Removal of P-Cresol Sulfate by Hemodialysis

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Protein-bound solutes are poorly cleared by dialysis. Among the most extensively studied of these solutes is p-cresol, which has been shown to be toxic *in vitro*. This study examined the form in which p-cresol circulates and quantified its removal by hemodialysis. HPLC analysis of plasma from hemodialysis patients contained a peak whose mobility corresponded to synthetic p-cresol sulfate (PCS) but no detectable unconjugated p-cresol. Treatment with sulfatase resulted in recovery of this peak as p-cresol, confirming its identity. Subsequent studies compared the removal of PCS and another protein-bound solute, indican, to the removal of urea during clinical hemodialysis treatments. PCS and indican were $94 \pm 1\%$ and $93 \pm 2\%$ bound to plasma protein, respectively. Protein-binding caused a predictable decrease in measured dialytic clearance, which averaged 20 ± 4 ml/min for PCS and 25 ± 5 ml/min for indican as compared with 260 ± 20 ml/min for urea. Volumes of distribution for the protein-bound solutes were greater than the plasma volume, averaging 15 ± 7 L for PCS and 14 ± 3 L for indican as compared with 37 ± 7 for urea. Solute reduction ratios were $20 \pm 9\%$ for PCS, $30 \pm 7\%$ for indican, and $69 \pm 5\%$ for urea. We conclude that p-cresol circulates in the form of its sulfate conjugate, PCS. PCS is poorly removed by hemodialysis because its clearance is limited by protein binding and the ratio of its volume of distribution to its clearance is high.

The adequacy of hemodialysis is now assessed by measuring urea removal. But this practice is not based on evidence that urea causes uremic toxicity (1–3). It is widely recognized, moreover, that the behavior of urea during dialysis is not representative of the behavior of all uremic solutes (4). One class of solutes which do not behave like urea are those that bind to plasma proteins, particularly albumin (5,6). The normal kidney can clear such solutes by tubular secretion, but protein binding impairs their clearance during hemodialysis such that plasma levels in dialysis patients, when expressed as multiples of normal, often exceed levels of urea (4). Accumulation of such solutes could contribute to residual uremic toxicity when urea removal is adequate.

Among the most extensively studied of protein-bound solutes has been p-cresol. This compound is one of a large number of phenols derived from degradation of the amino acid phenylalanine and from phenol compounds in vegetable foods. Many phenols accumulate in uremia and there has been longstanding interest in their contribution to uremic toxicity (7–10). Interest has focused on p-cresol because its plasma levels are higher than those of most uremic solutes and because *in vitro* studies have shown that it has toxic effects (11–14). Recent studies have confirmed that protein binding limits the removal of p-cresol by dialysis and associated high p-cresol levels with poor outcomes in dialysis patients (15–17).

The goal of our study was to better understand the removal of p-cresol by hemodialysis. An initial, and unexpected, finding was that p-cresol is present in plasma largely in the form of its sulfate conjugate, p-cresol sulfate (PCS). We found that PCS, like p-cresol, binds avidly to plasma proteins. Our

results suggest that acid hydrolysis of PCS during deproteination of plasma samples has led to the reporting of high plasma p-cresol levels in previous studies of uremic patients. We therefore proceeded to measure the removal of PCS and another protein-bound solute, indican, by hemodialysis. The clearances of PCS and indican were much lower than the clearance of urea. We found further that the volumes of distribution for PCS and indican greatly exceed the plasma volume. Conventional hemodialysis is ineffective in reducing the plasma levels of these solutes because their volumes of distribution are large relative to their dialytic clearance rates.

Materials and Methods

Analytic Methods

PCS and indican in plasma and dialysate were assayed by HPLC using an Agilent 1100 Series system (Agilent, Palo Alto, CA). Plasma samples were deproteinized by the addition of 3 parts methanol to 1 part plasma. The free concentration was measured in plasma ultrafiltrates obtained using Microcon YM-30 separators (Millipore, Billerica, MA). Because concentrations of indican and PCS in dialysate are low, dialysate was concentrated eight-fold by rotary evaporation before assay by HPLC.

