Standard glucose-based peritoneal dialysis (PD) solutions have unfavorable effects on the peritoneum and contribute to metabolic abnormalities. A PD regimen in which solutions with an alternative osmotic agent (icodextrin, amino acids) and solutions with a bicarbonate/lactate buffer are combined may reduce those effects.

In a prospective crossover study, we randomized new continuous ambulatory peritoneal dialysis (CAPD) patients to one of two groups. One group used 4 exchanges of standard PD (SPD) solution (Dianeal: Baxter Healthcare BV, Utrecht, Netherlands) daily. The second group used 1 exchange of Nutrineal (Baxter Healthcare BV), 1 exchange of Extraneal (Baxter Healthcare BV), and 2 exchanges of Physioneal (Baxter Healthcare BV) daily (NEPP). After 30 weeks of treatment, each group switched over to the other regimen for 24 weeks. Statistical analysis used analysis of variance (ANOVA) for repeated measurements.

Of the 74 patients enrolled into the study, 50 completed the full study period (24 NEPP–SPD, 26 SPD–NEPP). With regard to daily ultrafiltration and dialysis efficacy (Kt/V), the NEPP regimen was as efficacious as the standard regimen. The NEPP regimen was found to be safe: body weight, blood pressure, decline in urine volume, residual creatinine clearance, and laboratory measurements did not differ statistically significantly from those measured in the standard regimen. The NEPP regimen was found to be safe: body weight, blood pressure, decline in urine volume, residual creatinine clearance, and laboratory measurements did not differ statistically significantly from those measured in the standard regimen. The NEPP regimen was well tolerated and was not accompanied by serious side effects. During the NEPP regimen, bicarbonate was found to be significantly higher in both groups. The NEPP regimen is a feasible treatment schedule for patients starting CAPD.

Clinical Effects of a Peritoneal Dialysis Regimen Low in Glucose in New Peritoneal Dialysis Patients: A Randomized Crossover Study

Key words
Amino acids, bicarbonate buffer, icodextrin, peritoneal dialysis solutions

Introduction
Peritoneal dialysis (PD) has become a well established renal replacement modality for patients with end-stage renal disease (ESRD). However, after 8 years of therapy, fewer than 5% of patients continue with PD (1). Drop-out from PD therapy is, for the most part, attributable to technique failure (2). Although ultrafiltration (UF) failure, the main cause of technique failure, can develop during the early course of treatment, its prevalence increases with time on PD (3).

Loss of UF is associated with exposure to hypertonic PD solutions (4) and with frequent episodes of peritonitis (5). In general, conventional PD solutions have several unfavorable characteristics (6) that contribute to damaging the peritoneum—for example, low pH, high lactate and high glucose levels (with associated hyperosmolarity), and presence of glucose degradation products (GDPs). In addition, absorption of glucose across the peritoneal membrane into the systemic circulation contributes to metabolic changes such as dyslipidemia, obesity, and protein malnutrition.

In an attempt to reduce unfavorable local and systemic effects of conventional PD solutions, alternative solutions with more physiologic characteristics have been developed. Glucose-based PD solutions with a combination of bicarbonate and lactate have the advantage of a more biocompatible buffer, neutral pH, and lower GDP content than do conventional PD solutions (7). Solutions that contain icodextrin or amino acids as the osmotic agent instead of glucose have the benefit of low GDP levels and absence of glucose. However, icodextrin can be used only once daily because it causes systemic accumulation of glu-
cose polymers and maltose, and it provides sustained UF only during a long dwell (8). Prescription of an amino-acid-based PD solution is limited to once or twice daily because of a slight increase in serum urea, a mild decrease in pH, and low UF potential (9).

A dialysis regimen composed of 1 exchange with icodextrin, 1 exchange with amino acids, and supplementary exchanges of glucose-containing bicarbonate/lactate–buffered fluids may possibly be the best available alternative regimen for minimizing glucose and GDP load. Our main aim in the present study was therefore to compare for efficacy, safety, and tolerability a conventional continuous ambulatory peritoneal dialysis (CAPD) regimen using glucose-containing, lactate-buffered solution with a regimen low in glucose and GDPs.

Patients and methods
From November 1999 until April 2003, we conducted an open-label, randomized, multicenter, crossover study that compared, in CAPD patients, an alternative regimen low in glucose [Nutrineal/Extraneal/Physioneal/Physioneal (NEPP): Baxter Healthcare BV, Utrecht, Netherlands] with a standard glucose-based CAPD regimen [Dianeal (SPD): Baxter Healthcare BV]. To be included in the study, patients had to be more than 17 years of age, in stable clinical condition with an estimated life expectancy of more than 1 year, and willing to comply with the study treatment schedule. Only incident PD patients were included. Women of childbearing potential were excluded unless taking adequate contraceptive precautions.

