Increasing Dialysate Flow and Dialyzer Mass Transfer Area Coefficient to Increase the Clearance of Protein-bound Solutes

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Abstract. Clinical hemodialysis systems achieve high single pass extraction of small solutes that are not bound to plasma proteins. But they clear protein-bound solutes much less effectively. This study examines the extent to which clearance of a protein-bound test solute is improved by increasing the dialyzer mass transfer area coefficient (KoA) and the dialysate flow rate (Qo). A reservoir containing test solutes and artificial plasma with albumin concentration approximately 4 g/dl was dialyzed with a standard clinical dialysate delivery system. The clearance of phenol red (Clpr) was compared with the clearances of urea and creatinine at a plasma flow rate (Qp) of 200 ml/min with varying values of KoA and Qo. Clpr increased from 11 ± 2 ml/min to 23 ± 2 ml/min when KoA for phenol red, KoAPR, was increased from 238 to 640 ml/min and Qo was increased from 286 ± 6 ml/min to 734 ± 9 ml/min. Increasing either KoAPR or Qo alone had lesser effects. Clearance values for phenol red were much lower than clearance values for the unbound solutes urea and creatinine, which ranged from 150 to 200 ml/min and were less affected by varying KoA and Qo. A mathematical model was developed to predict Clpr from values of Qp, Qo, the fraction of phenol red bound to albumin (94% ± 1%) and KoAPR. The model accurately predicts the pattern of measured results and shows further that Clpr can be made to approach Qp only by very large increases in both KoAPR and Qo.

Protein-bound solutes are poorly cleared during conventional hemodialysis (1–4). Accumulation of such solutes may contribute to residual uremic disorders when levels for dialytic clearance of urea and other small, unbound solutes are considered “adequate” (3–5). The dialytic clearance of an unbound solute is determined by the blood flow rate, Qp, the dialyzer mass transfer area coefficient, KoA, for that solute, and the dialysate flow rate, Qo (6). In conventional practice, KoA and Qo are set to achieve values for urea clearance close to Qp. Further increases in KoA and Qo cannot significantly increase urea clearance and are therefore generally not contemplated.

The study presented here examined whether the clearance of protein-bound solutes could, by contrast, be improved by increasing KoA and Qo relative to the plasma flow rate, Qp. In vitro dialysis experiments measured the clearance of phenol red, a small solute strongly bound to albumin, from artificial plasma. As expected, the clearance of phenol red was much lower than the clearances of the unbound solutes, urea and creatinine. Phenol red clearance could, however, be increased by increasing both KoA and Qo. We developed a mathematical model to predict the clearance of a protein-bound solute from Qp, Qo, KoA, the albumin concentration, and the association constant, KoA, that describes the strength of solute binding to albumin. Mathematical modeling accurately predicted the pattern of measured results and showed further that clearance values for tightly protein-bound solutes can be made to approach Qp by significantly increasing KoA and Qo.

Materials and Methods

Clearance Measurements during In Vitro Dialysis

Model Dialysis System. Clearances of phenol red, urea, and creatinine were measured during dialysis in vitro. Fluid representing a patient’s plasma was placed in a continuously stirred 4.5 L reservoir and dialyzed with a Fresenius D machine (Fresenius, Gurnee, IL). The reservoir and dialysate fluids were prepared to have identical free electrolyte concentrations, which approximated Na 140 mEq/L, K 4.0 mEq/L, HCO3− 24 mEq/L, Mg 2.0 mEq/L, Ca 2.5 mEq/L, and PO4 4.0 mg/dL. Fluid preparation comprised addition of small amounts of KCl, MgCl2, and CaCl2 to Fresenius 6615 acid concentrate and NaH2PO4 to 1.0 M NaHCO3 concentrate solutions. These solutions were then mixed and appropriately diluted by the dialysis machine. Addition of reagent phenol red, urea, and creatinine to the reservoir provided concentrations of approximately 3.0 mg/dL, 120 mg/dL, and 12.0 mg/dL, respectively, at the beginning of each dialysis run. Dialysis experiments encompassed the combinations of flow rates and dialyzers shown in Table 1. For each of these combinations, four clearance
Table 1. Dialyzer and flow combinations^a

<table>
<thead>
<tr>
<th>Dialyzer</th>
<th>Dialysate Flow (Q_d) ml/min</th>
<th>Reservoir Fluid Flow (Q_p) ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>F6</td>
<td>750</td>
<td>200</td>
</tr>
<tr>
<td>Optiflux F200NR</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>Optiflux F200NR</td>
<td>750</td>
<td>200</td>
</tr>
</tbody>
</table>

^a Dialyzers were purchased from Fresenius. Measured flow rates varied slightly in individual experiments as reported in Table 2.

Dialysis experiments were performed with BSA (Sigma, St. Louis, MO) added to the reservoir fluid (4.0 g/dl), and three clearance experiments were performed without added albumin. Up to five experiments were performed with the same batch of albumin-containing fluid, and up to two experiments were performed with the same dialyzer. Each new batch of albumin-containing fluid was purified by dialysis for 2 h before test solutes were added for clearance experiments.