To develop a PCS assay, PCS was synthesized as described by Feigenbaum and Neuberg (18) and purified by recrystallization from ethanol. In this procedure, p-cresol dissolved in pyridine is converted to PCS by the slow addition of chlorosulfonic acid. PCS is crystallized out of the reaction mixture by addition of ethanol and then purified by recrystallization from 90% ethanol, 10% water. The identity of the synthetic compound was confirmed by electrospray ionization mass spectrometry (ESI-MS) as illustrated in Figure 1. Fluorescence spectra of synthetic PCS were then examined using a luminescence spectrometer (LS50B, Perkin-Elmer, Wellesley, MA). An HPLC assay using detection at the optimum fluorescence values of excitation (214 nm) and emission (306 nm) was performed at room temperature on a reverse phase C18 column of 4.6 mm X 15 cm. Buffer flow was 1 ml/min using methanol (A) and ammonium formate (50 mM, pH 4) (B) with a gradient from 35%A/65%B to 75%A/25%B over 15 min. Under these conditions, PCS appeared at 5.2 min and standard curves from aqueous synthetic PCS at 1, 2, 4, and 8 mg/dl processed like plasma samples had average $r2$ values of 0.9998 ± 0.0001. Recovery of PCS with the assay was $100 \pm 1\%$ of PCS added to normal plasma to achieve concentrations similar to those found in patients on dialysis (n = 4) and $100 \pm 6\%$ of PCS (n = 4) added to fresh dialysate to achieve concentrations similar to those found in spent dialysate. Repeat PCS assay of plasma from dialysis patients in the same HPLC assay yielded $94 \pm 7\%$ of the original value (n = 6) and repeat assay on plasma stored frozen at —80°C for 4 to 7 wk yielded $95 \pm 11\%$ of the original value (n = 6). Values for PCS concentration are given in mg/dl of PCS (mol wt, 188 daltons). P-cresol in the same assay appeared at 9.9 min. The sensitivity of this assay for p-cresol, as determined by adding reagent p-cresol to samples of plasma from normal subjects, was <0.01 mg/dl, with recovery of $101 \pm 7\%$ at 0.0125 mg/dl (n = 3).

Indican was measured with fluorescence detection using a protocol based on those of Niwa *et al.* (19) and Fagugli *et al.* (15), using excitation 295 nm and emission 390 nm. Buffer flow was 1 ml/min employing methanol (A) and ammonium formate (50 mM, pH 4) (B) with a gradient from 15%A/85%B to 80%A/20%B over 25 min. Under these conditions, indican appeared at 8.4 min and standard curves

from aqueous reagent indican at 0.25, 0.5, and 1.0 mg/dl and had average $r2$ values of 0.99999 ± 0.00001. Recovery with the assay was 100 ± 1% of indican added to normal plasma to achieve concentrations similar to those found in patients on dialysis (n = 4) and 100 ± 6% of indican (n = 4) added to fresh dialysate to achieve concentrations similar to those found in spent dialysate. Repeat assay of plasma from dialysis patients in the same HPLC assay yielded 99 ± 1% of the original value (n = 6) and repeat assay on plasma stored frozen at —80°C for 4 to 7 wk yielded 97 ± 10% of the original value (n = 6).

Methanol deproteinization was used as described above to avoid hydrolysis of PCS by acid. Additional samples were reassayed to assess the extent to which previously described acid precipitation methods convert PCS to p-cresol. Five predialysis samples were treated with HCl as described by De Smet *et al.* (20). To 150 µl of sample, 22.5 µl of 6N HCl was added, after which 150 mg of NaCl was added. Four predialysis samples were treated with H2SO4 as described by Bammens *et al.* (21). Two hundred microliters of serum were diluted with 180 µl of water and the pH was lowered to 1.0 by the addition of concentrated H2SO4, after which the mixture was heated to 90°C for 1 h. P-cresol was measured after ethylacetate extraction (1:1) of the acidified plasma mixtures and of the same predialysis plasma samples. The sensitivity of the plasma assay for p-cresol using ethyl acetate extraction, as determined by adding p-cresol to normal plasma, was <0.01 mg/dl plasma, with recovery of 113 ± 4% for 0.0125 mg/dl (n = 3).

