified from brain and pituitary tissues in bovines (11). Along with vascular endothelial growth factor, bFGF is well known to be a strong angiogenic factor (12). Our findings in the present study suggest the possibility that bFGF released by HPMCs may participate in the development of peritoneal fibrosis and neovascularization, which may lead to peritoneal dysfunction.

Previous studies have demonstrated that a high glucose concentration and a hyperosmolar peritoneal dialysate have an adverse influence on mesothelial cells in vivo and in vitro (13,14). The present study assessed the effect on HPMCs of incubation with 1.5% (84 mmol/L) glucose and 4.0% (222 mmol/L) glucose, which are the glucose concentrations found in commercial peritoneal dialysates. We confirmed a concentration-dependent increase of bFGF protein secretion by HPMCs in the presence of high glucose levels.

Fluids for CAPD containing 40 mmol/L lactate, 15 mmol/L lactate and 25 mmol/L bicarbonate, and 34 or 39 mmol/L bicarbonate are currently available in Europe. In the present study, we assessed effects of lactate and bicarbonate at a total concentration of 40 mmol/L. Several reports have described clinical experience with bicarbonate-buffered and lactate-buffered PD solutions. Feriani et al. (15) found that use of bicarbonate-buffered dialysate for CAPD improved acid–base status, replenished bicarbonate stores, and normalized the rate of protein catabolism as compared with lactate-buffered dialysate (16). The present study suggests that lactate-containing CAPD solutions may also be more likely to induce fibrosis and neovascularization than bicarbonate-containing solutions.

Advanced glycation end-products are thought to be another cause of peritoneal dysfunction. Accumulation of AGEs has been detected in the connective tissues, mesothelium, and vessel walls of CAPD patients (17). Miyata et al. (18) showed that AGE levels in serum and PD fluid were significantly increased in CAPD patients. How AGEs influence HPMCs is unclear, but various effects could occur owing to binding with the receptors for AGE expressed by those cells (19). Proteins that are AGE-modified can stimulate cell activation and increased expression of extracellular matrix proteins, vascular adhesion molecules, cytokines, and growth factors. Depending on the cell type and the concurrent signaling, those changes are associated with chemotaxis, angiogenesis, oxidative stress, cell proliferation, and programmed cell death (20). Our findings in the present study raise the possibility that AGE-modified proteins could cause loss of HPMCs and increased release of bFGF.
**Conclusion**

In an attempt to prevent peritoneal dysfunction, various changes have been made to CAPD fluid, including the addition of icodextrin, sodium bicarbonate, or amino acids; adjustment to neutral pH; and adoption of a twin-bag system and connectors sterilized by ultraviolet light. To improve the viability of HPMCs and to reduce secretion of bFGF by those cells, our data suggests the usefulness of a fluid with low glucose content, low lactate, low AGEs, and a high level of bicarbonate. Although our *in vitro* data support the benefits of a low-glucose, high-bicarbonate solution without lactate or AGEs, further investigations are required to determine the ideal dialysate for CAPD patients.

**References**


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Sodium sieving is a measure of free water transport. However, its assessment is disturbed when a large difference exists between sodium concentrations in plasma and in dialysate—that is, when the diffusion rate is high. Based on previous findings concerning similarity in the mass transfer area coefficients (MTACs) of sodium and urate, we developed a model that corrects for high diffusion. The model enables us to predict the dialysate sodium concentration resulting from diffusion alone at any time point during a dwell. The correction was based on knowledge of the intraperitoneal volume at any time point during the dwell, which can be calculated by using a volume marker (reference method). However, in a peritoneal equilibration test (PET), only the drained volume after 4 hours is available, and urate concentration is not routinely measured. Therefore, our objective in the present study was to investigate whether a diffusion correction using the MTAC of creatinine and the drained volume at the end of the dwell would be as accurate in estimating maximum sodium sieving as the reference method is.

We analyzed standardized 4-hour dwells in 28 patients, 19 with stable PD and 9 with ultrafiltration failure. The dialysate consisted of a 3.86% glucose-containing solution to which dextran 70 was added as a volume marker. The correlation coefficient between the PET correction method and the reference method was 0.92 in all patients (0.90 in stable patients and 0.95 in the patients with ultrafiltration failure (p < 0.01 for all)). We conclude that a diffusion correction for sodium can be made using PET data. A diffusion correction yields a better estimate of sodium sieving than does the sole use of the lowest dialysate-to-plasma (D/P) sodium irrespective of diffusion rate.