Dialysis experiments with albumin in the reservoir fluid were carried out over 2 h. Reservoir fluid temperature ranged from 32 to 41°C during experiments, but average temperature did not vary among the different conditions. The ultrafiltration rate was set at zero. Immediately before and after each experiment, the albumin binding of phenol red was assayed by operating the system in the ultrafiltration mode and measuring phenol red concentration in simultaneous samples of ultrafiltrate and reservoir fluid. Plasma and dialysate flow rates were measured volumetrically at the beginning and end of each experiment. A reservoir fluid sample was collected at the beginning of each experiment to confirm the intended electrolyte concentrations had been achieved. Additional reservoir fluid samples were collected at 0, 10, 20, 30, 45, 60, 90, and 120 min for measurement of clearance values. Reservoir pH measured at each sample collection ranged from 6.9 to 8.0.

Chemical Assays and Clearance Calculations. Phenol red was assayed by a method similar to that described by Hirata-Dulas et al. (7). Protein was removed from 0.3-ml aliquots of albumin-containing fluid by addition of 0.9 ml acetone and centrifugation at 3000 × g for 15 min. Then 1.0-ml aliquots of deproteinized supernatant were alkalinized by addition of 1.0 ml of 1.0 M NaOH, and phenol red was assayed by measuring absorption at 560 nm. We used 50-mm cuvettes to increase accuracy of measurements in ultrafiltrate. For experiments without albumin, phenol red was assayed directly after alkalinization of reservoir fluid samples. Creatinine was measured with a Beckman Creatinine Analyzer 2, and urea was measured with a commercial kit (ThermoDMA, Arlington, TX). Electrolyte concentrations were measured by means of standard autoanalyzer techniques in the clinical laboratory. Clearance values for urea and creatinine were calculated from the best fit slope of log values for concentration at 0, 10, 20, 30, and 45 min and from measured reservoir volume. Clearance values for phenol red were calculated in the same way for experiments without albumin and with the addition of 60-, 90-, and 120-min concentration values for experiments with albumin. Values for R^2 for the slope determinations averaged 0.998 ± .002, 0.999 ± .001, and 0.992 ± .011 for urea, creatinine, and phenol red, respectively.

Modeling the Effect of Protein Binding on Dialytic Clearance

We modeled the clearance of protein-bound solutes by combining solute mass balances with a diffusion equation for transport across the dialyzer membrane. The dialyzer was considered to have a dimensionless length of unity (0 ≤ x ≤ 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 differential mass transfer rate, dM, for a solute across an infinitesimal length of the dialyzer, must obey the conservation of mass and can be expressed as

\[ dM = Q_p \cdot dC_p = Q_d \cdot dC_d \]  (1)

where \( Q_p \) is the plasma flow rate, \( C_p \) is the solute concentration on the plasma side of the membrane, \( Q_d \) is the dialysate flow rate, and \( C_d \) is the solute concentration on dialysate side of the membrane. Integration of equation (1) along the length of the dialyzer yields the mass transfer rate, \( M \), given by

\[ M = Q_p \cdot (C_{p,1} - C_{p,0}) \]  (2)

where \( C_{p,x} \) denotes the plasma solute concentration at position \( x \) along the dialyzer (0 ≤ x ≤ 1). The mass transfer of solute into the dialysate is similarly expressed as

\[ M = -Q_d \cdot C_{d,0} \]  (3)

where the solute concentration in the dialysate entering the dialyzer at \( x = 1 \) is zero.

The effect of protein binding is introduced by considering the solute on the plasma side to be partitioned between a bound form, \( C_{pf} \), and an unbound (free) form, \( C_{pf} \), so that

\[ C_p = C_{pf} + C_{pb} \]  (4)

The equilibrium relationship between the bound and unbound forms of the solute is expressed as

\[ K_A = \frac{C_{pb}}{C_{pf} \cdot (C_{alb} - C_{pb})} \]  (5)

\( C_{alb} \) is the total albumin concentration, and the solute binds reversibly to albumin at a single site with an association constant \( K_A \). Rearranging equation (5) yields

\[ C_{pf} = \frac{C_{pb}}{(C_{alb} - C_{pb}) \cdot K_A} \]  (6)

The extent of binding is commonly described by the fraction of the solute, \( f \), unbound at given concentrations of solute and binding protein and can be expressed as

\[ f = \frac{C_{pf}}{C_{pf} + C_{pb}} = \frac{C_{pf}}{C_p} \]  (7)

Substituting equation (6) into equation (7) yields

\[ f = \frac{1}{1 + (C_{alb} - C_{pb}) \cdot K_A} \]  (8)