Urea was measured using a commercial kit (#1770–50, ThermoDMA, Arlington, TX). Reagents including bovine P-glucuronidase type B-10, and sulfatase type H-1 powder from *Helix pomatia* (#S9626–10KU) were obtained from Sigma (St. Louis, MO).

Clinical Studies

Indican and PCS clearances were measured during the third session of the week in five stable outpatients (4 male/1 female) receiving hemodialysis three times weekly using Fresenius F70NR kidneys, which are not reused and have a surface area of 1.6^{m2} provided by polysulfone fibers. Solute levels were measured in plasma samples obtained pre- and postdialysis and in dialysate collected during the treatment (average 178 ± 12 L). The average ultrafiltration rate was 21 ± 6 ml/min. The immediate postdialysis blood sample was collected after reducing the arterial flow to 100 ml/min for 10 s. The arterial access was then left in place and an additional blood sample was obtained at 30 min postdialysis to assess rebound of solute concentrations. Urea levels were measured in simultaneous arterial and venous samples obtained at 30, 90, and 150 min for measurement of urea clearance. To determine values for the solute free fraction f, solute levels were measured in Microcon 30 ultrafiltrates of the plasma samples obtained pre- and postdialysis. The mean of these values was used in modeling the clearances of the protein-bound solutes. The volume of distribution for urea was calculated from the pre- and postplasma solute levels, the dialysate solute content, and the ultrafiltration rate using a single compartment variable-volume model (22). For the protein-bound solutes, however, a constant volume was assumed because the effect of ultrafiltration on volume of distribution cannot be predicted. Clearance values for urea, expressed in ml/min of plasma, were calculated from urea concentrations measured in the three simultaneous arterial and venous samples and from values for blood flow and

hematocrit using standard formulas. Clearance values for PCS and indican cannot be obtained from simultaneous arterial and venous concentrations because the concentration differences across the kidney are too small. Clearance values for these solutes were therefore obtained from solute removal as measured in the total dialysate collection and pre- and postdialysis plasma levels. Separate experiments were performed to confirm that the solute concentrations in Microcon 30 ultrafiltrates accurately represented the free concentration of solute available for diffusion across the kidney membrane. Three patients were shifted to ultrafiltration mode after 150 min and ultrafiltered at the rate of 1000 ml/h for 30 min. Samples of plasma and ultrafiltrate were then collected for determination of the solute free fractions. Solute free fractions obtained using ultrafiltrate obtained during dialysis (indican, $7 \pm 3\%$; PCS, $8 \pm 4\%$) were very close to those measured simultaneously using a Microcon 30 (indican, $7 \pm 2\%$; PCS $6 \pm 3\%$). Additional measurements showed that the Microcon filters did not absorb PCS (recovery in filtrate $99 \pm 1\%$).

The clearance values calculated from blood and dialysate solute content were compared with those predicted by a recently described model for the dialysis of protein-bound solutes (23,24). Input values for the model include the blood flow Qb, the dialysate flow Qd, the ultra-filtration rate Qf, hematocrit, the free solute fraction f, and the dialyzer mass transfer area coefficient K_oA for the solute of interest. K_oA values for indican and urea were measured in separate *in vitro* clearance experiments (n = 6). In these experiments, the solutes were dissolved in a buffered saline solution and dialyzed out of a 4-L reservoir using the F70NR dialyzer with reservoir fluid flow 400 ml/min and dialysate flow of 800 ml/min. Values for K_oA of 665 ml/min for indican and 731 ml/min for urea were obtained from measured solute clearance values using the equation derived by Michaels (25). The K_oA for PCS (mw 187) was assumed to be the same as that for indican (mol wt 213 daltons).