Patients were randomized to one of two treatment groups: NEPP–SPD or SPD–NEPP. During the first 6 weeks, which functioned as a stabilization period, patients were taught CAPD according to their allocated PD regimen: NEPP for the NEPP–SPD patients and SPD for the SPD–NEPP patients. Patients were then continuously treated using the allocated regimen for another 24 weeks. Subsequently, patients were switched to the alternative PD regimen: SPD for the NEPP–SPD patients, and NEPP for the SPD–NEPP patients. The patients were treated on the new regimen for an additional 24 weeks.

The NEPP regimen consisted of 2 exchanges of the glucose-containing, bicarbonate-buffered PD solution Physioneal, 1 exchange of the amino-acid solution Nutrineal, and 1 exchange of the icodextrin-based solution Extraneal for the overnight dwell. The SPD regimen consisted of 4 exchanges of the glucose-containing, lactate-buffered solution Dianeal. In cases of clinically inadequate UF, Dianeal or Physioneal exchanges of higher glucose concentration were prescribed, together with restriction of daily fluid intake. A maximum daily fluid intake of 500 mL plus the volume of 24-hour UF and urine output was advised. When the preceding measures were not sufficient to achieve adequate UF in the course of the NEPP regimen, the Nutrineal exchange could be replaced by a Physioneal exchange. In cases of failure to achieve a weekly Kt/V of 1.85, an increase in the dialysis prescription by increasing the dwell volume or adding an extra Physioneal or Dianeal exchange was recommended. If a patient developed hypersensitivity to Extraneal, that exchange could be replaced by a Physioneal exchange.

The ethics committees of the 4 participating hospitals (VU University Medical Center, Amsterdam; the Red Cross Hospital, The Hague; Leijenburg Hospital, The Hague; and Medical Center Alkmaar, Alkmaar, Netherlands) approved the study. Written informed consent was also obtained from all patients.

Clinical visits were made every 6 weeks. During each visit, clinical data such as blood pressure, body weight, adverse effects, and prescribed drugs were assessed, and fasting early-morning blood samples were taken. Every 12 weeks, 24-hour urine and dialysate samples were obtained. At home, patients kept a diary and recorded the glucose concentration and UF volume for every dwell.

Unless immediately assayed, blood, plasma, and dialysate samples were stored at –80°C until analysis. Routine laboratory hematology tests were performed using a Sysmex SE9000 analyzer (Sysmex UK, Milton Keynes, U.K.). Chemistry tests were performed using either a Vitros 950 (Ortho-Clinical Diagnostics, Beere, Belgium), Synchron LX 20 Pro (Beckman Coulter, Fullerton, CA, U.S.A.), or Hitachi 747 or 911 (Boehringer Mannheim, Mannheim, Germany) analyzer. Blood hemoglobin, sodium, potassium, calcium, phosphate, albumin, glucose, and lipids were measured at each visit. Values for low-density lipoprotein cholesterol were calculated according to the Friedewald formula (10), provided that triglyceride levels did not exceed 5 mmol/L. Patients with hyperlipidemia and patients who, because of logistics reasons, were unable to have blood samples drawn in a fasting state were excluded from the lipids analyses.
Statistics
Results are expressed as mean ± standard deviation. A per-protocol analysis was done. Changes during the first study period (first 6 weeks compared with all 30 weeks) and the second study period (first 30 weeks compared with all 54 weeks) have been tested within and between the groups using analysis of variance (ANOVA) for repeated measures.
Analyses were carried out using SPSS 11.0 for Windows (SPSS, Chicago, IL, U.S.A.). A p value < 0.05 was considered significant.

Results

Patients
Between November 1999 and January 2001, we enrolled 74 patients (39 NEPP–SPD, 35 SPD–NEPP) into the study. Of the 74 patients, 50 completed the 54-week study period (24 NEPP–SPD, 26 SPD–NEPP). Patient characteristics did not differ between the two groups at baseline (Table I).

Reasons for withdrawal from the two treatment groups (NEPP–SPD/SPD–NEPP) during the first study period were renal transplant (1/1), patient wish (2/2), death (1/2), peritonitis (1/2), low Kt/V (1/0), inability to perform CAPD (3/0), and somatic reasons (2/0). Reasons for withdrawal during the second study period were protocol violation (1/0), renal transplant (2/1), UF failure (1/0), and dyslipidemia (1/0).

Clinical effects of the NEPP regimen
During the two study periods, systolic and diastolic blood pressure did not change statistically significantly within the two groups, nor did blood pressure differ significantly between the two groups (Tables II and III). The use of antihypertensive medications declined, but not significantly, in both groups during both study periods [medications per day, first period: NEPP–SPD 2.8 ± 1.2 → 2.4 ± 1.2 (p = 0.3), SPD–NEPP 2.5 ± 1.3 → 2.0 ± 1.4 (p = 0.9); second period: NEPP–SPD 2.4 ± 1.2 → 2.2 ± 1.2 (p = 0.5), SPD–NEPP 2.0 ± 1.4 → 1.8 ± 1.5 (p = 0.6)]. Use of antihypertensive drugs did not differ between the groups.

