

# The clearance of protein-bound solutes by hemofiltration and hemodiafiltration

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*Background.* Hemofiltration in the form of continuous venovenous hemofiltration (CVVH) is increasingly used to treat acute renal failure. Compared to hemodialysis, hemofiltration provides high clearances for large solutes but its effect on protein-bound solutes has been largely ignored.

*Methods.* Standard clinical systems were used to remove test solutes from a reservoir containing artificial plasma. Clearances of the protein-bound solutes phenol red ( $C_{PR}$ ) and indican ( $C_{IN}$ ) were compared to clearances of urea ( $C_{UREA}$ ) during hemofiltration and hemodiafiltration. A mathematical model was developed to predict clearances from values for plasma flow  $Q_p$ , dialysate flow  $Q_d$ , ultrafiltration rate  $Q_f$ , filter size and the extent of solute binding to albumin.

*Results.* When hemofiltration was performed with  $Q_p$  150 mL/min and  $Q_f$  17 mL/min, clearance values were  $C_{PR}$   $1.0 \pm 0.1$  mL/min;  $C_{IN}$   $3.7 \pm 0.5$  mL/min; and  $C_{UREA}$   $14 \pm 1$  mL/min. The clearance of the protein-bound solutes was approximately equal to the solute-free fraction multiplied by the ultrafiltration rate corrected for the effect of predilution. Addition of  $Q_d$  42 mL/min to provide HDF while  $Q_p$  remained 150 mL/min resulted in proportional increases in the clearance of protein-bound solutes and urea. In contrast, the clearance of protein-bound solutes relative to urea increased when hemodiafiltration was performed using a larger filter and increasing  $Q_d$  to 300 mL/min while  $Q_p$  was lowered to 50 mL/min. The pattern of observed results was accurately predicted by mathematical modeling.

*Conclusion.* In vitro measurements and mathematical modeling indicate that CVVH provides very limited clearance of protein-bound solutes. Continuous venous hemodiafiltration (CVVHDF) increases the clearance of protein-bound solutes relative to urea only when dialysate flow rate and filter size are increased above values now commonly employed.

Increasing molecular size limits solute transport by diffusion more than solute transport by convection. Henderson et al [1, 2] established that the clearance of solutes with size ranging from about 500 to 15,000 D can therefore be increased by employing hemofiltration instead of hemodialysis. Clinical studies, however, have so far failed to establish that hemofiltration is more beneficial than hemodialysis over the long term. Hemodialysis thus remains the predominant modality for end-stage renal disease (ESRD) treatment. Hemofiltration, however, is increasingly employed in the treatment of acute renal failure. In this setting, hemofiltration is usually prescribed as continuous venovenous hemofiltration (CVVH) or continuous venovenous hemodiafiltration (CVVHDF). It has been suggested, though not proven, that clearance of large solutes is particularly important when renal failure develops in patients with sepsis, shock, or other conditions which precipitate multiorgan failure [3, 4]. Because they are continuous therapies, CVVH and CVVHDF may also facilitate control of extracellular fluid volume in patients with acute renal failure, who often receive large amounts of intravenous fluid and have low blood pressure.

Remarkably, most studies of CVVH and CVVHDF have failed to consider the effect of these treatments on protein-bound solutes. The kidney clears many substances which are bound to plasma proteins and in particular to albumin. Such solutes accumulate in renal failure and there is increasing evidence that some of them are toxic [5–8]. Protein binding limits convective as well as diffusive transport of solutes across artificial kidney membranes. We have recently developed a model which describes the clearance of protein-bound solutes during hemodialysis [9]. The current study examined the clearance of protein-bound solutes during hemofiltration and hemodiafiltration. A mathematical model was developed to describe solute clearances and the predictions of this model were tested in vitro.

We found that small protein-bound solutes are poorly cleared by hemofiltration. In particular, our results indicate that CVVH may clear protein-bound solutes less effectively than intermittent hemodialysis and that the clearance of such solutes can be increased by adding dialysis to provide CVVHDF. Dialysate flow rates much higher than those conventionally used in CVVHDF, however, are required to make the clearance of protein-bound solutes approach the clearance of unbound solutes.

## METHODS

### Clearance measurements during *in vitro* CVVH and CVVHDF

**CVVH.** Clearances of phenol red, indican, urea, and creatinine were measured during CVVH *in vitro*. Fluid representing a patient's plasma was placed in a continuously stirred 1.0 L reservoir and CVVH was performed using a Prisma system (Gambro, Lakewood, CO, USA). The reservoir fluid contained bovine albumin (Sigma A-7906) (Sigma Chemical Co., St. Louis, MO, USA) at 4.0 g/dL and had electrolyte concentrations which approximated sodium 149 mEq/L, potassium 4.0 mEq/L, magnesium 2.0 mEq/L, calcium 2.5 mEq/L, and  $\text{PO}_4$  47.5 mg/dL with the pH adjusted to 7.4. Reagent phenol red, indican, urea, and creatinine were added to the reservoir to provide concentrations of approximately 3.0 mg/dL, 2.0 mg/dL, 120 mg/dL, and 12.0 mg/dL, respectively, at the beginning of each CVVH run. Replacement fluid contained electrolytes in the same concentrations as reservoir fluid but no albumin or test solutes. CVVH was performed over 150 minutes (four runs) in the predilution mode using a Prisma M60 Set which includes a 0.6 m<sup>2</sup> kidney composed of AN69 hollow fibers with wall thickness 50  $\mu\text{m}$ . The "plasma" (reservoir fluid) flow rate set at 150 mL/min and ultrafiltration and replacement fluid flow rates set equal at 16.7 mL/min. The ultrafiltrate volume was measured at the end of each experiment. The free solute fraction was calculated as the average of the solute concentration in the effluent line divided by the solute concentration in the reservoir in samples obtained at 10 and 150 minutes. Additional reservoir fluid samples were obtained at 0, 10, 30, 60, 90, and 120 minutes for clearance calculations.

**CVVHDF.** CVVHDF *in vitro* was first performed using the Prisma system with the same kidney and with the same reservoir and replacement fluids as used in CVVH experiments. The dialysate had the same composition as the replacement fluid. The dialysate flow rate was set at 41.7 mL/min while the plasma flow was again set at 150 mL/min and the ultrafiltration and replacement flow rates at 16.7 mL/min. The reservoir volume was increased

to 2.0 L and CVVHDF was performed over 75 minutes (four runs). The total volume of ultrafiltrate combined with dialysate was measured at the end of each experiment. Batches of albumin-containing fluid were purified by dialysis for 2 hours before test solutes were added for clearance experiments. This was done to reduce possible interference with solute binding to albumin by contaminants in the reagent albumin. The free fraction of each solute was calculated as the average of measurements made at 5 and 75 minutes. Microcon YM-30 tubes (Millipore, Billerica, MA, USA) were used to obtain ultrafiltrate from reservoir fluid for these measurements, since ultrafiltrate in the effluent line was mixed with dialysate. Additional reservoir fluid samples were obtained at 0, 5, 15, 30, 45, 60, and 75 minutes for clearance calculations.