Samples were obtained from a larger group of chronic hemodialysis patients to assess the relative plasma concentrations of p-cresol and PCS. Samples were obtained immediately before the third treatment of the week in 19 patients who had been on dialysis for an average of 3.7 ± 3.6 yr. Plasma levels and 24-h urinary excretion rates for PCS and indican were measured in four normal adult subjects (1 female/3 male) for comparison with the values obtained in dialysis patients.

Values are expressed as the mean $\pm$ SD throughout.

Results

Initial studies assessed the relative abundance of p-cresol and PCS in the plasma of patients with ESRD. Assay using the PCS protocol described in the methods revealed an average PCS level of 3.9 ± 1.5 mg/dl and no detectable p-cresol.

To confirm that the compound measured in predialysis samples was PCS, samples from five patients were assayed before and after incubation for 24 h at 37°C with sulfatase type H-1 powder from *Helix pomatia* as described by Niwa *et al.* (8). Assay before sulfatase treatment revealed an average PCS concentration of 4.2 ± 1.0 mg/dl and no detectable p-cresol. After sulfatase treatment, the original PCS peak was no longer present, and a new p-cresol peak appeared, which corresponded to a recovery of $95 \pm 13\%$ of the original PCS peak as p-cresol (Figure 2).

To allow comparison of our results with those of other studies, additional samples were reassayed

using previously reported methods in which acid treatment has been used to free bound solutes from plasma proteins. Five predialysis samples in which the mean PCS level was 4.2 ± 1.0 mg/dl were deproteinized with 6N HCl as described by De Smet *et al.* (20). We found that acid treatment in this manner yielded a recovery of $44 \pm 12\%$ of the original PCS peak as p-cresol. Ethyl acetate extracts (ethyl acetate:plasma 2:1) of the same predialysis samples yielded no detectable p-cresol. In four additional predialysis samples, the pH was lowered to 1.0 by the addition of concentrated H_2SO_4 using the alternate procedure of Bammens *et al.* (21). Acid treatment in this manner yielded a recovery of $73 \pm 4\%$ of the original PCS peak as p-cresol, while again no p-cresol was detected by ethyl acetate extraction in the same samples.

The results described above indicated that p-cresol is present in the plasma of dialysis patients largely in the form of PCS. Further studies, therefore, assessed the dialytic removal of PCS. Clinical parameters of the patients studied are summarized in Table 1. Blood flow during the runs averaged 379 ± 21 ml/min as recorded by the machine. Measured dialysate flow was 804 ± 15 ml/min with the nominal dialysate flow set at 800 ml/min.

Results of clearance studies are summarized in Table 2. As expected, urea was removed effectively by hemodialysis. The predialysis concentration of 57 ± 10 mg/dl fell to 18 ± 4 mg/dl for a solute reduction ratio of 0.69 ± 0.05. Urea nitrogen removed as measured in the dialysate was 18.0 ± 2.1 x 10^3 mg, and the postdialysis urea volume of distribution calculated using a single compartment variable volume model was 37 ± 7 L. The urea clearance calculated using this model was 251 ± 33 ml/min while the urea clearance calculated from arterial-venous concentration differences measured across the kidney three times during each run was 260 ± 20 ml/min. These measured values were slightly lower than the clearance value of 303 ± 11 ml/min predicted from values for Qb, ^{Qd,} Qf, hematocrit, and K_oA.

