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Ultrafiltration and effective peritoneal blood flow during peritoneal dialysis in the rat

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Ultrafiltration and effective peritoneal blood flow during peritoneal dialysis in the rat. The dependence between maximum net ultrafiltration rate (nUFR) created by 15% dextrose dialysis solution and effective peritoneal capillary blood flow (EPBF) estimated by the diffusive mass transport coefficient (K_{BD}) and peritoneal clearance (C_p) of CO_2 gas was evaluated during 30 minute, 15 ml peritoneal dialysis exchanges in anesthetized rats (N = 18). The values of K_{BD} for CO_2 suggested a mean EPBF of 1.9 ± 0.1 (SEM) ml/min for isosmotic exchanges and 2.7 ± 0.2 ml/min for hyperosmotic ones with a mean maximum nUFR of 0.43 ± 0.01 ml/min. C_p of CO_2 measured after the first five minutes of dwell underestimated EPBF. In normally hydrated rats, maximum nUFR was achieved when the peritoneal filtration fraction was 32 ± 2%. This value is similar to the glomerular filtration fraction in rats of 30%. Thus, our results indicate the following relationships: EPBF = (≈ 3 × maximum nUFR)/(1 − hematocrit). EPBF was about six times greater than maximum nUFR and exceeded about 57 times nUFR obtained under isosmotic conditions. These differences between EPBF and nUFR suggest normal EPBF is not a major limiting factor for maximum ultrafiltration achieved during peritoneal dialysis.

Combined capillary hydrostatic, interstitial oncotic and dialysis solution osmotic pressures favor transcapillary ultrafiltration during peritoneal dialysis while plasma oncotic and interstitial hydrostatic pressures oppose these forces [1]. The net ultrafiltration rate (nUFR), however, also depends on lymphatic absorption [2]. Pharmacological agents can induce changes in transperitoneal water movement [3]. There is inferential evidence that nUFR can be also a plasma-flow-dependent process [4–6] as has been shown for glomerular filtration when filtration pressures were under conditions of equilibrium and disequilibrium [7, 8].

According to previous studies [9–11], peritoneal short-dwell clearances and mass transfer area coefficients of gases such as hydrogen, carbon dioxide and xenon are estimates of effective peritoneal capillary blood flow (EPBF). Investigations of glomerular filtration [7, 8] revealed that capillary blood flow can be estimated using filtration fraction, that is ultrafiltration rate divided by plasma flow. Five years ago Ronco et al [6] postulated that EPBF can be also calculated when both ultrafiltration rate and filtration fraction are known. They reported evidence that the plateau in ultrafiltration rate with 2.5% dextrose dialysis solution occurs when filtration fraction approaches 50%, assuming that filtration pressure equilibrium is reached in the peritoneal capillaries when the rising protein concentration generates an oncotic pressure able to balance the high osmotic pressure of dialysis solution. Thus, effective plasma flow through peritoneal capillaries actually participating in the exchange process under these conditions should approach two times nUFR and total EPBF should be estimated as (≈ 2 × nUFR)/(1 − hct), where hct = hematocrit value at the midpoint of the study period. Attempts to increase filtration fraction over 50% by increasing the mean osmotic gradient would result in little or no increase in ultrafiltration rate since oncotic pressure rises sharply with only small increments in filtration fraction [6]. Thus, dialysis solutions with a dextrose concentration over 2.5% should also yield filtration fractions of about 50%. According to Levin et al [12], 15% dextrose dialysis solution yields in rats maximum nUFR with short dialysis cycles. The mean EPBF, calculated by Levin et al [12] using maximum nUFR, was 1.5 ml/min. This value, however, was not compared with other estimates of EPBF.

The purpose of our studies is to compare EPBF estimated in rats using peritoneal clearances and diffusive mass transport coefficients (K_{BD}s) for CO_2 gas as well as maximum nUFR obtained with 15% dextrose dialysis solution. If the concept that filtration pressure equilibrium limits maximum nUFR near a plasma filtration fraction of 50% is correct, then the short-dwell clearances of CO_2 gas and K_{BD} values for CO_2 should be near to (2 × maximum nUFR)/(1 − hct), supporting the hypothesis that they all estimate EPBF and that maximum nUFR is blood flow limited. If results with these different approaches do not agree, then a peritoneal filtration fraction should be calculated as maximum nUFR/[EPBF × (1 − hct)], assuming as per previous studies [10] that peritoneal transfer parameters of CO_2 gas estimate EPBF.

