Removal of the Protein-Bound Solutes Indican and P-Cresol Sulfate by Peritoneal Dialysis

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Background and objectives: Protein-bound solutes are poorly cleared by peritoneal dialysis. We examined the hypothesis that plasma concentrations of bound solutes would therefore rise as residual renal function is lost.

Design, setting, participants, & measurements: Clearances of urea indican and p-cresol sulfate were measured in peritoneal dialysis patients with and without residual function.

Results: In patients with residual function, protein binding restricted the peritoneal indican and p-cresol sulfate clearances to 0.3 ± 0.1 ml/min, as compared to the peritoneal urea clearance of 5.5 ± 1.1 ml/min. The urinary indican and p-cresol sulfate clearances of 2.7 ± 2.5 and 1.3 ± 1.0 ml/min were closer to the urinary urea clearance of 3.9 ± 2.2 ml/min, reflecting the superior ability of native kidney function to clear bound solutes. Urinary clearance thus provided the majority of the total indican and p-cresol sulfate clearances of 3.0 ± 2.5 and 1.6 ± 1.0 ml/min in patients with residual function but the minority of total urea clearance of 9.4 ± 2.2 ml/min. Loss of residual function lowered the total clearances for indican and p-cresol sulfate to 0.5 ± 0.2 and 0.4 ± 0.2 ml/min, whereas the urea clearance fell only slightly. However there was only a modest increase in the plasma indican level and no increase in the plasma p-cresol sulfate level in patients with no residual function because reduction in the daily removal of these solutes accompanied the reduction in their total clearance rates.

Conclusions: Reduction in the removal of indican and p-cresol sulfate kept plasma levels from rising markedly when residual function was lost.
rotary evaporation or vacuum drying and resuspension in water. Recoveries of indican and PCS were 95 ± 1% and 97 ± 1% when the compounds were added at a concentration of 0.02 mg/dl and 104 ± 3% and 99 ± 1% when the compounds were added at 0.2 mg/dl (n = 4). Stabilities of indican and PCS in dialysate were assessed by repeating the assay on samples that were maintained at 37°C for 4 h and on samples that were maintained at room temperature for 24 h. Values were 100 ± 1 and 99 ± 2% of original values for indican and PCS, respectively, at 37°C and 99 ± 1 and 97 ± 6% of the original values for indican and PCS at 24°C.

Additional studies were performed to ensure that the presence of protein in the dialysate did not affect the results. Indican 0.02 mg/dl and PCS 0.03 mg/dl were added to unused dialysate, and the solution was then assayed with and without addition of albumin (BSA; CalBiochem, San Diego, CA) at a concentration of 1 g/L. Values that were obtained after addition of BSA were 102 ± 3 and 100 ± 1% of values that were obtained before addition of BSA for indican and PCS, respectively (n = 3). Recoveries of indican and PCS added to urine at concentrations of 1 mg/dl were 101 ± 6 and 93 ± 3%, respectively (n = 4). Stabilities of indican and PCS in urine were assessed on urine samples (n = 4) that were maintained at 37°C for 4 h and on samples that were maintained at room temperature for 24 h. Values were 105 ± 3 and 99 ± 11% of original values for indican and PCS, respectively, at 37°C and 101 ± 5 and 98 ± 6% of the original values for indican and PCS at room temperature.

As described previously, the assay used for PCS also detects p-cresol down to a level of <0.01 mg/dl, and standards including reagent p-cresol as well as PCS were used for this study (5). No p-cresol was detected in plasma from the dialysis patients or the normal control subjects. Separate measurements confirmed that added p-cresol was recovered from spent dialysate after 24 h at 37°C (recovery 113 ± 11% of original values for indican and PCS, respectively (n = 4). They did not test whether p-cresol was recovered after concentration by vacuum drying, but peaks corresponding to p-cresol were noted in only three of 23 unconcentrated peritoneal dialysate samples, two of 11 hemodialysate samples, and 12 of 18 urine samples from PD patients. Assuming that these peaks represented p-cresol would have increased the total removal of p-cresol and PCS, expressed as mg/d PCS, by only 0 ± 1 mg/d in the PD patients with no residual renal function, 1 ± 2 mg/d in the PD patients with residual renal function, and 2 ± 6 mg/d in the HD patients.

Plasma, dialysate, and urine levels of urea nitrogen (ureaN) and creatinine and plasma albumin levels were measured by routine methods in the clinical laboratory. Dialysate albumin and total protein concentrations were measured using commercial kits (Kamiya Biomedical, Seattle, WA; and Bio-Rad, Hercules, CA). Dialytic clearances were calculated by dividing the solute that was removed in the 24-h dialysate collection by the plasma level. Values are expressed as the mean ± SD throughout. Statistical significance was evaluated by the unpaired t test or by ANOVA and the least significant difference as required.