Results for the protein-bound solutes PCS and indican were quite different from those for urea. The free fractions for both solutes declined during the course of dialysis, with the free faction of PCS falling from $7.8 \pm 1.3\%$ at the beginning of treatment to $4.7 \pm 1.4\%$ at the end of treatment, and the free fraction of indican falling from $7.6 \pm 1.5\%$ to $5.4 \pm 2.2\%$. Protein binding limited solute removal so that the solute reduction ratios for PCS and indican were only 0.27 ± 0.11 and 0.30 ± 0.07. Solute removal measured in the dialysate was 161 ± 62 mg for PCS and 119 ± 59 mg for indican. Volumes of distribution calculated from solute removal and plasma concentrations were similar for the two protein bound solutes, averaging 15 ± 7 L for PCS and 14 ± 3 L for indican. Calculated clearance values for the protein-bound solutes were on the order of one tenth of the clearance value for urea, averaging 20 ± 4 ml/min and 25 ± 5 ml/min, respectively. These clearance values, which were calculated from the measured solute removal and plasma concentrations, were again slightly lower than those predicted from values for f, KoA, $_{Qb,}$ ^{Qd,} Qf, and hematocrit (23,24).

Low clearance rates for the protein-bound solutes reflected limited removal of these solutes during passage of blood through the artificial kidney. Because removal of these solutes was limited, arterial-venous concentration differences were hard to detect. Moreover, ultrafiltration raises the plasma protein concentration as blood passes through the kidney. This tends to increase the concentration of protein-bound solutes in venous blood samples and conceal their removal by dialysis. In our study, in which the ultrafiltration rate averaged $9 \pm 2\%$ of the plasma flow rate, the arterial-venous concentration differences

averaged only —1 ± 1% for PCS and —2 ± 2% for indican as compared with —75 ± 3% for urea.

Measurements were made postdialysis to assess possible rebound of solute levels. The urea nitrogen rose from 18 ± 4 mg/dl at the end of treatment to 22 ± 5 mg/dl at 30 min posttreatment. The levels of PCS and indican, however, did not change significantly. The plasma albumin declined from 4.8 ± 0.7 at the end of dialysis to 4.6 ± 0.6 g/dl at 30 min postdialysis. Based on the assumption that this decline was caused by refilling of the vascular compartment, there was negligible movement of the protein-bound solutes into the plasma over 30 min postdialysis, with the calculated solute flux being only 2 ± 4 mg for PCS and 2 ± 2 mg for indican.

Plasma levels of PCS and indican were much lower in four normal subjects than in the dialysis patients, averaging 0.38 ± 0.33 mg/dl and 0.10 ± 0.04 mg/dl, respectively. Twenty-four– hour urine excretion in the normal subjects averaged 78 ± 34 mg for PCS and 69 ± 23 mg for indican.

Discussion

The first finding of this study was that p-cresol is present in the plasma in the form of PCS. We found, in the course of developing our assays, that the reagent p-cresol could be quantitatively recovered from normal plasma by extraction with the organic solvent ethyl acetate. But no p-cresol was found in ethyl acetate extracts of uremic plasma. Deproteination of uremic plasma with methanol, however, yielded a peak that corresponded to the peak seen when synthetic PCS was added to normal plasma. When uremic plasma was treated with sulfatase, this peak disappeared and new peak appeared which could be quantitatively accounted for by the deconjugation of PCS to p-cresol.

The finding that uremic plasma contains no detectable p-cresol is at variance with the results of many previous reports (4,8,13– 17,21). We believe this is because acid has been used to deproteinize plasma in these studies. Our results are in accord, however, with those of Wengle and Hellstro¨m (26,27), who first employed gas chromatography/mass spectroscopy to assay serum phenols. They reported that p-cresol does not circulate unconjugated but can be released by acid hydrolysis of a conjugate, which they presumed to be the sulfate. Variable hydrolysis of PCS during acid deproteination presumably accounts for some of the wide variability in p-cresol levels subsequently reported in dialysis patients. We found that a higher portion of PCS is recovered as p-cresol when deproteination is performed with sulfuric acid as described by Bammens *et al.* (21) than when deproteination is performed with hydrochloric acid as described by De Smet *et al.* (20). Consistent with this difference, the former group of investigators has reported higher average p-cresol levels in dialysis patients than the latter (14– 17,21).