Here we assume only the free portion of the solute contributes to the concentration gradient driving diffusion across the membrane. Fick’s first law of diffusion states that the flux of a solute is directly proportional to the concentration gradient. The differential mass transfer rate across the membrane, \( dM \), can therefore be expressed as

\[ dM = -k \cdot dA \cdot (C_{pf} - C_d) \]  (9)

where \( k \) is a constant characteristic of the membrane and \( A \) is the membrane area. Combination of equation (3) with equation (9) yields
\[
\frac{dC_d}{C_p^f - C_d} = -\frac{k \cdot dA}{Q_d}
\]

and combination of equation (1) with equation (9) yields

\[
\frac{dC_p}{C_p^f - C_d} = -\frac{k \cdot dA}{Q_p}
\]

Rewriting equation (7) in differential form and rearranging yields

\[
dC_p = \frac{1}{f} \cdot dC_p^f
\]

so that equation (11) becomes

\[
\frac{dC_p^f}{C_p^f - C_d} = -\frac{k \cdot dA}{Q_p}
\]

Subtracting equation (10) from equation (13) yields

\[
\frac{d(C_p^f - C_d)}{C_p^f - C_d} = -\frac{k \cdot dA}{Q_p} \cdot \left( \frac{f}{Q_p} - \frac{1}{Q_d} \right)
\]

Integration of equation (14) from the plasma inlet \((x = 0)\) to the plasma outlet \((x = 1)\) leads to

\[
\ln\left( \frac{C_p^f - C_d}{C_p^f - C_d} \right) = k_A \cdot \frac{f}{Q_p} \cdot \left( \frac{f}{Q_d} - \frac{1}{Q_d} \right)
\]

where the mass transfer area coefficient \(k_A\) is the integral of \(k \cdot dA\) along the dialyzer length. Equation (15) is obtained by using the simplifying assumption that \(f\) is constant along the length of the dialyzer. As revealed by inspection of equation (8), this assumption is practically true when any change in \(C_{pb}\) along the dialyzer is small relative to the concentration of albumin. In general, this will be the case for any tightly bound solute whose total concentration is significantly less than the concentration of the binding protein. A more general solution is possible in other cases, but requires more extensive analysis.

Equations (2), (3), and (15) were solved for \(M\), \(C_p\), and \(C_d\) as a function of the plasma solute concentration at the plasma inlet, \(C_{p,0}\), to yield

\[
C_p = C_{p,0} \cdot \left( \frac{f - \theta}{\phi f - \theta} \right)
\]

\[
C_d = C_{p,0} \cdot \phi \cdot \left( \frac{f - \theta}{\phi f - \theta} \right)
\]

\[
M = C_{p,0} \cdot Q_p \cdot \left( \frac{1 - f - \theta}{\phi f - \theta} \right)
\]

where \(\phi\) and \(\theta\) are defined as

\[
\phi = \exp(K_A \cdot \left( \frac{f}{Q_p} - \frac{1}{Q_d} \right))
\]

and

\[
\theta = \frac{Q_p}{Q_d}
\]

In equation (18), \(M\) is equal to \(C_{p,0}\) multiplied by a constant. In words, the amount of solute removed is proportional to the concentration of solute in the plasma entering the dialyzer, and the dialyzer can therefore be assigned a clearance given by

\[
Cl = \frac{M}{C_{p,0}} = Q_p \cdot \left( 1 - \frac{f - \theta}{\phi f - \theta} \right)
\]

If \(C_{alb}\), \(C_{p,0}\), and \(K_A\) are known quantities for the system, then equation (5) can be rewritten to give

\[
K_A = \frac{Q_p - Q_d}{C_{p,0} \cdot (C_{alb} - (C_p - C_d))}
\]

where all quantities are known except \(C_{p,0}\). Solving for \(C_{p,0}\) in equation (22) and substituting into equation (7), \(f\) can be expressed as

\[
f = \frac{C_p - C_{alb} - \frac{1}{K_A} \cdot \left( \frac{C_{alb} - C_p}{K_A} + \frac{1}{K_A} \right)^2 + 4C_p}{2C_p}
\]

Substituting the value for \(f\) from equation (23) into equation (21) gives the clearance of a protein-bound solute as a function of \(C_p\), \(C_{alb}\), \(K_A\), \(Q_p\), \(Q_d\), and \(K_f\).

The model described above predicts the effects of varying \(K_A\) and \(Q_d\) on the clearance of solutes with different degrees of protein binding. The general predictions of the model are presented graphically in Results. The study presented here tests the ability of the model to predict clearances of the protein-bound test solute, phenol red, during in vitro dialysis experiments. For this purpose, it was necessary to obtain phenol red \(K_A\) values for the individual dialyzers used. These \(K_A\) values were obtained from measured values for phenol red clearance, \(Cl_{PR}\), plasma flow rate, \(Q_p\), and dialysate flow rate, \(Q_d\), in dialysis experiments performed without albumin by the equation

\[
K_{oA} = \frac{Q_p \cdot Q_d}{Q_p - Q_d} \ln\left( \frac{1 - Cl_{PR}/Q_p}{1 - Cl_{PR}/Q_d} \right)
\]

as described by Michaels (8).