To test the effects of a higher dialysate flow and larger kidney size, *in vitro* CVVHDF was performed using a Fresenius D machine and F6 kidney (Fresenius, Gurnee, IL, USA). The F6 kidney provides a 1.3 m<sup>2</sup> surface area composed of polysulfone hollow fibers with wall thickness 40  $\mu\text{m}$ . As such, it provides  $K_oA$  values for various solutes which are approximately threefold greater than those provided by the Prisma M60 set, as further described in the results below. The dialysate flow rate for CVVHDF experiments performed with the F6 was set at 300 mL/min while the plasma flow was set at 55 mL/min and the ultrafiltration and fluid replacement rates were again set at 16.7 mL/min. CVVHDF was performed over 75 minutes (four runs). Solute free fractions were measured as described above and samples were collected at 0, 15, 30, 45, 60, and 75 for clearance calculations.

**Chemical assays and clearance calculations.** Creatinine was measured with a Beckman Creatinine Analyzer 2 and urea was measured using a commercial kit (1770-50) (ThermoDMA, Arlington, TX, USA). Indican was measured by high-performance liquid chromatography (HPLC) (Agilent 1100) (Agilent, Palo Alto, CA, USA). Plasma samples were deproteinized by addition of 900  $\mu\text{L}$  of methanol to 300  $\mu\text{L}$  of plasma. This method which is derived from that of Lagana et al [10] provided indican recovery of  $102 \pm 2\%$  and phenol red recovery of  $108 \pm 7\%$  (four runs at 1.0 mg/dL and 1.5 mg/dL, respectively). Samples of the resulting supernatant were assayed by fluorescence detection (excitation 250 nm, emission 410 nm) following processing on a C18 column using gradients of 50 mmol/L ammonium formate and methanol as described by Lesaffer et al [11]. For CVVH and CVVHDF, phenol red was assayed using a method modified from Hirata-Dulas et al [12] as previously described [9]. For CVVHDF with higher dialysate flow, phenol red was measured by HPLC using the same protocol as for indoxyl sulfate but employing ultraviolet absorption at

433 nm rather than fluorescence for detection. Clearance values were calculated from the best fit slope of log values for concentrations and from measured reservoir volume corrected for the volume of samples removed. Clearance values for various treatments were compared by one-way analysis of variance (ANOVA) with post hoc least significant difference testing for multiple comparisons.

Values for  $K_{oA}$  for the M60 and F6 filters were determined in separate experiments in which dialysis was performed without albumin in the reservoir. In these experiments (three for each filter), the Fresenius D machine was used with the ultrafiltration rate set at zero. For the M60 filter,  $Q_p$  was set at 200 mL/min and  $Q_d$  at 300 mL/min and for the F6 filter,  $Q_p$  was set at 300 mL/min and  $Q_d$  at 500 mL/min. Clearances for urea, creatinine, indican, and phenol red were measured as the average of the arterial venous (A-V) extraction multiplied by  $Q_p$  measured four times over 30 minutes during each experiment.  $K_{oA}$  values for each solute were then obtained from measured values for clearance,  $Q_p$ , and  $Q_d$  using the equation described by Michaels [13].

### Modeling the effect of protein binding on solute clearance

*Hemofiltration.* The flux of a solute which is not protein-bound during hemofiltration can be expressed as:

$$J_s = J_v \cdot (1 - \sigma) \cdot C_p \cdot \gamma \quad (\text{equation 1})$$

where  $J_v$  is the volume flux,  $\sigma$  is the reflection coefficient for the solute,  $C_p$  is the solute concentration in plasma, and  $\gamma$  is a factor which relates the solute concentration in plasma to the solute concentration in plasma water  $C_{pw}$ :

$$C_{pw} = \gamma \cdot C_p \quad (\text{equation 2})$$

This correction factor can be estimated as:

$$\gamma = \frac{1}{1 - \theta} \quad (\text{equation 3})$$

where  $\theta$  is the plasma protein concentration in g/dL multiplied by 0.011 [14]. To model the clearance of protein-bound solutes, we introduced two modifications in equation 1. First, the model was limited to small solutes for which  $\sigma$  is effectively zero. For modern membranes, this includes solutes with size less than 2000 D, and thus includes almost all the protein-bound solutes which have been shown to accumulate in uremia [7, 15]. Second, it was assumed that only the portion of a solute not bound to protein is filtered. The free solute concentration  $C_{pf}$  available for filtration is thus represented by the following:

$$C_{pf} = f \cdot C_p \quad (\text{equation 4})$$

where  $f$  is the fraction of solute which is not bound to proteins. Solute flux can then be expressed as:

$$J_s = J_v \cdot f \cdot C_p \cdot \gamma \quad (\text{equation 5})$$

When hemofiltration is performed with replacement fluid added to the plasma before it enters the kidney, values for  $f$ ,  $C_p$ , and  $\gamma$  must be corrected for the effects of this ‘‘predilution.’’ The corrected solute concentration  $C_p'$  is:

$$C_p' = \frac{C_p \cdot Q_p}{Q_p + Q_r} \quad (\text{equation 6})$$

and the corrected plasma protein concentration  $\theta'$  used to correct  $\gamma$  is

$$\theta_p' = \frac{\theta_p \cdot Q_p}{Q_p + Q_r} \quad (\text{equation 7})$$

where  $Q_p$  is the plasma flow rate and  $Q_r$  is the replacement fluid addition rate. The effect of predilution on  $f$  is less obvious but while predilution reduces the total solute concentration and protein concentration in proportion, it tends to increase the free fraction of a protein-bound solute. The magnitude of this effect can be calculated assuming that solute binding to albumin (or any other binding protein) is described by an association constant  $K_A$  such that:

$$K_A = \frac{C_p - C_{pf}}{C_{pf} \cdot (C_{alb} - C_p + C_{pf})} \quad (\text{equation 8})$$

This being the case,  $f$  can be expressed as:

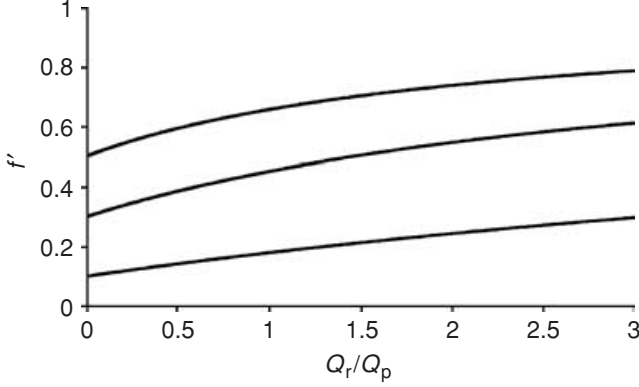
$$f = \frac{1}{1 + (C_{alb} - C_p + C_{pf}) \cdot K_A} \quad (\text{equation 9})$$

and the value  $f'$  corrected for predilution is then given by equation 10 (see **Appendix**).