The finding that p-cresol sulfate and not p-cresol accumulates in uremia is not surprising in light of our knowledge of the metabolism of other aromatic waste products. P-cresol is presumably converted to PCS in the liver, which performs numerous sulfation reactions (28,29). Indican, for example, is pro- duced by hepatic oxidation and sulfation of indole, which, like p-cresol, is made by gut bacteria. Sulfation generally increases the solubility and often increases the albumin binding of organic compounds. Sulfation may also decrease toxicity. In this regard, Vanholder *et al.* (12) have found that the respiratory burst activity of phagocytes is inhibited by p-cresol but not by PCS. It may be necessary to re-evaluate other reported *in vitro* toxicities of p-cresol in light of the current findings. The conclusion

that p-cresol measured in uremic plasma has derived from PCS would not, however, lessen the significance of the association of p-cresol levels with morbidity in dialysis patients (16,21).

Our results do not prove that PCS is the only form in which p-cresol circulates. Evidence has recently been presented that p-cresol infused into rats is excreted partly in the form of the glucuronide (30). Niwa *et al.* (31) found that indoxyl sulfate and indoxyl-glucuronide are both present in the serum of uremic humans, but that the sulfate greatly predominates. Niwa *et al.* (8) further detected p-cresol in ultrafiltrates of uremic plasma treated with glucuronidase, but in this study glucuronidase treatment was followed by acidification to pH 1 which may have hydrolyzed PCS. We could not detect p-cresol in uremic plasma which was incubated with glucuronidase without acid treatment (unpublished observations). Because we were not able to synthesize p-cresol–glucuronide, however, we could not test the recovery of p-cresol using this procedure, and our results must be regarded as inconclusive.

Having established that uremic plasma contains PCS and not p-cresol, we evaluated the removal of PCS during hemodialysis and compared it to the removal of indican. Our goal was to determine if we could account for the clearance of PCS *in vivo* based on its measured binding to plasma proteins. We found that our model for the clearance of protein-bound solutes predicted the relation of PCS and indican clearances to urea clearance with reasonable accuracy, but overestimated the clearance of each solute. We suspect that the model overestimates clearance largely because of errors in the input values for K_oA. Previous studies have shown that K_oA values *in vivo* may be 20% lower than values obtained *in vitro* during dialysis of saline solutions (32,33). Reduction of our *in vitro* K_oA values by 20% would bring the predicted values for the clearance of urea, indican, and PCS to 280 ± 10, 27 ± 7, and 26 ± 3 ml/min, respectively. Other factors could also contribute to the difference between observed and predicted clearance values. Of note, the observed clearances of the protein-bound solutes were greater than the values for Q_p multiplied by the solute free fractions. This means that solutes must dissociate from protein as blood passes through the artificial kidney. In modeling the clearance of protein-bound solutes, we have assumed that their binding to protein is rapidly reversible. Slower dissociation from protein would lower solute clearances below predicted values. In general, dissociation rates for bound solutes are fast in relation to the transit time of blood through artificial kidneys (34,35), but dissociation rates for PCS and indican have not been measured. Lastly, it should be noted that predicted clearance rates for protein-bound solutes are very sensitive to changes in the solute free fraction f. We found that values for f fell during the course of hemodialysis. This presumably reflects increased avidity of protein binding as the levels of competing bound solutes are reduced (36). In modeling the clearance of protein-bound solutes, we used the mean of the values for f obtained at the beginning and the end of treatment. Our ability to predict clearances might be improved if we profiled f more accurately.