Methods

Studies in rats

The animal model. This was similar to that described in the paper of Levin et al [12]. In brief, eighteen male Sprague-

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Dawley rats, 265.5 to 432.7 g, were anesthetized with 50 mg/kg of subcutaneous pentobarbital sodium solution (Nembutal, Abbott Laboratories, North Chicago, Illinois, USA). Rats were placed supine on a heating pad at 37°C and body temperature was monitored with a rectal temperature probe (Yellow Springs Instruments, Inc., Model 402). An endotracheal tube was inserted and the respiration rate was monitored using a TCT-IR Transducer (Grass Instruments Co., Quincy, Massachusetts, USA). The external jugular vein was exposed and cannulated for intravenous administration. Blood pressure was monitored through a cannula in the femoral artery with a pressure transducer (P 23/D, Gould Statham, Hato Rey, Puerto Rico) connected to a polygraph (Low-Level D.C. Preamplifier in a Grass Instruments Co., Model 7 Polygraph, Grass Instruments Co.). Blood samples for laboratory determinations were also taken from this artery as well as from the tail vein. A continuous electrocardiogram was recorded using subcutaneous electrodes with an EKG-Pulse preamplifier in a Model 7 polygraph (Grass Instruments Co.).

A peritoneal catheter was advanced into the peritoneal cavity through a midline incision 1 cm below the xiphoid process with the tip placed in the right lower quadrant of the abdomen.

The animals were hydrated through the venous cannula with warmed (37°C) lactated Ringer's solution (Baxter Company, Deerfield, Illinois, USA). The infusion rate was chosen to replace urine output and fluid losses due to peritoneal ultrafiltration. During exchanges with 15% dextrose solution pork insulin (Squibb-Novo, Bagsvaerd, Denmark) was given intravenously in Ringer's solution (15 to 30 U/1 ml of the solution). Insulin infusion provided 15 to 92 mg protein into the blood stream.

Dialysis solutions. The near isosmotic peritoneal dialysis solution was specially prepared and contained sodium 144 mEq/liter, potassium 0.6 mEq/liter, magnesium 1.5 mEq/liter, calcium 3.5 mEq/liter, chloride 118 mEq/liter, lactate 35 mEq/liter and dextrose 0.83%. The osmolality of this solution was 306 mOsm/kg and equal to normal rat serum osmolality [13]. The very hyperosmotic dialysis solution was Travenol Dianeal PD-2 with dextrose added to bring the anhydrous dextrose concentration to 15% and osmolality to 1,144 mOsm/kg. Both solutions were adjusted to a pH of 7.2 to 7.3 with sterile 1.0 N sodium hydroxide to avoid local CO_2 generation within the peritoneal cavity subsequent to the instillation of a relatively acid solution. Before each dialysis the CO_2 pressure, pH and osmolality of both dialysis solutions were checked.

Peritoneal dialysis protocols. Experiments were carried out according to two dialysis protocols. During the isosmotic-hyperosmotic protocol five exchanges were performed using isosmotic dialysis solution then followed by three to five exchanges with hyperosmotic solution. The hyperosmotic-isosmotic protocol was identical with the exception of solution order. Each protocol was used in nine rats.

Two consecutive in and out exchanges were performed at the beginning of the study (exchanges 1 and 2) as well as when dialysis solution osmolality was changed (exchanges 6 and 7). Exchanges 3 to 5 and 8 to 10 lasted 30 minutes each (inflow—approximately 15 seconds, dwell—25 min, outflow—approximately 4 min 45 sec). Instillation volume was always 15 ml. Investigations were performed only in 30-minute dialysis cycles, thus dialysate flow rate was unchanged during the studies to eliminate its influence on the rate of CO_2 removal [14].

Arterial blood samples were taken prior to each of 30 minute dialysis cycle (exchanges 3 to 5 and 8 to 10) as well as at the end of the study. Venous blood samples were obtained at the same time but in the final exchanges not in every case because of technical problems; at the beginning of exchange 10 venous blood was obtained only in 4 of 12 rats in which this exchange was performed. Total blood volume removed per animal for sampling approximated 0.5 ml. The CO_2 pressure, glucose concentration and hct were immediately determined; the serum osmolality was estimated after obtaining the hct value. CO_2 pressure was determined in arterial and venous blood; all other estimations were carried out in arterial blood.