Key words
Sodium sieving, diffusion correction, free water transport

Introduction
The dialysate concentration of sodium decreases during the initial phase of a dialysis dwell with a 3.86% or 4.25% glucose-based dialysis solution (1). The minimum value is usually reached after 1 – 2 hours. Because the plasma sodium concentration is unchanged, the dialysate-to-plasma ratio (D/P) of sodium decreases. This so-called sieving of sodium in a situation with a high ultrafiltration rate is caused by free water transport—that is, water transport without concomitant transport of sodium, probably through intracellular water channels. Impaired channel-mediated water transport leads to a decrease of sodium sieving.

The diffusion rate of sodium is usually low owing to the relatively small concentration gradient between plasma and dialysate sodium and to the low mass transfer area coefficient (MTAC) of sodium, which averages 8 mL/min (2,3). A relatively high diffusion rate is present when the concentration gradient of sodium is high (for example, in patients with hypernatremia) or when high MTAC values are present owing to a large vascular peritoneal surface area. The latter condition is the most prevalent cause of ultrafiltration failure (1).

A high sodium diffusion rate blunts the decrease in D/P sodium, falsely suggesting impaired free water transport. A model has therefore been developed to correct for diffusion (4). The model is based on the similarity of the MTACs of sodium and urate, as found in previous studies (2). Using the MTAC of urate, the dialysate sodium concentration caused solely by diffusion can be predicted at any time point during the dwells. The calculations require the use of an intra-
peritoneally administered volume marker, multiple samples of dialysate, and determination of urate.

In the present study, we investigated whether a diffusion correction could also be applied during a peritoneal equilibration test (PET) with 3.86% or 4.25% glucose, using only the dialysate values of sodium and creatinine, the plasma concentrations of those solutes, and the drained volume after 4 hours.

Patients and methods

Rationale
In the case of an ideal correction for diffusion of sodium from the circulation to the dialysate, the D/P sodium should, when no or negligible free water transport occurs, remain constant after reaching its minimum value. Analysis of the results obtained with a reference method showed that that situation was more or less the case (4). It also showed that replacing MTAC urate with MTAC creatinine led to only a slight overcorrection for diffusion. We therefore used the simplified Garred model (5) to calculate the MTAC creatinine from plasma and dialysate concentrations of creatinine and the drained volume after 4 hours. The resulting MTAC creatinine was used to predict the dialysate sodium concentration at 4 hours, when only diffusion would have occurred.

Patients
We randomly selected 28 patients from our standard peritoneal permeability analysis (SPA) database (6). The SPAs were done using a 3.86% or 4.25% glucose-based dialysis solution (Dianeal: Baxter BV, Utrecht, Netherlands). Of the 28 patients, 9 had ultrafiltration failure (UFF), defined as net ultrafiltration <400 mL in 4 hours using a 3.86% or 4.25% glucose-based dialysate (7). None of the patients had peritonitis at the time of the study or in the previous four weeks.

Calculations
The following equation can be used to predict the dialysate sodium concentration attributable solely to diffusion at 4 hours:

\[
\text{MTAC Na}^+ = (V_t / \tau) \ln \left[ \frac{V_0 (P\text{Na}_0 - D\text{Na}_0)}{V_t (P\text{Na}_t - D\text{Na}_t)} \right] \tag{1}
\]

where \(V_0\) is the intraperitoneal volume at time 0, \(V_t\) is the intraperitoneal volume at 240 minutes, \(P\text{Na}_0 - D\text{Na}_0\) is the sodium concentration gradient at time 0, \(P\text{Na}_t - D\text{Na}_t\) is the sodium concentration gradient at 240 minutes, \(D\text{Na}_0\) is the dialysate sodium concentration before inflow, and \(D\text{Na}_t\) is the dialysate sodium concentration after drainage.

The MTAC creatinine was calculated using the simplified Garred method:

\[
\text{MTAC creatinine} = (V_t / \tau) \ln \left[ \frac{V_0 (P\text{creat}_0 - D\text{creat}_0)}{V_t (P\text{creat}_t - D\text{creat}_t)} \right] \tag{2}
\]

Then, based on the assumption

\[
\text{MTAC Na}^+ = \text{MTAC creatinine}, \tag{3}
\]

the MTAC Na\(^+\) in equation (1) was replaced by MTAC creatinine:

\[
\text{MTAC creatinine} = (V_t / \tau) \ln \left[ \frac{V_0 (P\text{Na}_0 - D\text{Na}_0)}{V_t (P\text{Na}_t - D\text{Na}_t)} \right] \tag{4}
\]

Equation (4) can be rearranged to read

\[
D\text{Na}_t = -\left\{ \frac{V_0 (P\text{Na}_0 - D\text{Na}_0) e^{MTACcreatinine(V_t/V_t)}}{V_t} \right\} + P\text{Na}_t. \tag{5}
\]

Using equation (5), the theoretical \(D\text{Na}_t\) by diffusion was calculated for each patient. The initial dialysate sodium concentration was subtracted from that value to yield the diffusion. The diffusion was then subtracted from the measured sodium concentration to correct for diffusion.