A final aim of our study was to calculate volumes of distribution for the protein-bound solutes. We found that PCS and indican had volumes of distribution which were several times the estimated plasma volume. The high ratios of distribution volume to clearance account for the limited removal of these solutes during hemodialysis. Protein-bound solutes restricted to the plasma space would be much more effectively removed. For example, if PCS had a 3-L distribution volume and a clearance of 20 ml/min, its plasma level would fall by approximately 75% during a 3.5-h hemodialysis treatment. Instead, we observed that routine hemodialysis reduced PCS and indican levels by only 20 to 30%. Our results for

indican are similar to the original findings of Niwa *et al.* (6) and Lesaffer *et al.* (17).

An unanswered question is where solutes like PCS and indican are distributed outside the plasma. In normal humans, about 60% of the albumin in the body is located outside the vasculature, so that a substance which bound exclusively to albumin would have a distribution volume of about two and a half times the plasma volume (37). It seems unlikely that the fraction of albumin outside the vasculature is increased enough in dialysis patients to account for the distribution volumes we obtained for PCS and indican. Of note, distribution volumes for other solutes that bind extensively to albumin, such as furosemide, have often been found to exceed the estimated albumin space in normal subjects (38). We calculated volumes of distribution based on the assumption of a single compartment because we observed no rebound at 30 min posttreatment. Measurements over a longer interval after dialysis might reveal the existence of an additional, slowly equilibrating compartment.

An obvious question is how the removal of protein-bound solutes can be increased. Fagugli *et al.* (15) tested the effect of dividing the total weekly dialysis time into six rather than three sessions. They found that increasing treatment frequency only slightly reduced the predialysis levels of indican and p-cresol, which is consistent with the predicted behavior of solutes for which the ratio of distribution volume to clearance is high. Our model predicts that the removal of protein-bound solutes can also be increased by raising Qd and K_oA above conventional levels (23). The increase in solute removal may be limited, however, by a decline in solute free fractions, as bound solutes are removed during aggressive treatment. Theoretically, the clearance of protein-bound solutes could also be increased by chemical treatment to increase the solute free fraction while blood flows through the dialyzer or by various sorbent techniques (39,40). Further evidence that protein-bound solutes contribute to uremic toxicity would presumably stimulate efforts to apply such methods in practice.

Acknowledgments

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Table 1. Hemodialysis patient characteristics

	Mean ± SD	Range
Age, yr	55 ± 12	38 to 67
Duration on dialysis, yr	3.2	0.9 to 5.2
Dry weight, kg	73 ± 11	53 to 80
Length of dialysis session, min	218 ± 14	209 to 243
Q_b, ml/min	379 ± 21	347 to 401
Q_d, ml/min	804 ± 15	795 to 830

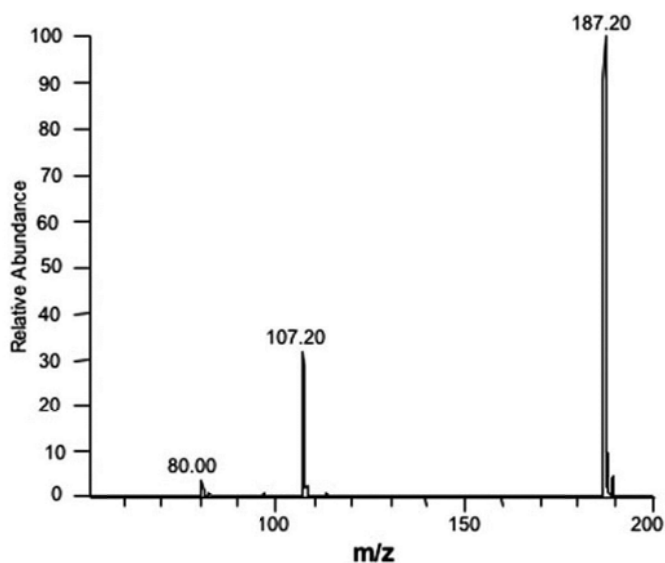Values are mean ± SD. Q_b indicates blood flow; Q_d, dialysate flow.