Dialysate samples for the determination of CO_2 partial pressure were taken after every five minutes of dwell: 3 ml of dialysate were withdrawn and the last 160 microliters sampled for CO_2; the rest was reinfused promptly. Dialysate from each exchange was collected and dialysate volume measured; the volume removed for CO_2 pressure estimations was added to obtain the total drain volume. A sample was taken for osmolality determination.

The isosmotic-hyperosmotic and the hyperosmotic-isosmotic dialysis protocols were additionally performed in three rats each, but instead of CO_2 pressure urea concentration was determined in arterial blood and dialysate samples.

Laboratory instruments. The partial pressures of CO_2 were measured with the use of a pH/blood gas analyser (Instrumentation Laboratory System 1303, USA). Urea was estimated with the urease method using a Synchron Clinical System AS™ 8 (Beckman Instruments, Inc., Fullerton, California, USA). Osmolality measurements were carried out using a Wescor 5100 B vapor pressure osmometer (Wescor Inc., Logan, Utah, USA). Blood glucose concentrations were estimated using Glucometer® II (Ames Division, Miles Laboratories, Inc., Elkhart, USA) and Chemstrip BG (Boehringer Mannheim Diagnostic Inc., Indianapolis, Indiana, USA). The hct was determined using an IEC Centrifuge Micro Hematocrit (Damon IEC Division) and a Spirocrit® (Sherwood, Medical Industries Inc., St. Louis, Missouri, USA).

Calculations and statistics

The following parameters were calculated per exchange: net ultrafiltration rate (nUFR), mean osmotic gradient (MOG) and effective peritoneal capillary blood flow (EPBF) according to the equations:

$$\text{nUFR} = (V_d - V_i)/t$$
$$\text{MOG} = 1/2 \times [(DS_{Osm} - P_{Osm}b) + (DI_{Osm} - P_{Osm}e)]$$
$$\text{EPBF} = (2 \times \text{maximum nUFR})/(1 - \text{hct})$$

where

V_d = drainage volume,
V_i = instillation volume,
t = total cycle time,
DS_{Osm} = dialysis solution osmolality,
DI_{Osm} = dialysate osmolality,
P_{Osm} = plasma osmolality at the beginning ($P_{Osm}b$) and at the end ($P_{Osm}e$) of the exchange.

For comparisons of nUFR, it was assumed that capillary hydrostatic and oncotic pressures were constant with consecutive exchanges using the same solution. Blood volumes removed for sampling were not large, water and electrolyte losses were replaced, and during the hyperosmotic exchanges high doses of insulin were also a source of protein. All this suggests that effective ultrafiltration pressure should be similar in exchanges performed with the solution of the same osmolality.

Calculations of dialysate-to-blood (D/B) ratios and peritoneal clearances (C_p) were performed after every five minute period of each dwell using separately the partial pressures of CO_2 obtained from arterial and venous blood as well as urea concentrations in arterial blood. The following formulae were used for calculations:

$$D/B = DI'/B'$$
$$C_p = DI'/B' \times V_d'/t'$$

where: DI' is the solute level in dialysate at the beginning of each five minute period or at the end of dwell (minus solute level in dialysis solution before instillation for C_p calculations); B' is the interpolated blood solute level at the beginning of each five minute period or at the end of dwell; V_d' is the interpolated dialysate volume at the beginning of each five minute period or at the end of dwell; and t' is the duration of dwell.

K_{BD} values for CO_2 were calculated for the first five minutes of dwell of exchange three of both protocols. In later exchanges, especially hyperosmotic ones, the D/B ratios for CO_2 gas exceeding unity were shown during dwell, indicating that peritoneal transfer of CO_2 gas was influenced by mechanism(s) other than diffusion from arterial blood. Full (A) and simplified (B) equations of Garred, Canaud and Farrell [15] as well as the simplified equation (C) of Lindholm, Werynski and Bergström [16] were used for calculation of K_{BD}s for CO_2 gas:

$$K_{BD} = \frac{V_D}{t} \times \left\{ \ln[V_D^o(C_B^o - C_D^o)] - \ln[V_D(C_B - C_D)] + \int_{C_B^o}^{C_B} \frac{dC_B}{C_B - C_D} \right\} \quad (A)$$