In both study groups, patient weight increased during the SPD regimen; however, the change was significant only in patients treated with the SPD regimen during the second study period (NEPP–SPD). During the NEPP regimen, a significant decline in body weight occurred in the NEPP–SPD group; weight in the SPD–NEPP group was stable.

The 24-hour urine volume declined in both groups. Net UF volume increased significantly in the SPD–NEPP group during both study periods, but no significant change in net UF was found in the NEPP–SPD group. Overnight UF was significantly higher during the NEPP regimen than during the SPD regimen in both study groups.

As expected, the daily glucose prescription was significantly lower during the NEPP regimen than during the SPD regimen. Residual creatinine clearance declined during the entire study period in both study groups, but the decline was statistically significant only in the SPD–NEPP group during the first study period. Weekly Kt/V declined significantly during the second study period in both groups.

Laboratory measurements
No differences in routine laboratory measurements were found between the two groups (Tables IV and V). Analyzing diabetic and nondiabetic patient subgroups separately, no consistent effects of the two regimens were found with regard to glucose, HbA1c, or lipids. The use of lipid-lowering medications did not differ significantly between or within the groups.

Table I Patient characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>NEPP–SPD</th>
<th>SPD–NEPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients [n (M/F)]</td>
<td>24 (13/11)</td>
<td>26 (16/10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.4±16.1</td>
<td>56.9±12.8</td>
</tr>
<tr>
<td>Diabetic patients (n)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Previous hemodialysis (n)</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>159±33</td>
<td>159±20</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>90±15</td>
<td>90±15</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76±15</td>
<td>71±15</td>
</tr>
<tr>
<td>Urine volume (mL/24 h)</td>
<td>1124±602</td>
<td>1386±649</td>
</tr>
<tr>
<td>Residual CCr (mL/min)</td>
<td>5.5±3.9</td>
<td>6.7±3.6</td>
</tr>
<tr>
<td>Causes of renal failure (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrosclerosis</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Undetermined cause</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

NEPP = dialysis regimen consisting of single exchanges of Nutrineal, Extraneal, Physioneal, and Physioneal (Baxter Healthcare BV, Utrecht, Netherlands); SPD = dialysis regimen consisting of four exchanges of standard Dianeal (Baxter Healthcare BV); M/F = male/female; BP = blood pressure; CCr = creatinine clearance.
during the first study period [mean medications per day: NEPP–SPD 0.3 ± 0.4 → 0.3 ± 0.5 (p = 0.6); SPD–NEPP 0.5 ± 0.5 → 0.4 ± 0.5 (p = 0.4)]. During the second study period, use of lipid-lowering medications did not differ statistically significantly within the groups [mean medications per day: NEPP–SPD 0.3 ± 0.5 → 0.4 ± 0.5 (p = 0.1); SPD–NEPP 0.4 ± 0.5 → 0.4 ± 0.5 (p = 0.1)], but medication use was significantly different between the groups (p = 0.007).

**Peritonitis episodes**

During the study, 27 peritonitis episodes were recorded in the 74 participating patients.

In the NEPP–SPD group, 10 peritonitis episodes occurred: 7 during the NEPP period (including 3 suspected sterile episodes) and 3 during the SPD period.

Three episodes of peritonitis occurred in patients that discontinued the study. In 2 of those patients, peritonitis was the reason for discontinuation.

In SPD–NEPP patients, 17 peritonitis episodes occurred: 8 during the SPD period and 9 during the NEPP period (including 3 suspected sterile episodes). Four peritonitis episodes occurred in patients that discontinued the study. In 1 of those patients, the peritonitis episode was the reason for discontinuation.

**Adverse events**

Icodextrin had to be discontinued in 5 NEPP–SPD patients and 3 SPD–NEPP patients. The reasons were (NEPP–SPD/SPD–NEPP) allergic skin reaction (1/0), sterile peritonitis (3/3), and nausea (1/0). Amino-acid