The magnitude of this correction, which is hard to appreciate by inspection of equation 10, is illustrated in Figure 1. It should be noted that the application of equations 8 to 10 to dialysis systems is based on the assumption that solute binding to protein is rapidly reversible. In using equation 10 to correct  $f$  for the effect of predilution, we assume that solute can dissociate from protein in the time it takes for plasma to flow to the kidney from the point in the circuit where predilution fluid is added. As revealed by Figure 1, the magnitude of this correction is small for the  $Q_r/Q_p$  ratios used in our experiments.

During hemofiltration, the free solute concentration does not change as plasma passes along the kidney. Equation 5 therefore not only describes local values for flux, expressed as solute transport and fluid flow per unit length along the kidney, but also can be used to calculate the total transport. Solute clearance is then given by:

$$Cl = J_v \cdot f \cdot \gamma \quad (\text{equation 11})$$



**Fig. 1. The predicted effect of predilution with a replacement fluid flow rate ( $Q_r$ ) on the free fraction ( $f'$ ) of protein-bound solutes.** Curves are drawn for solutes whose free fractions are 0.10, 0.30, and 0.50 in plasma before the addition of replacement fluid.  $f'$  increases as  $Q_r$  increases relative to  $Q_p$ . In clinical practice with  $Q_r$  less than  $Q_p$ , the effect is modest but still large enough so that predilution causes less reduction in the clearance of protein-bound solutes than in the clearance of unbound solutes.

with appropriate substitution of  $f'$  and  $\gamma'$  if predilution is employed.

**Hemodiafiltration.** In hemodiafiltration, solute concentrations vary along the length of the kidney and local fluxes must be integrated to obtain total fluxes. For these calculations, the kidney was considered to have a dimensionless length of unity ( $0 \leq x \leq 1$ ) with the plasma (or reservoir fluid) inlet and dialysate outlet at  $x = 0$  and the plasma outlet and dialysate inlet at  $x = 1$ , providing countercurrent flow. The transfer of solute along an infinitesimal length of the kidney during hemofiltration with countercurrent flow dialysis must then satisfy the conservation of mass:

$$-J_s \cdot dx = d(Q_p C_p) = d(Q_d C_d) \quad (\text{equation 12})$$

and the transfer of fluid must satisfy the conservation of volume:

$$-J_v \cdot dx = dQ_p = dQ_d \quad (\text{equation 13})$$

where  $Q_p$  is the volumetric flow of plasma,  $Q_d$  is the volumetric flow of dialysate,  $C_p$  is the solute concentration in the plasma, and  $C_d$  is the solute concentration in the dialysate. The flux of an unbound solute being transported by both diffusion and convection can further be described by the equations 14 to 17 as developed by Villarreal, Klein, and Holland [16] and further elaborated by Waniewski et al [17]:

$$J_s = -k \cdot (C_{pw} - C_d) + J_v \cdot (1 - \sigma) \cdot \bar{C} \quad (\text{equation 14})$$

where  $k$  is the membrane permeability, here expressed per unit length along the kidney, and

$$\bar{C} = C_{pw} \cdot (1 - \phi) + C_d \cdot \phi \quad (\text{equation 15})$$

with

$$\phi = \frac{1}{\text{Pe}} - \frac{1}{\exp(\text{Pe}) - 1} \quad (\text{equation 16})$$

where  $\text{Pe}$ , the Peclet number, is a dimensionless quantity which represents the ratio of convective to diffusive transport and is given by:

$$\text{Pe} = \frac{(1 - \sigma) \cdot J_v}{k} \quad (\text{equation 17})$$

The same modifications used in the case of pure hemofiltration were again used to adapt these equations to describe the flux of protein-bound solutes. The model was restricted to small solutes for which sigma is effectively equal to zero and the effective solute concentration on the plasma side was assumed to be the unbound solute concentration:

$$C_{pf} = f \cdot C_p \quad (\text{equation 18})$$

When convection and diffusion are combined,  $f$  will vary along the length of the filter. The local value for  $f$  can be expressed as:

$$f_x = \frac{C_{p,x} - C_{alb,x} - \frac{1}{K_A} + \sqrt{\left(C_{alb,x} - C_{p,x} + \frac{1}{K_A}\right)^2 + \frac{4C_{p,x}}{K_A}}}{2C_{p,x}} \quad (\text{equation 19})$$

where  $K_A$  is the association constant described in equation 8 and where  $C_{p,x}$  is the local solute concentration and  $C_{alb,x}$  is the local albumin concentration which is in turn given by

$$C_{alb,x} = \frac{C_{alb,0} \cdot Q_{p,0}}{Q_{p,0} - \int_0^x J_v \cdot dx} \quad (\text{equation 20})$$

In applying equations 8 and 19, we again assume that solute binding to protein is rapidly reversible. Specifically, we assume that the time required for solute to dissociate from albumin is short in comparison to the time required for plasma to transit the artificial kidney. In the current study, the plasma transit time for experiments with the M60 kidney was approximately 20 seconds and the plasma transit time for experiments with the F6 kidney was approximately 60 seconds. We do not know the rate constants for the dissociation of phenol red and indican from albumin. Bilirubin, however, which is much more tightly bound, can dissociate from plasma albumin in a fraction of a second [18, 19]. Moreover, the blood transit time through the native kidney, which effectively removes

many protein bound solutes, is less than 5 seconds. We therefore modeled protein bound solute clearance based on the assumption of rapid dissociation. To the extent that this assumption is untrue, the real clearance of protein bound solutes will be less than that predicted by the model. In the extreme case where no solute dissociates from albumin as plasma transits the kidney, the clearance of a protein bound solute would be equal to the clearance of an unbound solute of the same size multiplied by  $f$ .

To determine solute transport using the above equations, we must first specify the profile of fluid transport along the kidney. The total fluid transport must add up to the ultrafiltration rate so that:

$$Q_f = \int_0^1 J_v \cdot dx \quad (\text{equation 21})$$

For the current model, we assumed that the transmembrane hydraulic pressure difference changes linearly along the kidney and that  $J_v$  is proportional to the local value for transmembrane hydraulic pressure,  $\Delta P_x$ . In this case, the local value for  $J_v$  is:

$$J_{v,x} = 2 \cdot Q_f \cdot \left[ \frac{\Delta P_0 \cdot (1 - x) + \Delta P_1 \cdot x}{\Delta P_0 - \Delta P_1} \right] \quad (\text{equation 22})$$

where  $\Delta P_0$  is the transmembrane pressure at the plasma inlet end of the filter and  $\Delta P_1$  is the transmembrane pressure at the plasma outlet end of the filter.