$$\bar{K}_{BD} = \frac{\bar{V}_D}{t} \times \{\ln[V_D^o(\bar{C}_B - C_D^o)] - \ln[V_D(\bar{C}_B - C_D)]\} \quad (B)$$

$$\bar{K}_{BD} = \frac{\bar{V}_D}{t} \times [\ln(\bar{C}_B - C_D^o) - \ln(\bar{C}_B - C_D)] \quad (C)$$

where

C_B^o = blood solute level at the beginning of exchange,
C_B = interpolated blood solute level after the first 5 minutes of dwell,
$\bar{C}_B$ = interpolated blood solute level in the midpoint of the first 5 minutes of dwell,
C_D^o = solute level in dialysis solution;
C_D = solute level in dialysate after the first 5 minutes of dwell,
V_D^o = instillation volume,
V_D = interpolated dialysate volume after the first 5 minutes of dwell,
$\bar{V}_D$ = interpolated dialysate volume in the midpoint of the first 5 minutes of dwell,
t = 5 minutes.

Although K_{BD}s for CO_2 gas were derived only from exchange 3 of both protocols in which the D/B ratios for CO_2 were the lowest, the results of CO_2 gas determination obtained in these exchanges were additionally analyzed to achieve the most credible parameters for the estimation of EPBF. If solute concentration in dialysate depends mainly on diffusion from blood, and changes in solute concentration in blood are connected mainly with its peritoneal excretion, then ln(1 − D/B) values plotted versus dwell time should approach a straight line. Spencer and Farrell [17] calculate K_{BD}s, when $\ln[V_D(C_B - C_D)]$ values plotted versus dwell time create a straight line. We have assumed a linear fall of logarithm values when a correlation of six experimental values from each dwell of exchange 3 and those calculated from a regression line revealed $r > 0.88$. With $r = 0.88$ statistical significance of a correlation is obtained at $P = 0.01$ for four degrees of freedom. An analysis of the D/B ratios and logarithm values resulted in a selection of two groups of rats in each protocol. Rats in which the D/B ratios for CO_2 gas were lower than unity during the entire dwell of exchange 3 and both ln(1 − D/B) and $\ln[V_D(C_B - C_D)]$ values, obtained during exchange 3 of both protocols, created straight lines plotted against dwell time were considered to have simple (mainly diffusive) CO_2 transport in the course of this exchange. Rats in which the linear fall in logarithm values could not be obtained over the entire dwell time were considered to have evidence for local CO_2 generation and/or other factors distorting peritoneal transport of CO_2 gas or the estimation of CO_2 pressure. Peritoneal transfer parameters of CO_2 gas representative for EPBF were derived only from the results obtained in rats with features of simple CO_2 transport.

With a linear fall in logarithm values, K_{BD}s can also be calculated from the slope of obtained lines [17]. Under hyperosmotic conditions K_{BD}s for CO_2 gas calculated after the first five minutes of dwell and from the slope yield similar values. Under isosmotic conditions K_{BD}s were higher in the first than in the later minutes of dwell and than those obtained from a slope using all values of 25 minute dwell. The possibility of change in K_{BD}s at different periods of dwell was also noted by Garred et al [15]. Because K_{BD}s for CO_2 gas should represent EPBF and exclude eventual increments in peritoneal resistence during dwell, we have chosen K_{BD}s for the first five minutes of dwell for further analysis.

Urea K_{BD}s were calculated from the slope $-K_{BD}/\bar{V}_D$ of a straight line obtained plotting $\ln[V_D(C_B - C_D)]$ versus dwell time.

Special studies

To increase the number of cases with evidence for simple CO_2 transport, we have performed three exchanges (two in and out followed by one lasting 30 min) using near isosmotic dialysis solution in three rats and very hyperosmotic solution in another