**TABLE II Clinical effects, NEPP–SPD a**

<table>
<thead>
<tr>
<th></th>
<th>NEPP 6 weeks</th>
<th>NEPP 30 weeks</th>
<th>SPD 54 weeks</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>145±29.3</td>
<td>134±33</td>
<td>128±25</td>
<td>0.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>85±13</td>
<td>82±10</td>
<td>79±13</td>
<td>0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75±15</td>
<td>75±13</td>
<td>77±12</td>
<td>0.007</td>
</tr>
<tr>
<td>Urine volume (mL/24 h)</td>
<td>1101±585</td>
<td>894±532</td>
<td>719±616</td>
<td>0.9</td>
</tr>
<tr>
<td>Net UF (mL/24 h)</td>
<td>1198±692</td>
<td>1126±817</td>
<td>1054±569</td>
<td>0.8</td>
</tr>
<tr>
<td>Overnight UF (mL/night)</td>
<td>609±259</td>
<td>500±290</td>
<td>125±204</td>
<td>0.2</td>
</tr>
<tr>
<td>Daily glucose prescription (g/day)</td>
<td>79±34</td>
<td>99±76</td>
<td>167±40</td>
<td>0.4</td>
</tr>
<tr>
<td>Residual CCr (mL/min)</td>
<td>5.8±3.4</td>
<td>4.3±2.9</td>
<td>4.2±4.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Weekly Kt/V</td>
<td>2.1±0.6</td>
<td>2.0±0.7</td>
<td>1.7±0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*a All data are presented as mean ± standard deviation of the mean (analysis of variance for repeated measurements). NEPP = dialysis regimen consisting of single exchanges of Nutrineal, Extraneal, Physioneal, and Physioneal (Baxter Healthcare BV, Utrecht, Netherlands); SPD = dialysis regimen consisting of four exchanges of standard Dianeal (Baxter Healthcare BV). BP = blood pressure; UF = ultrafiltration; CCr = creatinine clearance.

**TABLE III Clinical effects, SPD–NEPP a**

<table>
<thead>
<tr>
<th></th>
<th>SPD 6 weeks</th>
<th>SPD 30 weeks</th>
<th>NEPP 54 weeks</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135±24</td>
<td>129±24</td>
<td>128±18</td>
<td>0.7</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83±12</td>
<td>78±15</td>
<td>79±12</td>
<td>0.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71±15</td>
<td>73±15</td>
<td>73±15</td>
<td>0.8</td>
</tr>
<tr>
<td>Urine volume (mL/24 h)</td>
<td>1368±649</td>
<td>855±680</td>
<td>704±493</td>
<td>0.1</td>
</tr>
<tr>
<td>Net UF (mL/24 h)</td>
<td>1038±626</td>
<td>1265±645</td>
<td>1555±664</td>
<td>0.002</td>
</tr>
<tr>
<td>Overnight UF (mL/night)</td>
<td>123±404</td>
<td>135±337</td>
<td>537±404</td>
<td>0.6</td>
</tr>
<tr>
<td>Daily glucose prescription (g/day)</td>
<td>148±44</td>
<td>158±43</td>
<td>96±50</td>
<td>0.3</td>
</tr>
<tr>
<td>Residual CCr (mL/min)</td>
<td>6.9±3.4</td>
<td>4.8±3.4</td>
<td>4.7±4.1</td>
<td>0.005</td>
</tr>
<tr>
<td>Weekly Kt/V</td>
<td>2.0±0.6</td>
<td>2.0±0.7</td>
<td>1.7±0.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*a All data are presented as mean ± standard deviation of the mean (analysis of variance for repeated measurements). SPD = dialysis regimen consisting of four exchanges of standard Dianeal (Baxter Healthcare BV, Utrecht, Netherlands); NEPP = dialysis regimen consisting of single exchanges of Nutrineal, Extraneal, Physioneal, and Physioneal (Baxter Healthcare BV). BP = blood pressure; UF = ultrafiltration; CCr = creatinine clearance.
solution was stopped in 1 NEPP–SPD patient because of skin eruptions, and in 1 SPD–NEPP patient because of low net UF.

**Discussion**

In the present study, we compared a low-glucose PD regimen with a conventional glucose-containing, lactate-buffered PD regimen for efficacy, safety, and tolerability.

**Efficacy**

With regard to daily UF and dialysis efficacy (Kt/V), the NEPP regimen was as efficacious as the standard regimen. The lower UF potential of the amino-acid...
solution was easily compensated by the higher UF during the long dwell with the icodextrin solution. Net UF was adequate as indicated by stable weight and blood pressure.

**Safety and tolerability**

The NEPP regimen has been found to be safe: body weight, blood pressure, decline in urine volume, residual creatinine clearance, and laboratory measurements did not differ statistically significantly from those recorded in patients on the standard regimen. During the NEPP regimen, body weight remained stable or even declined; during the SPD regimen, weight gain was seen. The most likely explanation for stable body weight during the NEPP regimen is the formation of less body fat because of the lower glucose load of the regimen (11). Another possible explanation for stable weight might be a better balanced fluid state during the NEPP regimen as a consequence of the higher UF rates in the nightly exchanges with icodextrin (12). However, the second explanation is not supported by better blood pressure control, as indicated by the similar blood pressure readings and number of antihypertensive drugs used during the NEPP regimen.

The NEPP regimen was well tolerated and was not accompanied by serious side effects. During the NEPP regimen, 2 patients experienced skin eruptions. In one patient, the skin eruptions were attributed to the use of the amino-acid solution; in the other patient, they were attributed to the icodextrin. Furthermore, in both groups, 3 patients had suspected episodes of sterile peritonitis during the NEPP regimen. Skin eruptions (13) and sterile peritonitis (14) have previously been reported during treatment with icodextrin. The occurrence of sterile peritonitis may possibly decline with recent changes by the manufacturer to the production process for icodextrin solution.