Using values for  $f_x$  provided by equations 19 to 22, equations 12 to 15 can be solved to yield total solute transport in terms of the boundary variables  $Q_{p,0}$ ,  $C_{p,0}$ ,  $C_{alb,0}$ ,  $Q_{d,1}$ , and  $Q_f$  and the constants  $K_A$  and  $K_oA$ . When predilution is employed, the value  $Q_r$  is also specified and the values:

$$C'_{p,0} = \frac{C_{p,0} \cdot Q_{p,0}}{Q_{p,0} + Q_r} \quad (\text{equation 23})$$

$$C'_{alb,0} = \frac{C_{alb,0} \cdot Q_{p,0}}{Q_{p,0} + Q_r} \quad (\text{equation 24})$$

and

$$Q'_{p,0} = Q_{p,0} + Q_r \quad (\text{equation 25})$$

are substituted for  $Q_{p,0}$ ,  $C_{p,0}$  and  $C_{alb,0}$ . For the present study,  $K_A$  was calculated from measured values for  $f$ ,  $C_p$ , and  $C_{alb}$  using equations 4 and 8. Predicted values for solute transport were obtained by solving the above equations using MATLAB (MathWorks, Natick, MA, USA). It should be noted that for the case of unbound solutes ( $K_A = 0$ ) and linear variation of  $J_v$  along the length of the filter, Waniewski et al [17] obtained an analytic solution to these equations. But we did not obtain an

**Table 1.** Solute clearances during continuous venovenous hemofiltration (CVVH)

Solute	Free fraction %	Measured clearance mL/min	Predicted clearance mL/min
Urea	100 ± 8	14 ± 1	16 ± 1
Creatinine	100 ± 3	14 ± 1	16 ± 1
Indican	25 ± 3 <sup>a</sup>	3.7 ± 0.5 <sup>a</sup>	4.3 ± 0.6 <sup>a</sup>
Phenol red	8.1 ± 1.0 <sup>a,b</sup>	1.0 ± 0.1 <sup>a,b</sup>	1.4 ± 0.2 <sup>a,b</sup>

Values are mean ± SD. Solute free fractions were assessed after predilution and clearance values were obtained with  $Q_p \approx 150$  mL/min,  $Q_r \approx 17$  mL/min, and  $Q_f \approx 17$  mL/min.

<sup>a</sup> $P < 0.05$  vs. value for urea; <sup>b</sup> $P < 0.05$  vs value for indican.

analytic solution which described the transport of protein-bound solutes. We are thus unable to provide a formula for clearance during CVVHDF analogous to the formula provided by equation 11 for CVVH or by the equations of Michaels [13] for hemodialysis using countercurrent flow.

Input values of the model described above include the transmembrane pressures  $\Delta P_0$  and  $\Delta P_1$  which are not routinely measured in clinical practice. In modeling clinical CVVHDF, however, the simplifying assumption of a uniform value for  $\Delta P$  along the length of the filter can be employed without introducing significant error. For instance, in a separate experiment in which an additional pressure transducer was added to the clinical system, we found that when CVVHDF was performed with the M60 at the flows used in this study,  $\Delta P_0$  was 88 mm Hg and  $\Delta P_1$  was 62 mm Hg. Use of a constant  $\Delta P$  value instead of these measured values produced changes in predicted solute clearance values of less than 1%. When CVVHDF was performed with the F6 and the flows used in this study, measured values for  $\Delta P_0$  and  $\Delta P_1$  were 17 mm Hg and was 15 mm Hg. Use of constant  $\Delta P$  values instead of these measured values again produced changes in predicted solute clearance values of less than 1%. In general, variation of  $\Delta P$  along the filter will affect clearances only when rapid ultrafiltration is combined with efficient dialysis, and the effect of  $\Delta P$  variation on clearance will diminish as solute binding increases. It should be noted that in such cases the pressure drop along the kidney may not be linear, as ultrafiltration simultaneously decreases blood flow and increases blood viscosity.

## RESULTS

Clearances measured during CVVH in vitro are summarized in Table 1. Clearance values for the unbound solutes urea and creatinine averaged  $14 \pm 1$  mL/min. Clearance values for the protein bound solutes were much lower, averaging  $3.7 \pm 0.5$  mL/min for indican and  $1.0 \pm 0.1$  mL/min for phenol red. The measured values

**Table 2.** Solute clearances during continuous venovenous hemodiafiltration (CVVHDF) with dialysate flow less than plasma flow

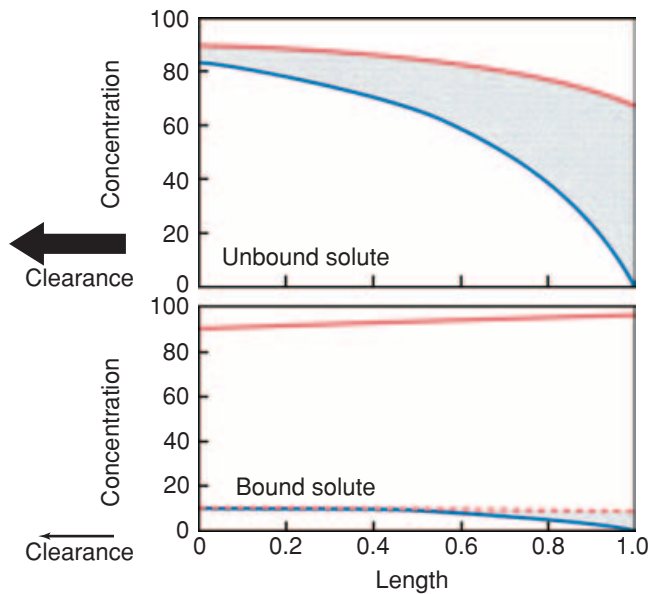
Solute	Free Fraction %	Measured clearance mL/min	Predicted clearance mL/min
Urea	100 ± 1	45 ± 2 <sup>a</sup>	52 ± 2
Creatinine	101 ± 1	42 ± 3 <sup>a</sup>	51 ± 2
Indican	18 ± 5 <sup>b</sup>	10 ± 2 <sup>a,b</sup>	9 ± 2 <sup>b</sup>
Phenol red	6.4 ± 0.9 <sup>b,c</sup>	2.7 ± 0.3 <sup>a,b,c</sup>	2.9 ± 0.4 <sup>b,c</sup>

Values are mean ± SD. Solute free fractions were assessed after predilution and clearance values were obtained using an M60 filter with  $Q_p \approx 150$  mL/min,  $Q_d \approx 42$  mL/min,  $Q_r \approx 17$  mL/min, and  $Q_f \approx 17$  mL/min.