Results

Results of experiments performed without albumin are summarized in Table 2. As expected, urea and creatinine clearance values approached \(Q_p\). Without albumin in the reservoir, the clearance of phenol red was only slightly less than the clearances of urea and creatinine. Values for phenol red clearance were combined with values for \(Q_p\) and \(Q_d\) to obtain \(K_A\) values by equation (24). The values obtained were not constant for the dialyzers used, but increased as \(Q_d\) was increased from 300 to 750 ml/min.

Results of experiments performed with albumin are summarized in Table 3. The concentration of albumin measured at the beginning of each experiment was 4.2 ± 0.2 g/dl. As expected, clearance values for urea and creatinine were not notably affected by the addition of albumin to the reservoir. Moreover, because urea and creatinine clearance values reached a large fraction of \(Q_d\), with the smaller dialyzer and lower dialysate flow, major increases in clearance could not be achieved by increasing dialyzer size and dialysate flow. The clearance of phenol red, in contrast, was markedly reduced by the addition of albumin to the reservoir, as illustrated in Figure 1. Moreover, when albumin was present, increases in dialyzer size and dialysate flow had a significant effect on phenol red clearance. Thus, the phenol red clearance of 23 ± 2 ml/min with an
and Methods and values for \( K_{oA\text{PR}} \) in Table 2.

Values for total albumin and phenol red concentrations were close to the value of 0.060 calculated from values for the unbound fraction, \( f \), phenol red binding, as further shown in Table 3. Measured on phenol red clearance was a predictable consequence of increasing both parameters together.

Increasing dialyzer size had much less effect on clearance than increasing dialyzer size without increasing \( Q_d \) as illustrated in Figure 5, the more avidly a solute binds to protein, given clearance. For example, if a solute is not bound to protein, a value of 2.8\,M\,^{-1} is the same. 

Increasing dialysate flow rate reduces the solute concentration in the dialysate. At very high flow, solute concentration in the dialysate approaches zero, maximizing the concentration gradient, as illustrated in Figure 4. But even when the concentration gradient is maximized by increasing \( Q_d \) far above \( Q_p \), Inspection of the gradients governing diffusion across the dialyzer reveals why high values for \( K_{oA\text{PR}} \) and \( Q_d \) are required to clear protein-bound solutes. Protein binding reduces the concentration of solute available for diffusion on the plasma side of the membrane, as illustrated in Figure 3. Clearance is therefore reduced relative to that of an unbound solute for which \( K_{oA} \) is the same. Increasing dialysate flow rate reduces the solute concentration in the dialysate. At very high flow, solute concentration in the dialysate approaches zero, maximizing the concentration gradient, as illustrated in Figure 4. But even when the concentration gradient is maximized by increasing \( Q_d \), the \( K_{oA} \) of a dialyzer designed to clear unbound solutes will not be adequate for efficient clearance of solutes that are protein bound. As illustrated in Figure 5, the more avidly a solute binds to protein, the larger the increases in \( Q_d \) and \( K_{oA} \) required to achieve any given clearance. For example, if a solute is not bound to protein, a clearance of 50% of \( Q_p \) can be obtained with \( K_{oA} \) and \( Q_d \) each set at \( \approx Q_p \). To get the same clearance with 80% of the solute bound to protein, \( K_{oA} \) and \( Q_d \) must be set at \( \approx 5 \times Q_p \). With 94% of the solute bound to protein, as was the case for

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**Table 2. Dialysis without albumin**

<table>
<thead>
<tr>
<th>Dialyzer</th>
<th>F6</th>
<th>F6</th>
<th>Optiflux</th>
<th>Optiflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Q_d ) ml/min</td>
<td>282 ± 9</td>
<td>207 ± 1</td>
<td>205 ± 2</td>
<td>208 ± 2</td>
</tr>
<tr>
<td>( Q_p ) ml/min</td>
<td>207 ± 1</td>
<td>195 ± 1</td>
<td>182 ± 9</td>
<td>219 ± 8abc</td>
</tr>
<tr>
<td>( C_{\text{urea}} ) ml/min</td>
<td>167 ± 4</td>
<td>184 ± 3</td>
<td>183 ± 10a</td>
<td>218 ± 3abc</td>
</tr>
<tr>
<td>( C_{\text{creatinine}} ) ml/min</td>
<td>153 ± 3</td>
<td>158 ± 1ab</td>
<td>191 ± 1abc</td>
<td></td>
</tr>
<tr>
<td>( Cl_{\text{PRmeasured}} ) ml/min</td>
<td>119 ± 1</td>
<td>238</td>
<td>291</td>
<td>476</td>
</tr>
</tbody>
</table>

Qₚ, plasma flow; \( Q_d \), dialysate flow; Cl, clearance; \( K_{oA\text{PR}} \), calculated dialyzer mass transfer area coefficient for phenol red.

\(^a\) \( P < 0.05 \) versus \( Q_d \approx 300 \) ml/min.

\(^b\) \( P < 0.05 \) versus Optiflux with \( Q_d \approx 300 \) ml/min.

\(^c\) \( P < 0.05 \) versus \( Q_d \approx 750 \) ml/min.