Decline in residual renal function was statistically significant only in the SPD–NEPP group during the first study period. The difference might be explained by the different PD regimens; however, no similar finding occurred during the second study period. It is therefore more conceivable that the decline seen in the SPD–NEPP group occurred because of a divergent cause in the natural decline of residual renal function. Alternatively, the decline may simply have resulted from the fact that, at the start of the study, residual renal function in the SPD–NEPP group was slightly higher than residual renal function in the NEPP–SPD group. Higher residual renal function is known to decline more steeply after initiation of dialysis.

No statistically significant differences in routine laboratory measurements were found between the NEPP and SPD regimens. Potassium and sodium tended to be lower during the NEPP regimen, which might be a result of dilution caused by the increased osmotic drive of the absorbed icodextrin polymers and their breakdown products in the systemic circulation (15). During the NEPP regimen, significantly higher bicarbonate was found in both groups. That finding may have been the result of the two exchanges with bicarbonate/lactate–buffered solution, as previously reported (16).

The lack of effect on lipids by the NEPP regimen—even when data for diabetic and nondiabetic patients were analyzed separately—may be explained by the small number of patients involved in the study. In addition, the use of lipid-lowering medications may have disturbed the expected relationship between the two regimens and patient lipid levels. Furthermore, the effects of icodextrin and amino-acid-based PD solutions are controversial: beneficial effects (17,18) and absence of effects (19,20) have both been reported.

**Conclusion**

The NEPP regimen is an efficacious, safe, and well tolerated treatment regimen for patients starting CAPD. Further analysis of the present study may reveal whether the NEPP regimen has advantages over a standard regimen with respect to unfavorable local and systemic consequences.

**Acknowledgments**

This work was supported by a grant from Baxter International (Netherlands) and the Dutch Kidney Foundation.

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Clinical Effects of a NEPP Regimen


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Conventional peritoneal dialysis solution (PDS) relaxes visceral and parietal peritoneal arterioles (microvessels) by unclear mechanisms. The present study was originally designed to investigate the mechanisms of PDS-mediated vascular reactivity. Surprisingly, our preliminary data indicated that PDS induces contraction in large vessels such as the aorta. That result contrasts with the relaxation observed in the microvasculature. We therefore extended the study to (1) determine the effect of PDS on the superior mesenteric artery (SMA), (2) confirm the PDS-induced contraction in the aorta, and (3) determine if a prostanoid and nitric oxide are involved in the observed PDS-induced vessel response.

Rat SMA rings with intact endothelium and aortic rings with and without endothelium were prepared and placed in baths filled with a non vasoactive physiologic salt solution (PSS), or with PSS plus mefenamic acid (MFA, a cyclo-oxygenase inhibitor), or PSS plus NG-monomethyl-L-arginine (L-NMMA, an inhibitor of nitric oxide synthase) under a force transducer. We recorded changes in tension throughout the protocols. After equilibration, the baths were filled with a conventional glucose-based PDS (Delflex 2.5%: Fresenius Medical Care, Bad Homburg, Germany) with and without MFA or L-NMMA for 30 minutes. The rings were then washed, contracted with phenylephrine, and relaxed with acetylcholine to verify the presence or absence of endothelium.

In both SMA and aorta, PDS induced contraction. That contraction was suppressed by MFA [SMA: 0.57 g vs. 0.13 g (± 0.035 g); aorta: 0.88 g vs. 0.27 g (± 0.035 g); p < 0.05 by analysis of variance (ANOVA)]. Aortic contraction induced by PDS was not altered by L-NMMA.

Conventional PDS induces contraction in large vessels, in contrast to its action of relaxation in microvessels. Vascular reactivity in large vessels involves the production of a constrictor prostanoid in the vascular smooth muscle. Peritoneal dialysis solutions do not induce NO in aortic endothelium. Peritoneal dialysis solution–induced, prostanoid-mediated contraction of smooth muscle may contribute to a worsening of hypertension and the premature uterine contractions observed in the rare cohort of pregnant uremic patients on peritoneal dialysis.

Key words
Peritoneal dialysis solution, vascular rings, vascular reactivity

Introduction
Conventional peritoneal dialysis solution (PDSs) dilates the visceral and parietal microvasculature by mechanisms that are possibly related to the hyperosmolality, low pH, and buffer anion system of the solution (1,2). Our recent intravital microscopy studies indicated that conventional PDS produces an instantaneous and sustained near-maximal vascular relaxation at all levels of the intestinal (visceral) microvasculature (3). That generalized vascular relaxation was independent of solution pH and similar in magnitude at all arteriolar levels (7 – 100 µm in diameter).