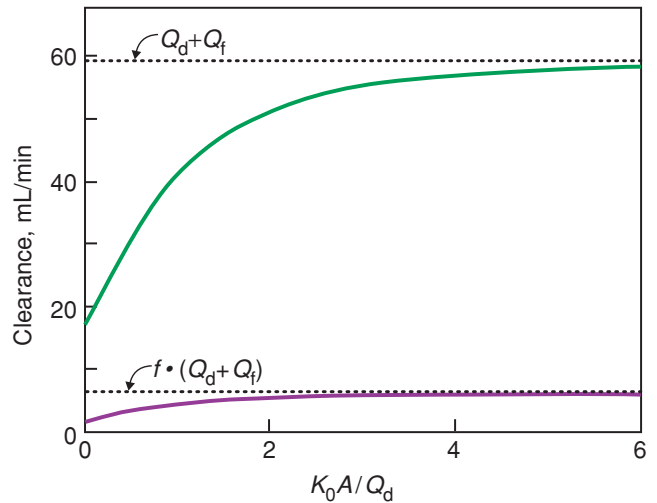
<sup>a</sup> $P < 0.05$  vs measured clearance during in vitro continuous venovenous hemofiltration (CVVH).

<sup>b</sup> $P < 0.05$  vs. value for urea.

<sup>c</sup> $P < 0.05$  vs. value for indican.



**Fig. 2.** Solute concentrations along the length of a dialyzer during continuous venovenous hemodiafiltration (CVVHDF) with  $Q_d < Q_p$ . In each panel, dialyzer length is on the horizontal axis with the plasma inlet and dialysate plus ultrafiltrate outlet at the left ( $x = 0$ ) and the plasma outlet and dialysate inlet at the right ( $x = 1$ ). Solute concentration is on the vertical axis. The total plasma solute concentration (red line) is set equal to 1.0 prior to predilution and the dialysate solute concentration (blue line) is set equal to 0.0 at the dialysate inlet. When there is protein binding, the concentration of unbound solute is depicted by the broken red line. The gradient driving diffusion is indicated by the shaded area. The top panel depicts the clearance of an unbound solute during CVVHDF performed with  $Q_p \approx 150$  mL/min,  $Q_f \approx 17$  mL/min, and  $Q_r \approx 17$  mL/min predilution,  $Q_d \approx 42$  mL/min, and  $K_0A \approx 150$  mL/min. The solute concentration in the slowly flowing dialysate plus ultrafiltrate stream approaches the solute concentration in the plasma entering the filter and the clearance is 49 mL/min. This is close to the theoretic maximum of 53 mL/min imposed by the sum of  $Q_d$  and  $Q_f$  corrected for the effect of predilution. The bottom panel depicts the clearance of a solute which is 90% bound to plasma protein. The solute concentration in the dialysate plus ultrafiltrate stream is limited by the free solute concentration in the plasma entering the filter and the clearance is 5.5 mL/min. The value is slightly higher than 10% of the unbound solute clearance because predilution increases the solute free fraction from 10% to 11%. The relative magnitude of the solute clearances is reflected by the size of the arrows on the left of the figure.

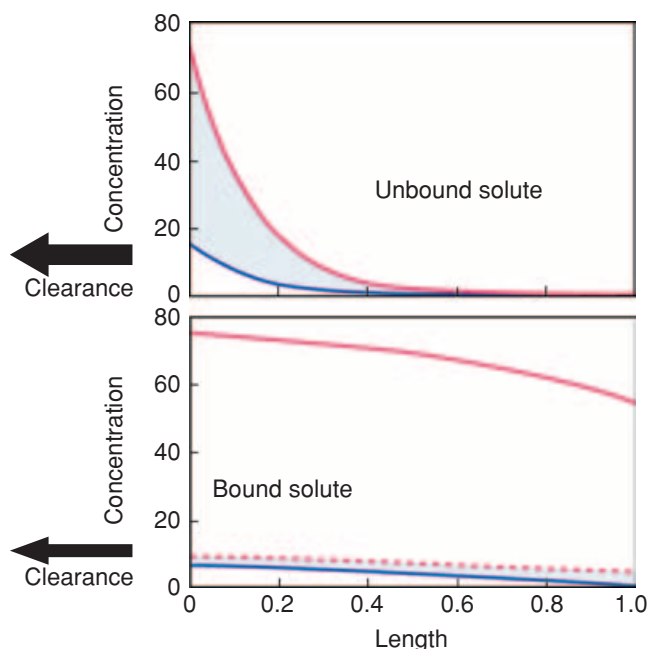


**Fig. 3.** The predicted effect of  $K_0A$  on solute clearances during continuous venovenous hemodiafiltration (CVVHDF) with  $Q_d < Q_p$ . The conditions represented are similar to those of our experiments with  $Q_d \approx 42$  mL/min and  $Q_f \approx 17$  mL/min except that the small effect of predilution is omitted for simplicity. As  $K_0A$  increases, clearance of an unbound solute (green line) approaches the theoretic maximum of  $Q_d$  plus  $Q_f$  and the clearance of a solute which is 90% protein bound (purple line) approaches the theoretic maximum of 10% of  $Q_d$  plus  $Q_f$ . Near maximal clearance values are obtained with small filters as long as  $Q_d$  is low.

were close to the values predicted by equation 11. Essentially, the predicted clearance rate for unbound solutes is the ultrafiltration rate corrected for the effect of predilution. The predicted clearance rate for bound solutes is obtained by multiplying this value by the plasma free fraction, with a further correction for the effect of predilution on the free fraction as described in the **Methods** section (equations 10 and 11) and Figure 1.

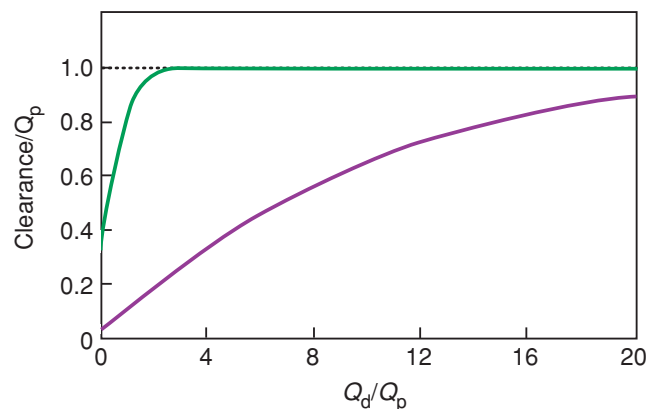
Clearances measured during CVVHDF performed with the dialysate flow lower than the plasma flow are summarized in Table 2. These experiments were performed using the maximal dialysate flow rate attainable with a commonly used CVVHDF system. The clearance rates of all solutes were significantly increased in comparison with values obtained during CVVH alone. Measured clearance values for the unbound solutes urea and creatinine averaged  $45 \pm 2$  mL/min and  $42 \pm 3$  mL/min, respectively. Clearance values for the bound solutes also increased but remained much lower than those for the unbound solutes. Overall, the proportional increase in clearance observed with the addition of dialysate to CVVH was similar for the unbound and bound solutes, and averaged approximately threefold.