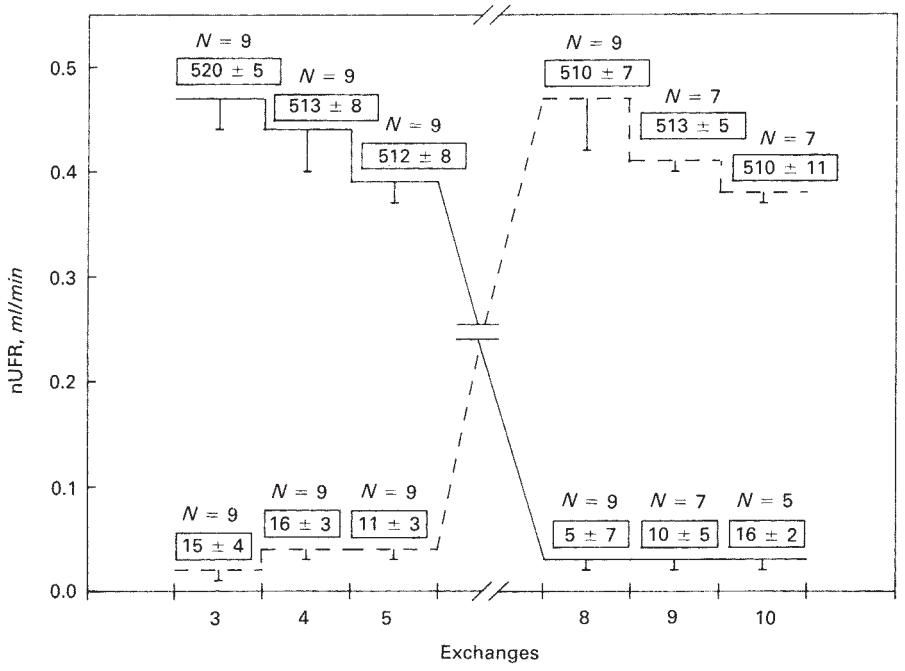

Fig. 1. *Net ultrafiltration rate (nUFR) and mean osmotic gradient (in rectangles, mOsm/kg) during dialysis solution exchanges with 25-minute dwell time.* Dialysis protocols: (- - -) isosmotic to hyperosmotic; (——) hyperosmotic to isosmotic.

eight animals. CO_2 gas pressure was determined during exchange 3 as in previously described protocols. Results obtained in this exchange, when they met criteria for simple CO_2 transport, were included so that the number of rats was nine for each subgroup with simple CO_2 transport in the two major protocols.

After an analysis of the results, nUFR, C_pCO_2 and K_{BD} for CO_2 values for estimation of EPBF were chosen and statistically analyzed.

Results are expressed as mean ± SEM. The Student's *t*-test for paired samples, the Mann-Whitney test or the Wilcoxon test were used for statistical analysis. Significance was established at levels of $P < 0.05$.

Results

Net ultrafiltration rate

The results of nUFR obtained at given MOG values are presented in Figure 1. The slight decrease in nUFR during subsequent hyperosmotic exchanges was not statistically significant. There were also no significant differences in nUFR when exchanges of dialysis solution of the same osmolality were performed at the beginning (exchanges 3 to 5) or at the end of the study (exchanges 8 to 10).

Peritoneal clearances and D/B ratios

Values of clearances are presented in Figures 2 through 4; those of D/B ratios are in Figure 5. The results obtained using CO_2 gas measurements, which are presented in these figures, were achieved in all rats investigated during both protocols.

The maximum values of C_pCO_2 and $C_p$urea were obtained after five minutes of dwell. In the later periods of dwell a gradual decrease in arterial and venous clearances was observed during dwell in all exchanges, as is shown in Figure 2 for exchange 3 of both protocols. However, when values obtained

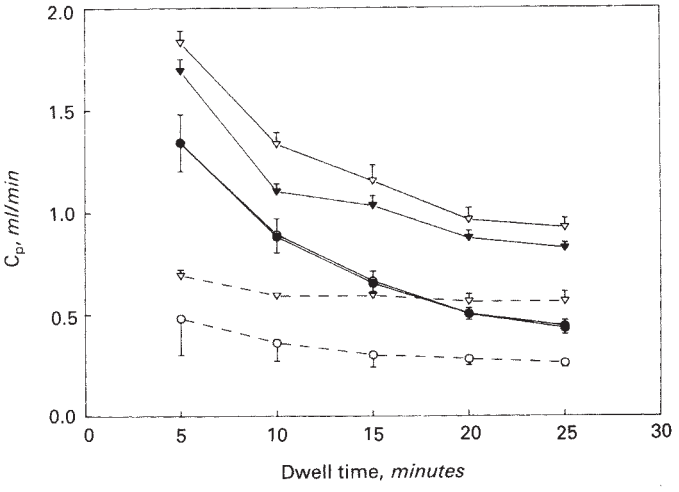

Fig. 2. *Peritoneal clearances (C_p) of CO_2 gas (solid lines) (N = 9 for each curve point) and urea (dashed lines) (N = 3 for each curve point) related to dwell time under near isosmotic and very hyperosmotic conditions.* Symbols are: (○) arterial isosmotic; (●) venous isosmotic; (▽) arterial hyperosmotic; (▼) venous hyperosmotic.

between 5 and 10 minutes of dwell were compared, a fall in clearances was significant in all exchanges only for arterial C_pCO_2. A decrease in $C_p$urea was significant only in the hyperosmotic exchange 3.