Statistics
Values are expressed as mean and standard deviation. The two patients groups were compared using the Student t-test. The paired t-test was used to analyze the differences between uncorrected and corrected dialysate sodium concentrations. A linear regression analysis was used to compare the diffusion-corrected acquired values for dialysate sodium concentration to the values calculated by the reference method. A Bland–Altman analysis was performed to investigate possible systematic errors (8).

Results
Table I summarizes the clinical and transport characteristics of the two groups of patients. Table II presents
the measured and the diffusion-corrected values for dialysate sodium concentration. The diffusion-corrected values were significantly lower than the uncorrected ones. Figure 1 shows the linear regression analysis between the two methods. The correlation coefficient for the whole group was 0.92. It was 0.90 in the stable patients and 0.95 in the patients with UFF. Stable patients had significantly lower values for both corrected and uncorrected dialysate sodium than did the UFF patients ($p < 0.05$). The Bland–Altman analysis (Figure 2) showed a mean overestimation of the PET corrected values of 1.8 mmol/L in comparison with the reference method. The individual data points were randomly distributed around the mean, which implies absence of systematic errors relative to the absolute values. The mean difference between the two methods of correction was of a similar magnitude in both groups.

**Discussion**

In the present study, we investigated whether a diffusion correction model that uses PET data only would be as accurate in estimating sodium sieving as the more complex method developed by Zweers *et al.* (4). A PET-based model would allow better assessment of sodium sieving in centers that use the PET to characterize peritoneal transport parameters. In a situation

### TABLE I Clinical and transport characteristics of the patients$^a$

<table>
<thead>
<tr>
<th></th>
<th>Stable</th>
<th>UFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients ($n$)</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>Female/male</td>
<td>6/13</td>
<td>4/5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 (27–69)</td>
<td>55 (20–76)</td>
</tr>
<tr>
<td>Duration of PD (months)</td>
<td>33 (2–69)</td>
<td>7 (3–119)</td>
</tr>
<tr>
<td>MTAC creatinine (mL/min)$^b$</td>
<td>7.5±2.2$^c$</td>
<td>11.4±4.7</td>
</tr>
<tr>
<td>Plasma Na$^+$ (mmol/L)</td>
<td>137.9±3.5</td>
<td>138.6±2.9</td>
</tr>
<tr>
<td>Ultrafiltered volume (mL/4 h)</td>
<td>706.8±203.1$^d$</td>
<td>263.3±316.3</td>
</tr>
</tbody>
</table>

$^a$ Age and duration of peritoneal dialysis (PD) are expressed as median and range; the other values are expressed as mean ± standard deviation.

$^b$ Simplified Garred.

$^c p < 0.05$.

$^d p < 0.001$.

UFF = ultrafiltration failure; PD = peritoneal dialysis; MTAC = mass transfer area coefficient.

### TABLE II Measured and corrected values for dialysate sodium [DNa$^+$ (mmol/L)]$^a$

<table>
<thead>
<tr>
<th></th>
<th>Stable</th>
<th>UFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNa$^+_{240}$</td>
<td>122.8±4.8</td>
<td>129.7±5.3</td>
</tr>
<tr>
<td>Reference method</td>
<td>115.0±3.4$^b$</td>
<td>121.3±4.4$^b$</td>
</tr>
<tr>
<td>PET correction method</td>
<td>116.8±3.9$^b$</td>
<td>123.9±5.2$^b$</td>
</tr>
</tbody>
</table>

$^a$ Values are expressed as mean ± standard deviation.

$^b p < 0.001$ as compared with DNa$^+_{240}$.

UFF = ultrafiltration failure; PET = peritoneal equilibration test.
of high diffusion rates, lack of correction could lead to a false assumption of impaired free water transport. A recent review (1) suggested that a diffusion correction would be useful when the concentration gradient of sodium exceeds 5 mmol/L.