Results

Table 1 summarizes the characteristics of the PD patients with residual function and without residual function. The two groups were similar in age and body size. There were more women and fewer patients with diabetes in the group with residual function, but these differences were NS. The serum albumin concentration and normalized protein nitrogen appearance were similar in the two groups. As expected, patients with residual function had been on dialysis for a shorter time. Their urine output averaged 0.9 ± 0.5 L/d. Despite a significantly lower 24-h drain volume, the patients with residual function had a higher total Kt/V for urea than the patients without residual function.

Results of clearance studies are summarized in Tables 2 and 3 and depicted in Figure 1. In patients with residual function, the blood urea nitrogen averaged 51 ± 15 mg/dl and the ureaN removal rate averaged 6.7 ± 2.1 g/d. The calculated dialytic clearance for urea was 5.5 ± 1.1 ml/min, and residual renal clearance was 3.9 ± 2.2 ml/min, yielding a total clearance of 9.4 ± 2.2 ml/min. In patients without residual function, the ureaN removal rate was nearly the same, averaging 6.5 ± 3.2 g/d. A reduction in the total clearance to 7.3 ± 1.7 ml/min despite an increase in the dialytic clearance was associated with a slightly higher average blood urea nitrogen of 61 ± 21 mg/dl. The dialysate-to-plasma (D/P) ratios for ureaN were similar between the two groups, averaging 0.7 ± 0.1 for those with residual function and 0.8 ± 0.1 for those without residual function. Creatinine followed the same pattern as urea. In patients with residual function, the serum creatinine averaged 7.7 ± 3.1 mg/dl and the creatinine removal rate averaged 1.1 ± 0.3 g/d. The calculated dialytic clearance was 3.3 ± 0.9 ml/min.

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<th>Table 1. Patient characteristics&lt;sup&gt;a&lt;/sup&gt;</th>
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<sup>a</sup>BSA, body surface area; PD, peritoneal dialysis; nPNA, normalized protein nitrogen appearance.

<sup>b</sup><i>p < 0.05</i> RRF versus NRRF.
and the residual renal clearance was 8.1 ± 4.5 ml/min, yielding a total clearance of 11.4 ± 4.3 ml/min. In patients without residual function, the creatinine removal rate was nearly the same, averaging 1.0 ± 0.4 g/d. Reduction in the total clearance to 4.9 ± 1.2 ml/min despite an increase in the dialytic clearance was associated with a higher plasma creatinine level of 13.3 ± 2.9 mg/dl. The D/P ratios for creatinine were again similar between the two groups, averaging 0.5 ± 0.1 for those with residual function and 0.5 ± 0.1 for those without residual function.

Results for the protein-bound solutes indican and PCS, as summarized in Table 3, were different from those for urea and creatinine. In patients with residual function, plasma indican averaged 1.8 ± 1.3 mg/dl, 7 ± 3% of which was unbound, and the removal rate averaged 45 ± 17 mg/d. The dialytic clearance was 0.3 ± 0.1 ml/min, and residual renal clearance was 2.7 ± 2.5 ml/min, yielding a total clearance of 3.0 ± 2.5 ml/min. In patients without residual function, the removal rate of indican was much lower, averaging 25 ± 16 mg/d. As a result, the plasma indican level was only slightly higher at 3.4 ± 1.4 mg/dl despite a markedly lower total clearance of 0.5 ± 0.2 ml/min. The percentage of unbound indican remained nearly the same at 8 ± 2%, and the calculated D/P
ratios for unbound indican were similar between the two groups, averaging 0.6 ± 0.2 for those with residual function and 0.7 ± 0.2 for those without residual function.

Results for PCS were similar to those for indican. In patients with residual function, the plasma PCS averaged 2.7 ± 1.6 mg/dl, 5 ± 2% of which was unbound, and the removal rate averaged 34 ± 34 mg/dl. The dialytic clearance was 0.3 ± 0.1 ml/min, and residual renal clearance was 1.3 ± 1.0 ml/min, yielding a total clearance of 1.6 ± 1.0 ml/min. In patients without residual function, the removal rate for PCS was again much lower, averaging 14 ± 6 mg/dl. As a result, the plasma PCS level was nearly the same, averaging 2.6 ± 1.3 mg/dl, despite a four-fold lower total clearance, averaging 0.4 ± 0.2 ml/min. The percentage of unbound PCS was only slightly higher at 7 ± 2%, and the calculated D/P ratios for unbound PCS were similar between the two groups, averaging 0.7 ± 0.2 for patients with residual function and 0.7 ± 0.3 for patients without residual function.