During the isosmotic-hyperosmotic protocol, arterial and venous C_pCO_2 as well as $C_p$urea did not show significant differences for respective values in three consecutive exchanges of dialysis solution of the same osmolality (Fig. 3). During the hyperosmotic exchanges in the hyperosmotic-isosmotic protocol, a significant increase in arterial C_pCO_2 (1.24 ± 0.06 ml/min vs. 1.39 ± 0.08 ml/min, $P = 0.048$) and $C_p$urea (0.59

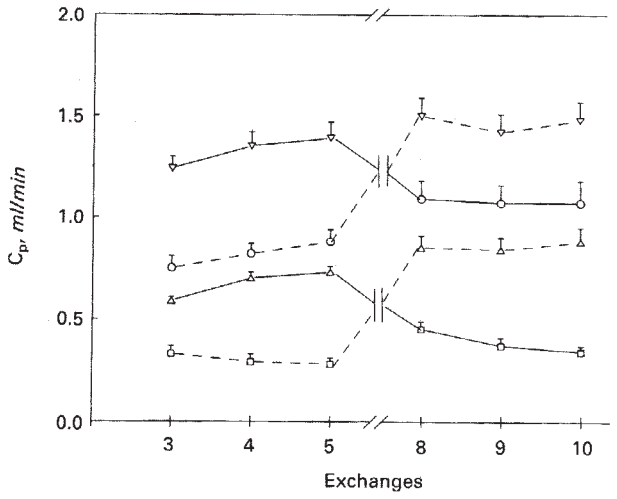

Fig. 3. *Arterial peritoneal clearances (C_p) in consecutive dialysis solution exchanges obtained during 25 minute dwell for CO_2 gas (mean ± SEM for curve points derived from 25 to 45 C_pCO_2 calculations) and for urea (mean ± SEM for each curve point achieved from 15 $C_p$urea calculations).* Symbols are: (○) isosmotic of CO_2; (▽) hyperosmotic of CO_2; (□) isosmotic of urea; (△) hyperosmotic of urea; (– –) isosmotic to hyperosmotic; (——) hyperosmotic to isomotic.

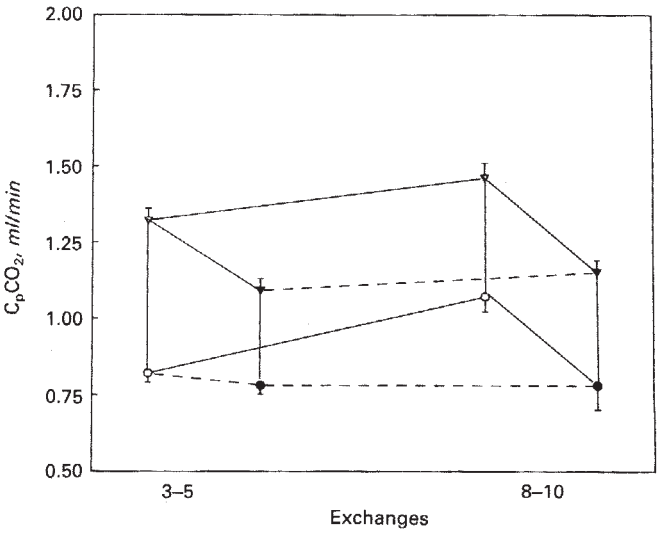

Fig. 4. *Arterial and venous peritoneal CO_2 gas clearances (C_pCO_2) under near isosmotic and very hyperosmotic conditions in exchanges 3 to 5.* Mean ± SEM obtained from 135 arterial isosmotic (○), 135 arterial hyperosmotic (▽), 135 venous isosmotic (●) and 90 venous hyperosmotic (▼) C_pCO_2 calculations) and in exchanges 8 to 10 (mean ± SEM derived from 115 arterial isosmotic, 105 arterial hyperosmotic, 20 venous isosmotic and 70 venous hyperosmotic C_pCO_2 calculations). Differences were statistically (——) significant or (– –) nonsignificant.