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**Table 3. Dialysis with albumin**

<table>
<thead>
<tr>
<th>Dialyzer</th>
<th>F6</th>
<th>F6</th>
<th>Optiflux</th>
<th>Optiflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Q_d ) ml/min</td>
<td>286 ± 6</td>
<td>745 ± 7</td>
<td>289 ± 6</td>
<td>734 ± 9</td>
</tr>
<tr>
<td>( Q_p ) ml/min</td>
<td>209 ± 1</td>
<td>207 ± 3</td>
<td>208 ± 2</td>
<td>203 ± 2</td>
</tr>
<tr>
<td>( Cl_{\text{urea}} ) ml/min</td>
<td>169 ± 7</td>
<td>193 ± 12abc</td>
<td>195 ± 14a</td>
<td>196 ± 14a</td>
</tr>
<tr>
<td>( Cl_{\text{creatinine}} ) ml/min</td>
<td>150 ± 4</td>
<td>175 ± 3abc</td>
<td>182 ± 8abc</td>
<td>194 ± 12abc</td>
</tr>
<tr>
<td>( Cl_{\text{PRmeasured}} ) ml/min</td>
<td>11 ± 1</td>
<td>16 ± 1abc</td>
<td>14 ± 1</td>
<td>23 ± 2abc</td>
</tr>
<tr>
<td>( f \times 10^7 )</td>
<td>5.8 ± 0.2</td>
<td>5.6 ± 0.4</td>
<td>6.1 ± 0.2</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>( Cl_{\text{PRpredicted}} ) ml/min</td>
<td>9 ± 1</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>24 ± 1abc</td>
</tr>
</tbody>
</table>

\( Cl_{\text{PRmeasured}} \), measured clearance of phenol red; \( Cl_{\text{PRpredicted}} \), predicted clearance of phenol red using the model described in Materials and Methods and values for \( K_{oA\text{PR}} \) in Table 2.

\(^a\) \( P < 0.05 \) versus \( Q_d \approx 300 \) ml/min.

\(^b\) \( P < 0.05 \) versus Optiflux with \( Q_d \approx 300 \) ml/min.

\(^c\) \( P < 0.05 \) versus \( Q_d \approx 750 \) ml/min.

Optiflux dialyzer and \( Q_d \approx 750 \) ml/min was approximately twice the phenol red clearance of 11 ± 1 ml/min obtained with an F6 dialyzer and \( Q_d \approx 300 \) ml/min. Of note, increasing dialyzer size without increasing \( Q_d \) and increasing \( Q_d \) without increasing dialyzer size had much less effect on clearance than increasing both parameters together.

Mathematical modeling confirmed that the effect of albumin on phenol red clearance was a predictable consequence of phenol red binding, as further shown in Table 3. Measured values for the unbound fraction, \( f \), of phenol red were similar in all experiments and were close to the value of 0.060 calculated from values for total albumin and phenol red concentrations and the reported \( K_A \) value of 2.8 × 10^4 M^-1 for phenol red binding to albumin (9). Combining the values for \( f \) with the values for phenol red \( K_A \) obtained in experiments without albumin allowed us to model phenol red clearance as described in Methods. The values obtained with the model agreed well with the observed values, and the model accurately predicted that increases in both \( K_A \) and \( Q_d \) would be required to achieve substantial increases in phenol red clearance.