Recent intravital microscopy study (4) of rat mesenteric arteries (250 – 350 µm) has shown that conventional lactate-buffered PDS dilates mesenteric arteries at a magnitude similar to that observed in the cremaster muscle and the cecal and intestinal arterioles (1–3). However, no significant vascular reactivity was observed when mesenteric arteries were suffused with a pH-neutral, bicarbonate-buffered PDS with low glucose degradation products (4). Thus, the
authors concluded that the vasoactive components of the conventional PDS are the glucose degradation products and, to a lesser extent, the lactate buffer anion system (4).

The present study was originally designed to investigate the molecular mechanisms responsible for the vascular relaxation induced by conventional PDS. The experiments were conducted with vascular rings from the aorta and superior mesenteric artery, studied in a standard tissue bath.

Vascular ring studies have the advantage of using multiple rings from each animal, allowing for the study of multiple inhibitors in a paired manner, and such studies are considered a “gold standard” for exploring the molecular mechanisms involved in vascular control.

The results of our current studies were completely surprising. In contrast to the data found in the literature on conventional PDS–mediated visceral and parietal microvascular relaxation, our data show that PDS produces contraction in large arteries by a vascular smooth muscle–derived constrictor mechanism.

**Material and methods**

**General animal care**

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN, U.S.A.) were housed in facilities approved by the American Association for Accreditation of Laboratory Animal Care and were maintained on standard rat diet and water ad libitum for at least 1 week before use. All animal care and experimental procedures conformed to Principles of Laboratory Animal Care by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals by the U.S. National Academy of Science as published by the National Institutes of Health (publication number 80-23, revised 1987) and were approved in advance by the Institutional Animal Care and Use Committee of the University of Louisville and the Louisville Veterans Administration. Experiments were performed on rats (215 – 235 g) that had been fasted overnight.

**Tissue preparation**

**SUPERIOR MESENTERIC ARTERY RINGS**

Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg). The superior mesenteric artery (SMA) was exposed and cleaned of adherent tissue. The straight segment of the SMA from its aortic origin to its first branch to the intestine—a portion approximately 5 – 7 mm long—was harvested. A mid-portion of the segment was cut to make a pair of 2-mm rings. Two stainless steel wires (each 0.3 mm in diameter) were passed through the lumen of each ring, and closed on themselves to form two wire triangles. One wire triangle was attached to a fixed glass hook and the other wire triangle was attached to a force transducer (Micro-Med, Louisville, KY, U.S.A.). The transducer was connected to a microprocessor-based tissue force analyzer (Micro-Med).

Each SMA ring with its two triangle attachments was suspended in a 17-mL tissue bath filled with a physiologic salt solution (PSS: 118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄, 12.5 mmol/L NaHCO₃, and 11.1 mmol/L glucose). The PSS was maintained at 37°C and was bubbled with a 95% O₂/5% CO₂ mixture via a frit tube to yield a pH of 7.4.

**AORTIC RINGS**

Immediately following SMA harvest, the thoracic aorta was excised from each rat and was cleaned of adherent extracellular tissue. The response of the aortic segments closer to the aortic arch and closer to the diaphragm to several agonists differs from the response of the middle segments (Unpublished data). We therefore used only the middle 8 mm of the thoracic aorta in the present experiments. One half of this thoracic aorta segment was denuded of endothelium by passing a fine glass rod about the size of the inner diameter of the aorta to and fro once through the lumen (endothelium denuded group). The other half of the thoracic aorta segment was not so treated (endothelium intact group). The presence or absence of viable endothelium in the aortic rings was tested by endothelium-dependent acetylcholine (ACH) relaxation.

The endothelium-intact and endothelium-denuded segments of the aorta were each cut to produce a pair of 2-mm rings. The aortic rings were mounted in a tissue bath in the same manner as the SMA rings were.

**Experimental protocols**

**PROTOCOL 1: SUPERIOR MESENTERIC ARTERY RINGS**

The SMA rings were stretched under an initial tension (preload) of 0.5 g in baths filled with PSS. Mefe-
namic acid (MFA) was added to the solution bathing one of each pair of rings to give final concentration of 40 µmol/L. The MFA remained in the solution from that point until the end of the protocol. The other ring of the pair acted as a “no inhibitor” control, but was otherwise treated identically to the ring with the added inhibitor.

The preload was adjusted every 10 minutes for 45 minutes for equilibration of preload and inhibitor. After 45 minutes of equilibration, the PSS or PSS+MFA was replaced with PDS or PDS+MFA. The changes in force level resulting from exposure to the PDS were recorded for 30 minutes. The rings were then washed with PSS three times over 25 minutes to bring the ring tension down to the level of the original preload.

Next, the rings were contracted with 1.0 µmol/L phenylephrine (PHE) for 15 minutes and relaxed with 3.0 µmol/L ACH for 10 minutes to demonstrate ACH-induced, endothelium-dependent relaxation as evidence for a viable endothelium.