Figure 1. Mass spectrum of synthetic p-cresol sulfate (PCS) acquired in negative electrospray ionization mode, showing a dominant peak matching the predicted PCS anion with mol wt 187.2 daltons. The smaller peak at 107.2 m/z corresponds to a p-cresol anion, presumably generated by fragmentation of PCS during electrospray ionization mass spectrometry (ESI-MS). No p-cresol was detected in HPLC analysis of the same material (see Figure 2.)
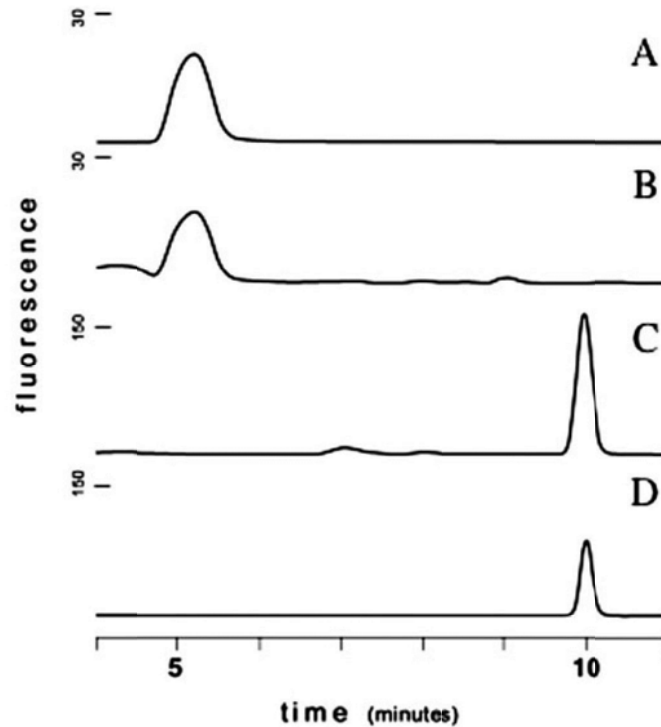
Figure 2. Elution of PCS and p-cresol by HPLC. (A) Elution of synthetic PCS. (B) PCS but not p-cresol is found in predialysis plasma from one of the study patients. (C) Loss of PCS after sulfatase treatment of the same plasma sample with appearance of a new peak. (D) The new peak corresponds to the elution of reagent p-cresol.

Table 2. Removal of urea and protein-bound solutes during hemodialysis

	Urea Nitrogen	PCS	Indican
Plasma level pretreatment, mg/dl	57 ± 10	4.3 ± 1.1	2.7 ± 1.3
Plasma level posttreatment, mg/dl	18 ± 4[a]	3.2 ± 1.2[a]	1.9 ± 0.8[a]
Plasma level 30 min posttreatment, mg/dl	22 ± 5[b]	3.1 ± 1.0	1.9 ± 0.9
Reduction ratio	0.69 ± 0.05	0.27 ± 0.11	0.30 ± 0.07
Unbound fraction pretreatment, %	—	7.8 ± 1.3	7.6 ± 1.5
Unbound fraction posttreatment, %	—	4.7 ± 1.4[a]	5.4 ± 2.2[a]
Unbound fraction 30 min posttreatment, %	—	5.3 ± 1.9	6.6 ± 3.0
Solute removed during dialysis, mg	$18.0 ± 2.1 \times 10^3$	161 ± 62	119 ± 59
Volume of distribution, L	37 ± 7	15 ± 7	14 ± 3
Measured clearance, ml/min	260 ± 20	20 ± 4	25 ± 5
Modeled clearance, ml/min	302 ± 11[c]	29 ± 3[c]	31 ± 8[c]

Values are mean ± SD. Values for indican and PCS in each case were not significantly different from each other but were significantly lower ($P < 0.05$, ANOVA and LSD) than values for urea nitrogen.

[a] $P < 0.05$ vs value pretreatment.

[b] $P < 0.05$ value 30 min posttreatment vs value posttreatment.

[c] $P < 0.05$ modeled vs measured.