A major aim of modeling the clearance of protein-bound solutes was to predict the effect of increasing \( K_A \) and \( Q_d \) above the levels examined experimentally. The predicted effect of increasing \( K_A \) and \( Q_d \) on the clearance of solutes with albumin binding equal to that of phenol red is illustrated in Figure 2. Values for solute clearance (\( Cl_{\text{solute}} \)), \( K_A \), and \( Q_d \) are expressed as multiples of \( Q_p \). It is apparent that \( Cl_{\text{solute}} \) can be made to approach the limiting value of \( Q_p \) but only at the expense of increasing \( K_A \) and \( Q_d \) far above \( Q_p \). Inspection of the gradients governing diffusion across the dialyzer reveals why high values for \( K_A \) and \( Q_d \) are required to clear protein-bound solutes. Protein binding reduces the concentration of solute available for diffusion on the plasma side of the membrane, as illustrated in Figure 3. Clearance is therefore reduced relative to that of an unbound solute for which \( K_A \) is the same. Increasing dialysate flow rate reduces the solute concentration in the dialysate. At very high flow, solute concentration in the dialysate approaches zero, maximizing the concentration gradient, as illustrated in Figure 4. But even when the concentration gradient is maximized by increasing \( Q_d \), the \( K_A \) of a dialyzer designed to clear unbound solutes will not be adequate for efficient clearance of solutes that are protein bound. As illustrated in Figure 5, the more avidly a solute binds to protein, the larger the increases in \( Q_d \) and \( K_A \) required to achieve any given clearance. For example, if a solute is not bound to protein, a clearance of 50% of \( Q_p \) can be obtained with \( K_A \) and \( Q_d \) each set at \( \approx Q_p \). To get the same clearance with 80% of the solute bound to protein, \( K_A \) and \( Q_d \) must be set at \( \approx 5 \times Q_p \). With 94% of the solute bound to protein, as was the case for
The goal of this study was to assess the dependence of protein-bound solutes poorly cleared by dialysis and have begun to analyze their potential toxicity. Recently identified specific protein-bound solutes poorly cleared by dialysis impair solute clearance (1,12). Vanholder and colleagues (3,4,13,14) and Niwa and colleagues (2,15) have reviewed modeling results that could be extended to solutes bound to other plasma components. The study presented here analyzes the dialytic clearance of solutes in this latter category. Solutes whose clearance is limited by binding to plasma proteins or other blood constituents. The study presented here agrees well with those predicted by mathematical modeling. Values for $K_A$ used to model phenol red clearance in the presence of albumin were obtained from experiments performed in the absence of albumin. These $K_A$ values were not constant for each dialyzer, but increased as $Q_d$ increased. Previous studies have suggested that increasing $Q_d$ increases $K_A$ by reducing concentration boundary layers adjacent to the dialysis membrane or by correcting maldistribution of dialysate flow (16). The magnitude of this effect may have been increased in the study presented here because the lower dialysate flow rate was below the range for which the dialyzers were designed and because flow rates were varied by 2.5 fold. Previous studies have also suggested that protein binding to dialysis membranes may be responsible in part for the finding that $K_A$ values are up to 20% lower in dialyzers exposed to blood than in dialyzers exposed only to salt solutions (17). Changes in $K_A$ of this magnitude would not significantly alter the relation of predicted to observed phenol red clearance values in the study presented here. It should be emphasized that the predictions of the model were tested only over a low range of values for $K_A$ and $Q_d$ in the study presented here. Testing the predicted effect of very high values of $K_A$ and $Q_d$, as illustrated in Figure 2, would require modification of current dialyzers.

The model of protein-bound solute clearance described in this study relies on several approximations. First, the model presumes that unbound solute fraction, $f$, constant along the length of the dialyzer. This will be the case when the solute bound solute clearance on dialyzer size and dialysate flow rates. Mathematical modeling suggests the clearance of protein-bound solutes is increased by increasing $K_A$ and $Q_d$ relative to $Q_p$. Effective clearance of tightly bound solutes, however, requires increasing $K_A$ and $Q_d$ far above the levels now used in clinical practice. This is because the adequacy of hemodialysis is currently assessed by measuring urea removal.

Dialysis is typically performed with values of $K_A$ and $Q_d$ in the range of two- to threefold higher than $Q_p$. This provides urea clearances in the range of 75% to 90% of dialyzer blood flow, because the dialyzer clears urea from red cells as well as from plasma (6). With urea clearance at this level, increases in $K_A$ and $Q_d$ can afford only limited improvement. The situation is different for protein-bound solutes. Because of protein binding, only a small fraction of the total solute in the plasma is available for diffusion across the dialyzer over any given segment of the dialyzer length. The limited quantity of free solute diffusing out of the plasma over any given segment tends to be replaced by dissociation of solute from the binding protein as plasma flows along the dialyzer length. The limitation imposed by protein binding can be offset only by increasing $K_A$ to maintain solute transport despite the limited effective concentration gradient and by increasing $Q_d$ so that the solute concentration in the dialysate does not rise toward the low free solute concentration in the plasma and reduce the gradient. Increases in either $K_A$ or $Q_d$ alone afford only limited increases in protein-bound solute clearance, as illustrated in Figure 4.

The effects of $K_A$ and $Q_d$ on phenol red clearance observed in the study presented here agreed well with those predicted by mathematical modeling. Values for $K_A$ used to model phenol red clearance in the presence of albumin were obtained from experiments performed in the absence of albumin. These $K_A$ values were not constant for each dialyzer, but increased as $Q_d$ increased. Previous studies have suggested that increasing $Q_d$ increases $K_A$ by reducing concentration boundary layers adjacent to the dialysis membrane or by correcting maldistribution of dialysate flow (16). The magnitude of this effect may have been increased in the study presented here because the lower dialysate flow rate was below the range for which the dialyzers were designed and because flow rates were varied by 2.5 fold. Previous studies have also suggested that protein binding to dialysis membranes may be responsible in part for the finding that $K_A$ values are up to 20% lower in dialyzers exposed to blood than in dialyzers exposed only to salt solutions (17). Changes in $K_A$ of this magnitude would not significantly alter the relation of predicted to observed phenol red clearance values in the study presented here. It should be emphasized that the predictions of the model were tested only over a low range of values for $K_A$ and $Q_d$ in the study presented here. Testing the predicted effect of very high values of $K_A$ and $Q_d$, as illustrated in Figure 2, would require modification of current dialyzers.