The finding that superimposing dialysis with  $Q_d < Q_p$  on hemofiltration increases the clearances of unbound and bound solutes in similar proportion is in accord with the predictions of the model. With dialysate flow much lower than plasma flow and with adequate membrane permeability, the model predicts that the solute



**Fig. 4. Solute concentrations along the length of a dialyzer during continuous venovenous hemodiafiltration (CVVHDF) with  $Q_d > Q_p$ .** The plasma total solute concentration (red line) and dialysate solute concentration (blue line) are depicted as in Figure 2. The top panel depicts the clearance of an unbound solute in CVVHDF performed with  $Q_p \approx 50$  mL/min,  $Q_f \approx 17$  mL/min, and  $Q_r \approx 17$  mL/min predilution,  $Q_d \approx 300$  mL/min, and  $K_oA \approx 600$  mL/min. Solute is rapidly removed from the plasma and the calculated clearance is 50 mL/min, which is the limiting value imposed by the plasma flow. The bottom panel depicts the clearance of a solute which is 90% bound to plasma protein while other parameters remain the same. The gradient driving solute diffusion into the dialysate plus ultrafiltrate stream is reduced by protein binding. Transport continues along the length of the dialyzer because the increased dialysate flow keeps the level in the dialysate plus ultrafiltrate compartment lower than the free level in the plasma. The bound solute clearance of 23 mL/min is almost half the clearance of the unbound solute, and could be made to approach even closer to the plasma flow by further increasing  $Q_d$  and  $K_oA$ .

concentrations in the mix of dialysate and ultrafiltrate leaving the kidney will be nearly equal to the free solute concentrations in the plasma entering the kidney, as illustrated in Figure 2. The clearance of each solute will thus approach the sum of the dialysate flow and ultrafiltration rates multiplied by the free solute concentration at the kidney inlet. Solute clearances will fall below this theoretic maximum if the kidney has a low mass transfer area coefficient, as shown in Figure 3. Dialysis experiments performed without albumin yielded  $K_oA$  values for the M60 set of  $186 \pm 12$  for urea,  $106 \pm 6$  for creatinine,  $108 \pm 10$  for indican, and  $61 \pm 6$  for phenol red. In our CVVHDF experiments with  $Q_d < Q_p$ , values for  $K_oA$  were thus greater than  $Q_d$ . When this is the case, as further shown in Figure 3, an increase in kidney size would not significantly increase solute clearances. When  $Q_d < Q_p$ , changes in  $Q_p$  will likewise have little effect on clearance. Our model predicts, for example, that reducing



**Fig. 5. The predicted effect of increasing  $Q_d$  and  $K_oA$  on solute clearances during continuous venovenous hemodiafiltration (CVVHDF).** The curves illustrate the clearance of an unbound solute (green line) and a solute which is 90% protein bound (purple line) as  $Q_d$  and  $K_oA$  are increased with  $K_oA$  equal to twice  $Q_d$  at every point. The clearance of both unbound and bound solutes is limited by the plasma flow rate,  $Q_p$ . But raising solute clearance to any desired fraction of  $Q_p$  requires much larger values of  $Q_d$  and  $K_oA$  when the solute is protein bound than when the solute is unbound. The ultrafiltration rate  $Q_f$ , which is set at one third of  $Q_p$  in this figure, has a significant effect on the clearance of small solutes only when  $Q_d$  and  $K_oA$  are relatively low.

$Q_p$  from 150 to 75 mL/min while  $Q_d$  is maintained at 42 mL/min would reduce the clearance of urea by less than 15% and the clearance of indican and phenol red by less than 5%.

Having found that superimposing dialysis with  $Q_d < Q_p$  on hemofiltration increases the clearance of bound and unbound solutes in nearly equal proportion, we sought a means to preferentially increase the clearance of bound solutes. The model predicts that this can be accomplished by increasing the dialysate flow above the plasma flow as long as membrane permeability is adequate. The results of in vitro CVVHDF experiments performed to test this prediction are summarized in Table 3. The measured clearances of urea and creatinine,  $52 \pm 3$  mL/min and  $54 \pm 3$  mL/min, respectively, were only slightly greater than observed in the previous experiment. But the measured clearances of indican and phenol red,  $31 \pm 2$  mL/min and  $10.2 \pm 0.4$  mL/min, respectively, were increased threefold. The ratio of bound to unbound solute clearances was thus greatly increased, in accord with the predictions of the model.

The solute concentration profiles associated with the increased clearance of bound solute when  $Q_d$  is greater than  $Q_p$  are depicted in Figure 4. Increasing the dialysate flow above the plasma flow reduces solute concentrations in the dialysate plus ultrafiltrate compartment. The unbound solute diffuses readily into the large quantity of dialysate plus ultrafiltrate, and its concentration rapidly approaches zero as the plasma flows through the kidney. Thus, for the unbound solute, transport is practically complete in the early part of the kidney and the

**Table 3.** Solute clearances during continuous venovenous hemodiafiltration (CVVHDF) with plasma flow less than dialysate flow

Solute	Free fraction %	Measured clearance mL/min	Predicted clearance mL/min
Urea	105 ± 1	52 ± 3 <sup>a</sup>	53 ± 1
Creatinine	99 ± 6	54 ± 3 <sup>a</sup>	53 ± 1
Indican	16 ± 1 <sup>b</sup>	31 ± 2 <sup>a,b</sup>	27 ± 1 <sup>b</sup>
Phenol red	5.8 ± 0.7 <sup>b,c</sup>	10.2 ± 0.4 <sup>a,b,c</sup>	9.0 ± 0.9 <sup>b,c</sup>

Values are mean ± SD. Solute free fractions were assessed after predilution and clearance values were obtained using an F6 filter with  $Q_p \approx 55$  mL/min,  $Q_d \approx 300$  mL/min,  $Q_r \approx 17$  mL/min, and  $Q_f \approx 17$  mL/min.

<sup>a</sup> $P < 0.05$  vs. measured clearance during in vitro continuous venovenous hemofiltration (CVVH) and CVVHDF with dialysate flow less than plasma flow.