The results of the present study show a good correlation between the two methods for correcting sodium sieving for diffusion. The PET correction model overestimated the true dialysate sodium by a mean of only 1.8 mmol/L. Also the 95% confidence interval ranged from –2.6 mmol/L to 6.2 mmol/L—and therefore from –2.8% to 5.0% of the mean \(\text{DNa}^+_{240}\) value. The slight overestimation was probably caused by using the MTAC creatinine, which is higher than that of sodium (2,3), or by the variability of diffusion rates throughout the dwell. An overestimation of dialysate sodium of that magnitude is unlikely to lead to a clinically important overestimation of impaired free water transport.

**Conclusion**

We conclude that a diffusion correction for the assessment of sodium sieving is possible using the widely applied PET when that test uses a 3.86% or 4.25% glucose-based dialysis solution. Diffusion correction allows a more precise estimation of peritoneal free water transport than does the lowest D/P sodium irrespective of diffusion rate. A diffusion correction especially avoids overestimation of impaired free water transport when the difference between the plasma and the dialysate sodium concentration exceeds 5 mmol/L.

**References**


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The transport capacity of any membrane depends on its surface area and permeability. In addition, peritoneal capillaries are probably barriers to solute transport. Although no decisive use of antihypertensive drugs has been reported in continuous ambulatory peritoneal dialysis (CAPD) patients with hypertension, those drugs are known to have various effects on vessels. In the present study, we used a charge-coupled-device (CCD) camera in renovascular hypertensive dogs with mild renal insufficiency to investigate the effects of various antihypertensive drugs on the peritoneal capillaries. Renovascular hypertension was induced in the dogs by placing silver clips on both renal arteries to create 90% occlusion. After confirmation of elevation of blood pressure (usually 20 days after the operation), each dog’s abdomen was opened while the animal was under general anesthesia. Using a CCD camera, the diameters of the small arteries of the peritoneum were measured after 3 days’ oral administration of a placebo (n = 5); or of 8 mg CS866, a selective angiotensin II type 1 receptor blocker (n = 5); or of 10 mg benazepril, an angiotensin-converting enzyme inhibitor (n = 5); or of 10 mg amlodipine, a calcium antagonist (n = 5). In dogs receiving CS866, blood pressure decreased to 128 ± 6 mmHg from 160 ± 6 mmHg (p < 0.01). A similar decrease in blood pressure was observed with the use of the other drugs. The diameter of the small vessels increased by 28% ± 6% in dogs receiving CS866 and by 24% ± 5% in dogs receiving benazepril, as compared with 3% ± 3% in dogs receiving the calcium antagonist. These data clearly demonstrate that blockade of the renin–angiotensin system produces an increase in solute clearance in hypertensive dogs with mild renal insufficiency and that such blockade may be applicable as therapy for hypertensive patients on CAPD.

Key words
ACE inhibitor, ARB, calcium antagonist, aquaporin

Introduction
Many physiologic and pharmacologic factors are known to contribute to the regulation of solute transport and ultrafiltration during peritoneal dialysis. Moreover, drugs are known to alter peritoneal transport of solutes and water (1). The alterations suggest an increase in peritoneal surface area or permeability resulting from vasodilation, attributable to dilation of the functional peritoneal capillaries combined with perfusion of more capillaries. Thus, many vasoactive drugs apparently can affect peritoneal transport rates. Although the contribution from baseline levels of angiotensin II to peritoneal transport rates in CAPD patients is uncertain, data from a limited number of studies have suggested that angiotensin-converting enzyme inhibitors (ACEi) increase peritoneal transport rates (2,3). In the present study, we used a charge-coupled-device (CCD) camera in renovascular hypertensive dogs with mild renal insufficiency to investigate the effects of various antihypertensive drugs on the peritoneal capillaries.

Materials and methods
All experimental procedures in the study were conducted according to the guidelines of the Animal Care and Use Committee of Saitama Medical School. In beagle dogs weighing between 10 kg and 15 kg, we exposed the bilateral renal arteries and placed external adjustable clips around the arteries to reduce the lumen by 90%. Three weeks later, the experiment was started.

After 3 days’ oral administration of a placebo (n = 5), or of 8 mg CS866 [a selective angiotensin II type 1 receptor blocker (ARB; n = 5)]; or of 10 mg benazepril [an ACEi (n = 5)], or of 10 mg amlodipine [a calcium antagonist (n = 5)], all dogs were anesthe-
tized with sodium pentobarbital (15 mg/kg, intravenously administered) and were intratracheally intubated for maintenance of constant respiration. A 7-Fr catheter was inserted through the right femoral artery to measure arterial pressure, and the left radial vein was catheterized for infusion of drugs. The omentum was then exposed through a midline incision.