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Figure 1. Effect of $K_A$ and $Q_d$ on the clearance of urea and of phenol red during in vitro dialysis with albumin. The curves represent reservoir solute concentrations calculated using the mean experimentally determined clearance value for each combination of $K_A$ and $Q_d$ values. $Q_p$ was 200 ml/min in each case. Red lines depict clearances of phenol red, which were increased by increasing dialyzer size and dialysate flow rate: (a) F6, $Q_d$ 300 ml/min, $C_{lpq}$ 11 ml/min; (b) Optiflux, $Q_d$ 300 ml/min, $C_{lpq}$ 14 ml/min; (c) F6, $Q_d$ 750 ml/min, $C_{lpq}$ 16 ml/min; (d) Optiflux, $Q_d$ 750 ml/min, $C_{lpq}$ 23 ml/min. Blue lines depict clearances of urea, which were higher and not much affected by increasing dialyzer size and dialysate flow rate: (e) F6, $Q_d$ 300 ml/min, $C_{lurea}$ 169 ml/min; (f) Optiflux, $Q_d$ 300 ml/min, $C_{lurea}$ 195 ml/min; (g) F6, $Q_d$ 750 ml/min, $C_{lurea}$ 193 ml/min; (h) Optiflux, $Q_d$ 750 ml/min, $C_{lurea}$ 196 ml/min. Clearances represented by (f), (g), and (h) approach the limiting value of $Q_p$ and are indistinguishable.

Discussion

Solutes poorly cleared by conventional dialysis presumably contribute to residual uremic illness in patients with ESRD. Such solutes can be divided into at least three categories (3,5,10,11). One category includes solutes retained within cells or some other body compartment. For such solutes, routine hemodialysis may provide only a modest removal from the body even if it affords a high plasma clearance rate. A second category includes molecules considerably larger than urea and therefore cleared less effectively. A third category includes solutes whose clearance is limited by binding to plasma proteins or other blood constituents. The study presented here analyzes the dialytic clearance of solutes in this latter category. The study focuses on a solute bound to albumin, but the modeling results could be extended to solutes bound to other plasma proteins, to lipids, or to red blood cells.

Early in the history of dialysis, it was recognized that protein binding impairs solute clearance (1,12). Vanholder and colleagues (3,4,13,14) and Niwa and colleagues (2,15) have recently identified specific protein-bound solutes poorly cleared by dialysis and have begun to analyze their potential toxicity. The goal of this study was to assess the dependence of protein-bound solute clearance on dialyzer size and dialysate flow rates. Mathematical modeling suggests the clearance of protein-bound solutes is increased by increasing $K_A$ and $Q_d$ relative to $Q_p$. Effective clearance of tightly bound solutes, however, requires increasing $K_A$ and $Q_d$ far above the levels now used in clinical practice. This is because the adequacy of hemodialysis is currently assessed by measuring urea removal.

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The model of protein-bound solute clearance described in this study relies on several approximations. First, the model presumes that unbound solute fraction, $f$, constant along the length of the dialyzer. This will be the case when the solute

phenol red in the study presented here, $K_A$ and $Q_d$ must each be set at $\approx 17 \times Q_p$. 
concentration is low compared with the concentration of binding protein. For instance, in the study presented here, the concentration of albumin was approximately 640 μM and the initial concentration of phenol red was approximately 90 μM. At these concentrations, $f$ would decline only from 0.060 to 0.056 along the dialyzer even if the clearance was increased to

![Figure 2](image-url)

Figure 2. Predicted effect of $K_oA$ and $Q_d$ on the dialytic clearance of a solute that is 94% bound to plasma protein. Values for clearance, shown on the vertical axis, can rise to approach $Q_p$, but only for large increases in $K_oA$ and $Q_d$. In the figure, values for clearance, $K_oA$, and $Q_d$ are all expressed as fractions of $Q_p$. The highest clearance obtained experimentally in the study presented here, using values of $Q_p \approx 200$ ml/min, $K_oA \approx 640$ ml/min, and $Q_d \approx 750$ ml/min, was approximately $0.12 \times Q_p$, as indicated by the asterisk.

![Figure 3](image-url)

Figure 3. Solute concentrations along the length of a dialyzer with and without protein binding. In each panel, dialyzer length is on the vertical axis with the plasma inlet and dialysate outlet at the bottom ($x = 0$) and the plasma outlet and dialysate inlet at the top ($x = 1$). Solute concentration is on the horizontal axis. The total plasma solute concentration (red line) is set equal to 1.0 at the plasma inlet ($x = 0$) and the dialysate solute concentration (broken black line) is set equal to 0.0 at the dialysate inlet ($x = 1$). When there is protein binding, the concentration of unbound solute is depicted by the broken blue line. Panel A depicts the clearance of an unbound solute with $K_oA$ and $Q_d$ both equal to 1.5 $\times Q_p$. The gradient driving diffusion, as indicated by the shaded area, is large and the clearance, as indicated by the fall in total plasma solute concentration, is 66% of $Q_p$. Panel B depicts the clearance of a solute that is 94% bound to plasma protein when the same values for $K_oA$ and $Q_d$ are used. The gradient driving diffusion, which is now the difference between the unbound solute concentration in the plasma and the solute concentration in the dialysate, is substantially reduced, thereby greatly lowering the clearance. The magnitude of the gradient can be better appreciated in Panel C, in which dialysate and plasma unbound solute concentrations under the same conditions are depicted by an expanded scale.
50% of \( Q_p \). Moreover, as long as solute concentration is low relative to that of the binding protein, clearance values do not change as solute concentration declines over time. Experimental results can thus be modeled on the basis of the assumption of a constant clearance as was done in the study presented here.