The 24-h protein and albumin content of the spent dialysate averaged 5.2 ± 2.3 and 4.3 ± 1.6 g for patients with residual function and 4.7 ± 2.1 and 3.4 ± 1.4 g for patients without residual function. On the basis of these values, the contribution of albumin to the dialytic removal of the protein-bound solutes was estimated to be very small. The mean albumin concentration in the dialysate for the two groups was 0.4 ± 0.2 g/L, or approximately 1% of the plasma level. Assuming that the association constants are the same in the plasma and dialysate, we would estimate that <20% of the indican and PCS removed in the dialysate was bound to albumin.

Removal rates for ureaN, indican, and PCS were measured in 11 anuric HD patients and 15 normal individuals for comparison with the values that were obtained in the PD patients, as illustrated in Figure 2. The predialysis plasma concentrations of ureaN, indican, and PCS in the HD patients averaged 55 ± 8, 3.1 ± 0.9, and 4.5 ± 1.3, mg/dl, respectively. The ureaN removal rate of 6.9 ± 2.4 g/d in the HD patients was not different from the ureaN removal rate in the PD patients. The ureaN removal rate in each of the three groups of dialysis patients was less than the rate of 12.3 ± 3.0 g/d observed in the normal individuals. The pattern was different for the protein-bound solutes. The indican removal of 72 ± 26 mg/d in the HD patients was greater than the indican removal of 45 ± 17 mg/d in PD patients with residual function, which was in turn greater than the value of 25 ± 16 mg/d in PD patients without residual function. The average indican removal of 59 ± 25 mg/d in normal individuals was significantly higher than the indican removal in PD patients without residual function but not different from the indican removal in PD patients with residual function and HD patients. The pattern of PCS removal was similar to that of indican but with more pronounced differences between the groups of dialysis patients. The PCS removal of 87 ± 35 mg/d in HD patients was markedly greater than the PCS removal of 49 ± 34 mg/d in PD patients with residual function, which in turn was greater than the value of 14 ± 6 mg/d in PD patients without residual function. The average PCS removal of 74 ± 42 mg/d in normal individuals was significantly higher than the PCS removal in PD patients without residual function but not significantly different from the PCS removal in PD patients with residual function and HD patients. Because ureaN removal was similar in the three groups of dialysis patients, the relation of indican and PCS production among these groups was not affected when solute production rates were factored by urea nitrogen removal. When expressed in this manner, however, indican and PCS production rates in the normal individuals were significantly below the values observed in HD patients and not significantly different from the values observed in PD patients (Figure 2).

**Discussion**

The native kidney clears many protein-bound solutes by secretion. Dialysis provides limited clearance of such solutes because only the unbound portion in the plasma contributes to the gradient driving diffusion into the dialysate (2,5,6,9,10). During HD, use of a high dialysate flow maximizes the gradient that drives diffusion, and increases the clearance of bound solutes (10). The clearance of bound solutes during contemporary HD, although still limited, can thus exceed their free fractions multiplied by clearance values for unbound solutes of the same size (5,10).

The relation of bound to unbound solute clearances during PD is different from that during HD. Bammens et al. (7) first described peritoneal p-cresol clearances averaging only 4.8 L/wk in a group of patients whose peritoneal creatinine clearance averaged 45 L/wk. A subsequent study confirmed that peritoneal clearances for p-cresol averaged only one tenth of peritoneal clearances for creatinine in a separate group of patients (8). The peritoneal clearance of p-cresol was not only very low in absolute terms but also lower relative to creatinine.
clearance than that observed by the same investigators during HD (6).

Peritoneal clearances of the two protein-bound solutes assessed in this study were similar to the peritoneal clearance of p-cresol reported by Bammens et al. (7,8). We found that the peritoneal clearances of indican and PCS averaged only 4.1 ± 1.8 and 3.3 ± 1.8 L/wk in patients whom peritoneal clearances of creatinine and urea averaged 41 ± 13 and 64 ± 17 L/wk, respectively. Measurement of the free as well as total plasma levels helped to explain the low clearance of the protein-bound solutes. The D/P free concentration ratios for indican and PCS were indistinguishable from the D/P concentration ratios for urea and slightly greater than the D/P concentration ratios for creatinine. This finding is compatible with the hypothesis that small bound solutes cross the peritoneal membrane as readily as unbound solutes but that only free solute is available for transport. It has been suggested that albumin leakage across the peritoneal membrane could contribute to the clearance of protein-bound solutes during PD (11,12).

Our calculations indicate, however, that loss of albumin in the dialysate does not contribute significantly to the clearance of solutes whose affinity for albumin is similar to that of indican and PCS.

The findings described suggest that the ratio of bound to unbound solute clearances obtained with PD cannot easily be increased. Volumes of dialysate and membrane mass transfer area coefficients are much lower for PD than for HD. By extending PD through much of the day, weekly peritoneal clearances of unbound solutes such as urea and creatinine can be made to approach the values obtained with conventional HD, but the increases in dialysate flow and membrane mass transfer area coefficient that are necessary to increase the diffusive clearance of bound relative to unbound solutes cannot be achieved with present peritoneal treatment.