**Visualization of the peritoneal circulation**

An intracavital needle-type CCD camera was used to observe the peritoneal circulation. The prototype CCD camera had been developed for assessment of cardiac or renal microcirculation (5). The system (VMS-1210: Nihon Koden, Tokyo, Japan) consists of a needle-type probe, a camera body with CCD sensor, a gradient index lens, and an optical fiber guide for illumination. The probe (4.5 mm in diameter) contained 18 optical fibers. The CCD had a 680×480-pixel image sensor and provided digital images to a Macintosh computer (PowerMac: Apple Computer, Cupertino, CA, U.S.A.) and a video recorder (EVO-9850: Sony, Tokyo, Japan).

After stabilization of the dog’s general condition under anesthesia, the CCD probe was inserted into the abdominal cavity. The probe was moved and swung gently to acquire clear images of the peritoneum.

**Measurements of vessel diameter**

Vessel diameter was measured as previously described (6). Briefly, sequential images of the peritoneal circulation were captured using a freeze-frame modality on the Macintosh computer, and the density in the gray scale mode was digitized along the scanning line across the vessel. A difference in the density value one quarter higher than noise level was identified as the inner diameter. A vessel segment approximately 15 µm in length was scanned, and the mean vessel diameter was determined by averaging at least 5 measurements during the plateau of the response. The spatial resolution of the CCD system is 0.87 µm.

**Experimental protocol**

After the CCD probe was inserted into the abdominal cavity, the experimental animals were allowed to equilibrate for 60 minutes before the experimental protocols were initiated. Each experimental period required 20 minutes to obtain a stable response in peritoneal circulation. After completion of protocol 1, a sample of the peritoneum was obtained. The sample was used to measure aquaporin mRNA.

**Measurements of AQP mRNA expression in the peritoneum**

Semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out to determine the levels of aquaporin mRNA in the peritoneal samples. The semi-quantitative RT-PCR was performed using these primer sets:

- aquaporin-1
  - sense primer CTTCGTCCTCAGCATCG
  - antisense primer TGAGCACAAGTGATGACC
- aquaporin-4
  - sense primer ATGGTGCTTCTAAGGCGT
  - anti-sense primer GAAGACAGACTTGGCGATGC

The initial denaturation step was conducted at 94°C for 5 min. The temperature profile of the PCR was 30 cycles at 94°C for 1 minute, 58°C for 2 minutes, and 72°C for 5 minutes. Values of aquaporin mRNA were normalized to corresponding values of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA—namely, aquaporin mRNA/GAPDH mRNA band density (7).

**Statistical analysis**

Values are expressed as mean ± standard error of mean of the experiments. Comparisons among groups were made using the Student t-test for unpaired data, with p < 0.05 required for significance.

**Results**

**Effects of antihypertensive drugs on blood pressure**

The three antihypertensive drugs—benazepril, CS866, and amlodipine—reduced both systolic and diastolic blood pressure significantly (Figure 1). No significant differences were observed among the drugs. Amlodipine and benazepril increased the heart rate significantly, but CS866 did not.

**Effects of antihypertensive drugs on the diameter of the vessels**

Administration of either benazepril or CS866 produced a significant dilator effect on the vessels (p <
Amlodipine did not dilate the vessels.

Effects of antihypertensive drugs on aquaporin-1 and aquaporin-4 of the peritoneum
Benazepril and CS866 both significantly reduced aquaporin-1 and aquaporin-4. Amlodipine induced a significant increase in aquaporin-1, but not in aquaporin-4 (Figure 3).

Discussion
The present study demonstrated that, in dogs with renovascular hypertension, ACEi and ARB produced vasodilation of the small vessels of the peritoneum.
and reduced the generation of aquaporins of the peritoneum. In comparison, amlodipine did not produce dilation of the vessels and increased the expression of aquaporin mRNA.

Blood flow to the visceral peritoneum derives predominantly from the mesenteric circulation. About 60% of the peritoneal surface can be ascribed to the mesentery of the esophagorectal viscera, with nearly 15% covering the liver and approximately 15% being parietal (8). The parietal peritoneum is perfused by vasculature of the abdominal wall. Mesenteric blood flow rates average about 10% of the cardiac output, that is, 40 mL/min/100g (9). The effective blood flow rate to the human peritoneum averages 60 – 100 mL/min (10). When mesenteric blood flow is doubled, clearances of small solutes such as urea increase by 30% – 50% (11), consistent with a resting blood flow that exceeds the maximal rate at which the capillary diffusion capacity can completely clear the perfusing blood. The suggestion is, therefore, that administration of antihypertensive drugs will produce alterations of solute transport.