PROTOCOL 2: AORTIC RINGS
The aortic rings were stretched to a preload of 1.0 g in baths filled with PSS. Either MFA or NG-monomethyl-L-arginine (L-NMMA) was added to the solution bathing one of the paired rings to give a final concentration of either 40 µmol/L (MFA) or 60 µmol/L (L-NMMA). The relevant inhibitor remained in the solution from that point until the end of the protocol. The other ring of the pair acted as a “no inhibitor” control, but was otherwise treated identically to the ring with the added inhibitor.

After 45 minutes of equilibration of the tissues to preload and inhibitor, the PSS and PSS+MFA or PSS+L-NMMA was replaced with PDS and PDS+MFA or PDS+L-NMMA. The changes in force level resulting from exposure to the PDS were recorded for 30 minutes. The rings were then washed with PSS, contracted with PHE, and relaxed with ACH to demonstrate ACH-induced relaxation as evidence for the presence (in endothelium-intact rings) or absence (in endothelium-denuded rings) of viable endothelium.

Experimental groups
A total of 12 SMA rings (6 pairs) and 36 aortic rings (18 pairs) were used. The 6 adjacent pairs of endothelium-intact SMA rings, the 12 adjacent pairs of endothelium-intact aortic rings, and the 6 adjacent pairs of endothelium-denuded aortic rings were divided into 4 groups, as follows:

- 6 pairs of endothelium-intact SMA rings were studied with and without MFA. [One ring from each pair was treated with MFA in the bath; the other was a “no inhibitor” control (protocol 1).]
- 6 pairs of endothelium-intact aortic rings were studied with and without MFA. [One ring from each pair was treated with MFA in the bath; the other was a “no inhibitor” control (protocol 2).]
- 6 pairs of endothelium-denuded aortic rings were studied with and without MFA. [One ring from each pair was treated with MFA in the bath; the other was a “no inhibitor” control (protocol 2).]
- 6 pairs of endothelium-intact aortic rings were studied with and without L-NMMA. [One ring from each pair was treated with L-NMMA in the bath; the other was a “no inhibitor” control (protocol 2).]

Protocol 1 was designed to test (A) the response of SMA to PDS, and (B) the effect of cyclo-oxygenase inhibitor (MFA) on the PDS-induced vascular response in SMA.

Protocol 2 was designed to test (A) the response of aorta to PDS, (B) the effect of endothelium on PDS-induced vessel contraction, (C) whether the effect of MFA on PDS-induced vessel contraction of aortic rings depended on endothelium, and (D) the effect of L-NMMA on PDS-induced vessel contraction in endothelium-intact aortic rings.

Drugs
We obtained PHE [phenylephrine hydrochloride: 1-(5-oxohexyl)-3,7-dimethylxanthine], ACH (acetylcholine hydrobromide), MFA, and L-NMMA from Sigma Chemical (St. Louis, MO, U.S.A.). All drugs were dissolved in distilled water except for MFA, which was dissolved in a bicarbonate buffer. The PDS (Delflex 2.5%: Fresenius Medical Care, Bad Homburg, Germany) was a conventional glucose-based solution.

Data analysis
The maximal force of contraction ($F_{max}$ in grams) with exposure to PDS, the $F_{max}$ with exposure to PHE, and the $F_{max}$ with exposure to ACH were determined for each ring from computer-stored, digitized raw data.
The $F_{\text{max}}$ for PDS was used for statistical analysis. The $F_{\text{max}}$ for PHE (pre-contraction), and the maximal relaxation to ACH were used to calculate ACH relaxation as a percentage. Presence of viable endothelium was accepted at ACH-induced endothelium-dependent relaxation > 60%, and absence of viable endothelium was accepted at ACH-induced endothelium-dependent relaxation < 5%.

Statistics
All data are presented as mean ± standard error of the mean. To determine the effect of inhibitors (MFA or L-NMMA) and endothelium on PDS-induced contraction, the contraction force in the presence of PDS was compared using two-way ANOVA within each pair of adjacent rings. Statistical significance was accepted at $p < 0.05$.

Results

Endothelium denudation
In the endothelium-denuded aortic ring preparation, a fine glass rod (a hematocrit microtube) was used to remove the endothelium by gently passing the rod to and fro once through the lumen of the aorta. All endothelium-intact rings demonstrated > 60% relaxation (81% ± 3.19%) and all endothelium-denuded rings demonstrated < 5% relaxation (2.7% ± 1.66%) in the presence of ACH. All experiments were performed using the timeline and protocol depicted in Figure 1.

Effect of PDS on vessel tension
In SMA (Figure 2, left bar) and aorta (Figure 3, left bar) alike, PDS induced vessel contraction. Both vessel types started to contract immediately after the PSS in the bath was replaced with PDS. Contraction reached plateau in about 20 – 25 minutes. Upon wash-out with PSS, vessel contraction immediately subsided. The tension level returned to baseline preload level in about 15 – 20 minutes.