As might be expected, residual renal clearance becomes relatively more important when dialytic clearance is low. Bammens et al. (7) included only patients with residual renal function in their original study of the clearance of p-cresol by PD. They found that residual function contributed more than two thirds of the total p-cresol clearance, although the residual GFR averaged only 4 ml/min. We obtained similar results in patients with residual renal function. The residual renal clearances of indican and PCS averaged 2.7 ± 2.5 and 1.3 ± 1.0 ml/min in patients whose residual GFR, estimated as the mean of the urea and creatinine clearances, averaged 6.0 ± 3.3 ml/min. The finding that the residual clearances of indican and PCS exceeded the GFR multiplied by the free solute fraction indicates that tubular secretion of these protein-bound solutes continues in the remnant kidney. On the basis of these results, we predicted that plasma levels of the protein-bound solutes would rise to high levels in PD patients without residual renal function and made an effort to include such patients in this study. The results were contrary to our expectation. We observed a marked reduction in the amount of the bound solutes removed from the body rather than the predicted large increases in plasma levels. The average plasma level of indican increased but not nearly in proportion to the reduction in clearance, and the average plasma level of PCS did not change, whereas the average clearance fell by 75%.

We did not identify a cause for the reduction in indican and PCS removal from the body in patients without residual function. An increase in extrarenal removal could explain the reduction in the amount of these solutes removed from PD patients without residual function, but in the case of PCS, we would have to assume that extrarenal removal was somehow triggered without any increase in the average plasma level. It is also possible that the remnant kidney is able to degrade these substances and continues to remove them from the circulation even when urine output has practically ceased. In general, however, removal rates for indican and PCS have been considered to reflect the production of these solutes, and we think it most likely that indican and PCS production were reduced in the PD patients who had lost residual function (13–15). Both indican and PCS are made in the colon, where bacteria act on tryptophan and on phenylalanine and tyrosine to produce indole and p-cresol, which are then conjugated with sulfate. The production of both compounds therefore depends on the delivery of amino acids to the colon and on the activity of colonic bacteria, which may ascend to the small bowel in uremic patients (16). Studies in normal individuals have shown that the production of p-cresol can be increased by dietary protein loading and medications that impair protein digestion and decreased by maneuvers that increase the delivery of carbohydrates and fiber to the colon (13,17–20), but we could not identify differences in protein intake, medications, or bowel history that would account for the lower indican and PCS removal observed in PD patients without residual renal function. Tracer studies may ultimately be required to identify the cause of reduced indican and PCS appearance in these patients.

It should be noted that most studies of protein-bound solutes in uremia have reported clearance values for p-cresol rather than PCS. It was recently shown, however, that the p-cresol circulates largely as PCS and that the previous detection of p-cresol was the result of inadvertent hydrolysis of the sulfate conjugate during sample preparation (5,21). We considered the possibility that reduced removal of indican and PCS in patients without residual renal function reflected impaired conjugation rather than reduced production of indole and p-cresol, but the very small amounts of p-cresol that we could detect in the urine and dialysate of the PD patients did not add significantly to the amount of p-cresol that was removed as PCS.

A final result of this study was the finding that indican and PCS removal was lower in both groups of PD patients than in a group of HD patients and that the removal rate for these solutes in the PD patients without residual function was less than their removal rate in normal individuals. An increase in the generation of p-cresol factored per gram of protein catabolism was previously documented in a mixed group of HD and PD patients and related to increased delivery of amino acids to colonic bacteria resulting from impaired assimilation of protein in the small intestine (22). In this study, rates of indican and PCS removal were significantly higher than normal when factored for protein catabolic rate in HD patients but not in PD patients. This suggests either that protein assimilation is less
impaired in PD patients or that some other factor limits amino acid conversion to indican and PCS in these patients. Because solute production rates were low, plasma levels for these solutes did not rise higher in PD patients than in HD patients even when residual function was lost. It should be noted that indican and PCS are not the only bound solutes that accumulate in uremia, although they are the most frequently studied (3). Poor dialytic clearance may cause plasma concentrations of some other bound solutes to reach high levels in PD patients without residual renal function, but further studies are required to address this question.

Acknowledgments
This study was supported by the National Institutes of Health (R33 DK071251), the VA Palo Alto Health Care System, and Satellite Research (Satellite Healthcare, Mountain View, CA) for assistance in recruiting participants. We are grateful to Dr. John Moran and Satellite Research (Satellite Healthcare, Mountain View, CA) for assistance in recruiting participants and provision of laboratory analyses.

Disclosures
None.

References