Several investigators have already evaluated the effects of calcium channel antagonists on peritoneal mass transport. In the anesthetized rat model, verapamil and diltiazem given locally increased modestly but significantly the peritoneal clearance of urea without enhancing protein loss (3). On the other hand, modest increases in urea clearance and glucose absorption and a marked exaggeration of protein loss followed intraperitoneal instillation of very large doses of captopril, an ACEi, in rats (12). Those increments, despite drug-induced systemic hypotension, may reflect increased blood flow, surface area, or permeability. Similarly, transport rates for glucose, creatinine, and β2-microglobulin were meaningfully increased after oral administration of hypotensive doses of enalapril in a study performed in CAPD patients (2). Yet similar doses of oral captopril significantly reduced peritoneal protein loss in diabetic CAPD patients, with only a small decrease in mean blood pressure (13). Whether that change in peritoneal permeability reflects the blockade of a baseline level of angiotensin II activity or the action of the drug on kinins is uncertain.

Rippe et al. (14), using computer simulations, first suggested that peritoneal membrane permeability is best described by a three-pore model. Large pores allow the transport of macromolecules and—

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...together with the small pores—the transport of low molecular weight solutes such as glucose, urea, and creatinine. An ultrasmall, water-only pore has been postulated to be selective for water and to be responsible for most osmotically induced water transport. During peritoneal dialysis, many physiologic and pharmacologic factors have been known to contribute to the regulation of solute transport and ultrafiltration. Moreover, drugs are known to alter peritoneal transport of solutes and water (1). Increasing blood flow to the peritoneum accelerates the rate of solute delivery to the membrane, augmenting transport of small, highly diffusible solutes (1). That finding suggests an increase in peritoneal surface area or permeability resulting from vasodilation, attributed to dilation of the functional peritoneal capillaries combined with perfusion of more capillaries. Apparently, then, many vasoactive drugs can affect peritoneal transport rates.

Although the extent to which baseline levels of angiotensin II contribute to peritoneal transport rates in CAPD patients is uncertain, a limited number of studies provided data indicating that ACE inhibitors increase peritoneal transport rates (2,12). Therefore the suggestion is that blockade of the renin–angiotensin system produces an increase in peritoneal transport of solutes in CAPD patients.

Recent evidence suggests that the function of the ultrasmall pores depends mainly on aquaporin in the peritoneal mesothelial cells. Aquaporin is not only constitutively expressed, but it can also readily be upregulated on exposure to osmotic agents. Few data have been generated regarding the relationship between vasoactive substances and expression of aquaporin in peritoneal mesothelial cells (or other tissues). Moreover, how osmotic agents stimulate aquaporin remains unresolved.

There are several possible explanations for why the blockade of angiotensin II binding on its specific receptor with ARBn increases net ultrafiltration. In general, in CAPD patients, the activity of the renin–angiotensin system is suppressed, and ACE inhibitors and ARBs seem to play a lesser role. However, blood pressure was markedly reduced with ARB, indicating the possibility that the tissue renin–angiotensin system was activated instead of circulating angiotensin II. If such activation occurs in the peritoneum, the mesenteric blood flow increases, leading to increases in the surface area and number of capillar-
ies. Then, capillary pressure decreases, and the osmotic pressure produced by the glucose peritoneal dialysate prevails. The newly produced gradient stimulates aquaporin, producing an increase in ultrafiltration. Second, the combined effect of reduced lymphatic reabsorption and of reduction in capillary pressure elevates net ultrafiltration.

Conclusion
The kinetics of solute and fluid removal from the peritoneal cavity during peritoneal dialysis has been studied extensively, yet the precise transport pathways remain incompletely understood. Moreover, the effects of antihypertensive drugs on solute and fluid removal have been less investigated in CAPD patients. The results of the present study suggest that the effects of antihypertensive drugs might be important for regulating both the cardiovascular system and peritoneal function. Studies looking at those effects would be needed for expansion of a CAPD program in the future.

The data from the present study indicate that blockade of the renin–angiotensin system may be applicable as therapy for hypertensive patients on CAPD.

References
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Antihypertensive Drugs and Peritoneal Vessels

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