<sup>b</sup> $P < 0.05$  vs. value for urea.

<sup>c</sup> $P < 0.05$  vs. value for indican.

clearance approaches the plasma flow rate. The concentration profile for the protein-bound solute is different. The gradient driving diffusion is reduced by protein binding even though the high dialysate flow keeps the solute concentration in the combined dialysate plus ultrafiltrate low. So the rate of solute transfer in the early part of the kidney is much lower than for the unbound solute. But as free solute diffuses through the membrane, bound solute dissociates from protein, replenishing the low free solute concentration available for diffusion and convection. The total concentration and the free concentration of the protein-bound solute fall gradually together as transport continues along the length of the kidney. In contrast to the result obtained when dialysate flow is only a fraction of plasma flow, the clearance of the protein-bound solute can now exceed the clearance of the unbound solute multiplied by the free fraction of the bound solute. Again, we are assuming that the time required for solute to dissociate from the binding protein is short compared to the transit time of plasma through the kidney.

To obtain a high relative clearance of protein-bound solutes during CVVHDF requires an increase in  $K_oA$  as well as in  $Q_d$ . Dialysis experiments performed without albumin yielded  $K_oA$  values for the F6 dialyzer of  $549 \pm 30$  mL/min for urea,  $353 \pm 24$  mL/min for creatinine,  $371 \pm 17$  mL/min for indican, and  $221 \pm 13$  mL/min for phenol red. In our CVVHDF experiments with  $Q_d > Q_p$ , estimated  $K_oAs$  were thus of approximately the same magnitude as  $Q_d$ . Theoretically, as long as  $K_oA$  is adequate, the clearance of even a tightly bound solute can be made to increase arbitrarily close to the limiting value imposed by the plasma flow, as illustrated in Figure 5. The figure shows modeled clearance values for an unbound solute and a protein-bound solute as  $Q_d$  is increased from zero (pure ultrafiltration) to greatly exceed  $Q_p$  while the  $Q_f$  remains constant and  $K_oA$  increases with  $Q_d$ . For an unbound solute, the clearance at  $Q_d = 0$  mL/min is equal to  $Q_f$ , and the clearance at low values for  $Q_d$  is close to the sum of  $Q_f$  and  $Q_d$ , as has been previously described [20–22]. As  $Q_f$  is increased, the clearance of the unbound

solute falls below the sum of  $Q_f$  and  $Q_d$  but still rapidly rises to approach  $Q_p$ . The clearance profile for a protein-bound solute is much different. For low values of  $Q_d$ , the clearance is close to the clearance of the unbound solute multiplied by the fraction of the bound solute which is free in plasma. As  $Q_d$  increases, the clearance of the bound solute increases more gradually than the clearance of an unbound solute, but keeps rising after the clearance of the unbound solute has reached the limiting value of  $Q_p$ . It should be noted that with high dialysate flow, the transport of small solutes is accomplished largely by diffusion, and turning ultrafiltration on and off has only a minor effect on solute clearance.

## DISCUSSION

The first aim of this study was to assess the clearance of protein-bound small solutes during hemofiltration. Results showed that for a given protein-bound solute, the clearance is approximately the ultrafiltration rate multiplied by the free solute fraction, corrected as necessary for the effects of predilution on solute concentration. Protein-bound solutes are thus poorly cleared by conventional CVVH. For instance, with an ultrafiltration rate of 30 mL/min, the predicted clearance of a solute which is 90% protein bound is only 3 mL/min using postdilution and slightly less than 3 mL/min using predilution.

The limitation imposed by protein-binding on solute clearance during hemofiltration is not hard to understand, but has received little attention. Hemofiltration was developed to improve the clearance of solutes whose diffusive transport during dialysis is limited by size [2]. Advocates of hemofiltration suggest that it is “more physiologic” than dialysis because it removes solutes by a process analogous to glomerular filtration [23, 24]. In this view, the infusion of replacement fluid takes the place of tubular reabsorptive function. But the absence of tubular secretory function, by which the normal kidney clears protein-bound solutes, is ignored.

The second aim of this study was to assess the clearance of protein-bound small solutes during hemodiafiltration when dialysis is superimposed on hemofiltration. We found that as long as dialysate flow is low compared to plasma flow, the superimposition of dialysis on hemofiltration increases the clearances of unbound and protein-bound solutes in proportion, with the clearance rate for a given solute being approximately the free solute fraction multiplied by the sum of the ultrafiltration and dialysate flow rates. The hemodiafiltration prescriptions now commonly employed in the treatment of acute renal failure thus do not provide greater relative clearance of protein-bound solutes than pure hemofiltration.

The final aim of this study was to identify means by which the clearance of protein-bound solutes can be increased relative to the clearance of unbound solutes.



**Table 4.** Modeled solute clearances during renal replacement therapy

Modality	Conventional hemodialysis	CVVH low $Q_f$	CVVH high $Q_f$	CVVHDF low $Q_d$	CVVHDF high $Q_d$	Low efficiency hemodialysis	Low efficiency hemodialysis
$Q_b$ mL/min	350	150	150	150	50	200	100
$Q_f$ mL/min	24	20	40	20	20	4	4
$Q_f$ mL/min	0	18	38	18	18	0	0
$Q_d$ mL/min	600	0	0	20	300	100	200
$K_oA_{urea}$ mL/min	800	200	200	200	600	600	600
$Cl_{urea}$ mL/min	270	19	34	38	45	100	90
$Cl_{90\%bound}$ mL/min	46	2.0	4.2	4.2	21	11	20

Abbreviations are: CVVH, continuous venovenous hemofiltration; CVVHDF, continuous venovenous hemodiafiltration. Modeled clearance values for urea ( $Cl_{urea}$ ) and a hypothetical solute of the same size which is 90% protein-bound ( $Cl_{90\%bound}$ ) during renal replacement therapy. The various prescriptions would provide the same net ultrafiltration if conventional hemodialysis was applied every other day and the other treatments were provided daily. The hematocrit was assumed to be 33% in each case.

We found that this can be accomplished by increasing the dialysate flow rate while restricting the plasma flow rate during hemodiafiltration. Indeed, by adequately increasing the dialysate flow, the clearance of small protein-bound solutes can be increased arbitrarily close to the clearance of small unbound solutes as long as kidney size is adequate. It should be noted that in current practice, when the dialysate flow exceeds the plasma flow, it also exceeds the ultrafiltration rate. In this setting, the contribution of ultrafiltration to the transport of protein-bound solutes is relatively small, and the clearance of protein-bound solutes is only slightly higher than that achieved by dialysis alone. The clearance of large solutes, in contrast, will still depend heavily on ultrafiltration.