A second approximation in the model relates to the binding of a single solute to a single site. On albumin, different solutes compete for binding at individual sites and some solutes bind to more than one site (9). Solute competition for protein binding sites has been described in renal failure, with ill-defined uremic toxins displacing solutes such as tryptophan and phenytoin from albumin (12). Presumably, values for \( f \) for some solutes may decline during dialysis as competing solutes are removed. For now, this problem must be approached by measuring solute binding at the beginning and end of dialysis, as the nature of the various competing solutes is not sufficiently well known to predict changes in binding.

Finally, in modeling the clearance of protein-bound solutes, we assume that dissociation of solutes from binding proteins is rapid compared with the transit time of plasma through the

Figure 4. Effect of increasing \( K_A \) and \( Q_d \) on concentrations of a protein-bound solute within the dialyzer. Panels A and D depict the effect of increasing \( Q_d \) to 15 \( \times \) \( Q_p \) while leaving \( K_A \) equal to 1.5 \( \times \) \( Q_p \). Increasing dialysate flow keeps dialysate solute concentration near zero, so that the gradient driving diffusion is close to the plasma unbound solute concentration, as shown in Panel A. Because the unbound solute concentration is low and because \( K_A \) has not been increased, however, the solute clearance is only 8% of \( Q_p \) as shown in Panel D. Panels B and E depict the effect of increasing \( K_A \) to 15 \( \times \) \( Q_p \) while leaving \( Q_d \) equal to 1.5 \( \times \) \( Q_p \). The increase in \( K_A \) allows increased solute diffusion despite a low initial gradient. However, without an increase in \( Q_d \), the dialysate solute concentration rapidly approaches the plasma unbound solute concentration. The concentration gradient along much of the dialyzer length is reduced nearly to zero, as shown in Panel B. Total solute clearance is only 9% of \( Q_p \) despite the large increase in \( K_A \), as shown in Panel E. Panels C and F depict the greater effect of combined increases in \( K_A \) and \( Q_d \), which are now both set equal to 15 \( \times \) \( Q_p \). The increase in \( Q_d \) keeps the dialysate concentration low while the increase in \( K_A \) allows diffusion, although the driving gradient is small compared with that for an unbound solute. The predicted clearance of a solute that is 94% bound to plasma protein rises to 46% of \( Q_p \), as illustrated in Panel F.
dialyzer. Values for the dissociation rate of phenol red from albumin are not available. In this regard, it is interesting to compare phenol red clearance in the normal kidney and the dialyzer. The normal human kidneys clear approximately 50% of phenol red from a renal plasma flow of approximately 600 ml/min even though phenol red binds strongly to albumin (18,19). Because phenol red clearance is less than renal plasma flow, Homer Smith (18) abandoned it in the 1930s in favor first of diodrast and then hippuran as a plasma flow marker. Nonetheless, phenol red clearance remained in use for many years as a test of renal function (19,20). High fractional clearance of protein-bound solutes like phenol red is achieved by active solute transport into the tubular lumen (21). Free solute levels in the renal interstitium are pumped down low enough so that a large portion of bound solute dissociates from plasma proteins during the few seconds that blood transits the kidney. It is tempting to speculate that the combination of active secretion and plasma protein binding represents an adaptation to allow excretion of toxic solutes while limiting free solute levels in the extracellular fluid (1,9,22).

The active transport that allows high clearance of protein-bound solutes in the normal kidney of course does not occur in dialysis systems. Several devices incorporating sorbents have been developed for the removal of such solutes (23,24). The ability of these systems to remove protein-bound solutes normally cleared by the kidney, however, has not been demonstrated. The study presented here suggests that dialytic clearance of protein-bound solutes can be increased by the simpler expedient of increasing $K_oA$ and $Q_d$. The value of increasing the clearance of protein-bound solutes from uremic patients remains to be determined. A number of protein-bound solutes are known to accumulate in renal failure and some of these solutes have toxic properties (10). The HEMO Study’s finding that increasing the clearance of large solutes has little beneficial effect provides increased impetus to study protein-bound solutes (25). At present, however, we do not know enough about these solutes to predict the effect of increased dialytic clearance on their extracellular fluid levels and on their putative toxic effects. In particular, the effect of increasing dialytic clearance on the time averaged concentration of a solute in patients maintained on intermittent dialysis depends heavily on the volume of distribution for that solute, and the volumes of distribution for protein-bound solutes that accumulate in uremic patients have not been well characterized. Moreover, dialytic clearance of protein-bound solutes is nonselective, so that valuable solutes could be lost as clearance is increased. Clinical studies will therefore be required to determine whether increasing dialytic clearance of protein-bound molecules has a beneficial effect in patients with ESRD.

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