Effect of MFA and L-NMMA on PDS-induced vessel contraction
The cyclo-oxygenase inhibitor MFA suppressed PDS-induced vessel contraction by about 65% – 80% in SMA and aorta alike, and in both endothelium-intact and endothelium-denuded rings (Figures 2, 3, and 4).

The L-NMMA did not alter PDS-induced vessel contraction (endothelium-intact aorta, Figure 5).

Discussion
The results of the present study completely surprised us. In contrast to the consistent finding of a relaxing effect of conventional PDS on microcirculation, PDS predominantly constricted large arteries through the action of a vascular smooth muscle–derived constrictor prostanoid. A possible candidate for this prostanoid is thromboxane A2, a cyclo-oxygenase product that is the major component of the constrictor prostanoids and that often appears in smooth muscle contraction mechanisms. However, the mechanism by which PDS induces the production of this vascular smooth muscle–derived constrictor prostanoid remains unknown.
MFA (a cyclo-oxygenase inhibitor) did not completely eliminate the PDS-mediated vascular contraction, indicating that another vascular smooth muscle–derived constrictor pathway remains active.

The unexpected vascular reactivity that we found was independent of vascular endothelium, given that the presence or absence of endothelium had no effect on the observed constriction response. Furthermore, MFA (a cyclo-oxygenase inhibitor) did not completely eliminate the PDS-mediated vascular contraction, indicating that another vascular smooth muscle–derived constrictor pathway remains active.
It is conceivable that hyperosmolality might have altered calcium mobilization in vascular smooth muscle. Previous studies by Wang and colleagues showed that vascular smooth muscle cells cultured in high concentrations of D-glucose (or its stereoisomer L-glucose) or in mannitol exhibit abnormal intracellular calcium mobilization, indicating that the observed phenomenon can be attributed to hyperosmolality and that it is not specific for a particular osmotically active solute (5).

The reasons for the disparity in the vascular response by arteries and microvessels to conventional PDS are not clear. It is well established that vascular endothelium modulates the tone of the underlying vascular smooth muscle through a balance of vasodilation mediators [NO, endothelium-derived hyperpolarizing factor (EDHF), and prostaglandin I2] and vasoconstrictors (endoperoxides, thromboxane A2, superoxide anions, and endothelin-1). Furthermore, depending on the vascular bed, vessels of similar size appear to use different mechanisms for endothelium-dependent regulation of vascular tone (6). Similarly, the relative contributions of agonist-stimulated NO and EDHF to endothelium-dependent relaxation appear to vary between the sexes (7), with arteriole size within the same vascular bed (8), and between arterioles from different vascular beds (6,9,10). Microvessels and large arteries are therefore likely to vary with regard to the mechanisms of local control of vascular tone as well as in their response to vasoactive agents.

The vascular ring technique used in the present study is a useful tool for dissecting the molecular mechanisms of vessel response, and the technique is typically used in ex vivo tissue-bath experiments. The technique does not account for neurohumoral effects on large vessels, which may override any local control of vascular tone. From this reasoning, the constriction of large arteries in response to conventional PDS may have an insignificant effect on peritoneal dialysis efficacy, but a greater effect on blood pressure and contractility of the smooth muscle of the uterus.

Given that NO and prostanoids are known to be involved in contraction–relaxation mechanisms in many vessels, including aorta and microvessels, we tested whether NO and prostanoids are involved in the PDS-induced vessel response. The fact that the observed vascular response in the present study is NO-independent and that it is exclusively accounted for by a vascular smooth muscle–derived mechanism indicates that basal production or flow-mediated release of NO from the vascular endothelia of large arteries is not a major component in the mechanism of local control of vascular tone in those vessels. That finding contrasts with many in vivo and ex vivo studies that show suppression of NO production results in substantial vasoconstriction in arteriolar segments and that almost one half of the basal smooth muscle contractile state (as judged by vasodilation) can be suppressed by increased shear forces within the physiologic range (11–13). It is well established that, in vascular beds such as the intestine, small arteries and large arterioles—rather than the terminal microvasculature—are the major contributors to intestinal vascular regulation and organ perfusion. Thus, relaxation of those small vessels in response to exposure to conventional PDS is detrimental in the number of perfused capillaries and in the modulation of the effective capillary surface area available for exchange during peritoneal dialysis.

Conclusions
Conventional PDS induces contraction in large vessels, which contrasts with the relaxation seen in microvessels. This PDS-induced large-vessel constriction involves production of a constrictor prostanoid in vascular smooth muscle. Peritoneal dialysis solutions do not induce NO in aortic endothelium. The PDS-induced, prostanoid-mediated contraction of smooth muscle may contribute to worsening of hypertension and to the premature uterine contraction observed in the rare cohort of pregnant uremic patients on peritoneal dialysis.

References


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