The current model also reveals one alternate theoretical means to increase the clearance of protein-bound solutes. As shown in Figure 1, if we assume solutes can rapidly dissociate from their binding proteins, the bound fractions will fall as the rate of predilution rises, and solute clearances can be increased during pure ultrafiltration by using predilution and increasing the replacement and ultrafiltration rates to greatly exceed the plasma flow. Because replacement fluid is more expensive than dialysate, however, this treatment would not be cost effective.

Solute clearances have the same dependence on plasma and dialysate flows during intermittent and continuous treatment. But hemofiltration and hemodiafiltration are most often prescribed continuously for the treatment of acute renal failure. Currently employed CVVH and CVVHDF regimens provide greater clearances of large solutes than intermittent hemodialysis while providing equal or greater clearances of urea, depending on the exact prescription [21, 25–27]. The current study suggests that in contrast, CVVH and CVVHDF may provide relatively low clearances of protein-bound solutes, as summarized in Table 4. The table presents clearance values for urea and a hypothetical solute PBS which is 90% bound to albumin. The clearance values have been calculated using the model described in the current study with the urea clearances corrected for the transport of urea out of red cells as described by Depner

[28]. Intermittent hemodialysis with  $Q_b \approx 350$  mL/min and  $Q_d \approx 600$  mL/min is predicted to provide a PBS clearance of  $\approx 42$  mL/min while CVVH with  $Q_f \approx 17$  mL/min is predicted to provide a PBS clearance of only 1.5 mL/min. Because volumes of distribution for protein-bound solutes have not been measured, it is not possible to compare the theoretic effects of intermittent and continuous treatment on plasma solute concentrations. Lesaffer et al [11] found that the concentrations of two solutes which are approximately 90% albumin bound fell much less than the concentration of urea during hemodialysis treatment in vivo. If albumin-bound solutes were restricted largely to the plasma space, their concentration would fall rapidly during dialysis despite the restriction imposed on clearance by albumin binding. The finding of Lesaffer et al [11] thus suggests that the volumes of distribution for protein-bound solutes may be considerably larger than the plasma volume, in which case CVVH with  $Q_f \approx 17$  mL/min would lower plasma protein-bound solute concentrations less effectively than intermittent dialysis performed 4 hours every other day. As further shown in Table 4, the predicted clearance of unbound and bound solutes during CVVH treatment will increase approximately in proportion if the ultrafiltration rate is increased to the recently recommended level of 35 mL/kg/hour, or about 40 mL/min for an averaged size person [29]. Similar clearances of bound solutes would be achieved by shifting to CVVHDF and providing the same total amount of fluid with half used as replacement fluid and half used as dialysate.

The restriction on the clearance of bound solutes relative to unbound solutes can be overcome only by using higher dialysate flows and larger kidneys than are now commonly employed for CVVHDF, as further summarized in Table 4. When high dialysate flows are employed, the clearance of small molecules is accomplished almost entirely by diffusion and ultrafiltration serves only to increase the clearance of large molecules. The predicted dependence of protein-bound solute clearances on dialysate flow and kidney size will be the same when intermittent treatment is prescribed. Recent studies have

described the treatment of acute renal failure using hemodialysis for 8 to 12 hours daily [30, 31]. These “low efficiency” regimens often restrict the clearance of urea and other small unbound solutes by limiting the dialysate flow rate [31]. Our model predicts that the clearance of protein-bound solutes would be greater if the clearance of unbound solutes were restricted by limiting the blood flow rate while maintaining a higher dialysate flow. For instance, as summarized in Table 4, the predicted clearance of a 90% protein-bound solute would be 10 mL/min during low efficiency dialysis with  $Q_b \approx 200$  mL/min and  $Q_d \approx 100$  mL/min and 18 mL/min during low efficiency dialysis with  $Q_b \approx 100$  mL/min and  $Q_d \approx 200$  mL/min, while both regimens would provide nearly the same urea clearance.

Several limitations of the current study should be acknowledged. We have assumed that solute dissociation from albumin is rapid compared to the plasma transit time through the kidney. The efficient extraction of protein bound solutes by the kidney suggests that this is indeed often the case, but measured dissociation rates are generally not available. Our model also assumes thorough solute mixing in both the plasma and the ultrafiltrate/dialysate compartments. To the extent that mixing is not complete, the assumption that kidneys can be characterized by single  $K_oA$  values is not justified, and measured clearances will fall below predicted values. The magnitude of this error may be expected to increase when kidneys are employed using flow rates for which they were not designed. In addition, the  $K_oA$  values we used in modeling were obtained from measurements of clearance from albumin-free solutions, and may have exceeded the  $K_oA$  values obtained when kidneys were perfused with 4% albumin [32, 33].

Despite these limitations, the current model accurately predicted the extent to which clearances of protein-bound solutes are restricted during hemofiltration and hemodiafiltration in vitro. Further studies are obviously required to determine whether the model predicts the behavior of protein-bound solutes in vivo. It should be noted that numerous solutes may compete for protein binding sites in uremic patients. The fractions of various solutes which are bound to protein may therefore increase unpredictably as solute concentrations fall during treatment. In general, clearance values would be expected to decline as protein-binding increases, but this requires testing in practice. A further interesting possibility is that some uremic solutes bind to other blood constituents such as lipids or red cells in vivo. If binding is rapidly reversible, such solutes, like protein-bound solutes, might be effectively cleared by the normal kidney but poorly cleared by hemofiltration.

To the extent that they apply in vivo, our findings suggest that conventional CVVH and CVVHDF regimens provide limited clearance of protein-bound solutes. Clinical studies, as far as we are aware, have yet to exam-

ine this issue. A recent clinical study has shown that addition of hemofiltration at rates of 5 to 15 L per hour increases p-cresol clearance in patients receiving intermittent hemodialysis [34]. The current model predicts this effect, but suggests that increases of the same magnitude could be more easily achieved by increasing dialysate flow rate and dialyzer size. A broader question is whether the clearance of protein-bound solutes is clinically important. Increasing evidence, however, links protein-bound solutes to uremic toxicity [6]. Limited clearance of such solutes could explain, at least in part, why the improved clearance of large solutes obtained with ultrafiltration based therapies has so far not been associated with a discernable improvement in patient outcome [35–37]. Clinical studies of modalities which increase protein-bound solute clearances will be required to address this question.

## APPENDIX

$$f' = \frac{c(c-1)(1-f) - (q+1)cf(cf-c+1) + \sqrt{[c(1-c)(1-f) + (q+1)cf(cf-c+1)]^2 + 4(q+1)c^3f(cf-c+1)(1-f)}}{2c^2(1-f)} \quad (\text{equation 10})$$

where  $c = C_p/C_{\text{alb}}$  and  $q = Q_r/Q_p$ .

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