± 0.02 ml/min vs. 0.73 ± 0.03 ml/min, $P = 0.0002$) was observed up to values which did not differ significantly from those obtained under hyperosmotic conditions in the isosmotic-hyperosmotic protocol (1.50 ± 0.09 ml/min, 1.42 ± 0.09 ml/min and 1.48 ± 0.09 ml/min in the consecutive exchanges 8 to 10 for C_pCO_2; 0.85 ± 0.06 ml/min, 0.84 ± 0.06 ml/min and 0.88 ± 0.07 ml/min in the consecutive exchanges 8 to 10 for $C_p$urea). During the course of the isosmotic exchanges of this protocol, C_pCO_2 were stable but higher than during the isosmotic exchanges of the isosmotic-hyperosmotic protocol (1.09 ± 0.09 ml/min vs. 0.75 ± 0.06 ml/min, $P = 0.0008$, 1.07 ± 0.09 ml/min vs. 0.82 ± 0.05 ml/min, $P = 0.0104$ and 1.07 ± 0.11 ml/min vs. 0.88 ± 0.06 ml/min, NS, for respective exchanges of both protocols, and 1.07 ± 0.05 ml/min vs. 0.82 ± 0.03 ml/min, $P = 0.0000$, for all values in the isosmotic parts of both protocols). $C_p$urea decreased (0.45 ± 0.04 ml/min vs. 0.34 ± 0.03 ml/min, $P = 0.0002$) to values not statistically different from those observed under isosmotic conditions of the isosmotic-hyperosmotic protocol (0.33 ± 0.04 ml/min, 0.29 ± 0.04 ml/min and 0.28 ± 0.03 ml/min in the isosmotic exchanges 3 to 5).

Figure 4 presents mean ± SEM values of C_pCO_2 obtained in exchanges 3 to 5 and 8 to 10 when the same dialysis solution osmolality and the same source of blood were used. With the same dialysis solution, mean arterial C_pCO_2 values (and the D/B ratios for CO_2) were higher in exchanges 8 to 10 than in 3 to 5. Venous C_pCO_2 did not differ significantly when mean C_pCO_2 values in exchanges 3 to 5 and 8 to 10 were compared, although venous D/B ratios were higher in exchanges 8 to 10 when very hyperosmotic dialysis solution was being used. Arterial C_pCO_2 (and the D/B ratios for CO_2) exceeded venous except in results from isosmotic exchanges in the isosmotic-hyperosmotic protocol in which the arterial and venous C_pCO_2 (and the D/B ratios for CO_2) were similar. The values of C_pCO_2 under very hyperosmotic conditions were significantly higher than those obtained in isosmotic exchanges. Mean D/B ratios for CO_2 were also higher under hyperosmotic conditions, but significant changes were observed less frequently.

In exchange 3, mean D/B ratios for CO_2 were below unity during all periods of dwell except for arterial values obtained after 25 minutes of dwell with the hyperosmotic solution (Fig. 5). In exchange 10, D/B ratios greater than unity were observed earlier in dwell, in some cases as early as 10 minutes, and reached a mean arterial value as high as 1.45 ± 0.08 for the hyperosmotic solution.

Diffusive mass transport coefficients

Under isosmotic conditions (the group with simple CO_2 transport, $N = 9$), K_{BD}s for CO_2 calculated using blood from the same source were slightly but significantly higher when the equation of Lindholm et al [16] was used for calculation (Table 1). There were no significant differences when results obtained with the full and simplified equations of Garred et al [15] were compared. There were also no significant differences when arterial and venous K_{BD}s for CO_2 gas, calculated using the same equation were compared. In the group with probable local CO_2 generation ($N = 3$) K_{BD} values for CO_2 (4.20 ± 0.29 ml/min) were about two times greater, when the simplified equation of Garred et al [15] was used for calculation.

In the hyperosmotic exchange 3 in rats of the group with simple CO_2 transport ($N = 9$), K_{BD}s for CO_2 obtained according to the equation of Lindholm et al [16] were significantly higher than K_{BD}s calculated using the full and simplified equations of Garred et al [15] when results obtained with blood from the same source were compared (Table 1). The K_{BD}s obtained with the full equation were slightly but significantly higher than those achieved with the simplified equation of Garred et al [15].

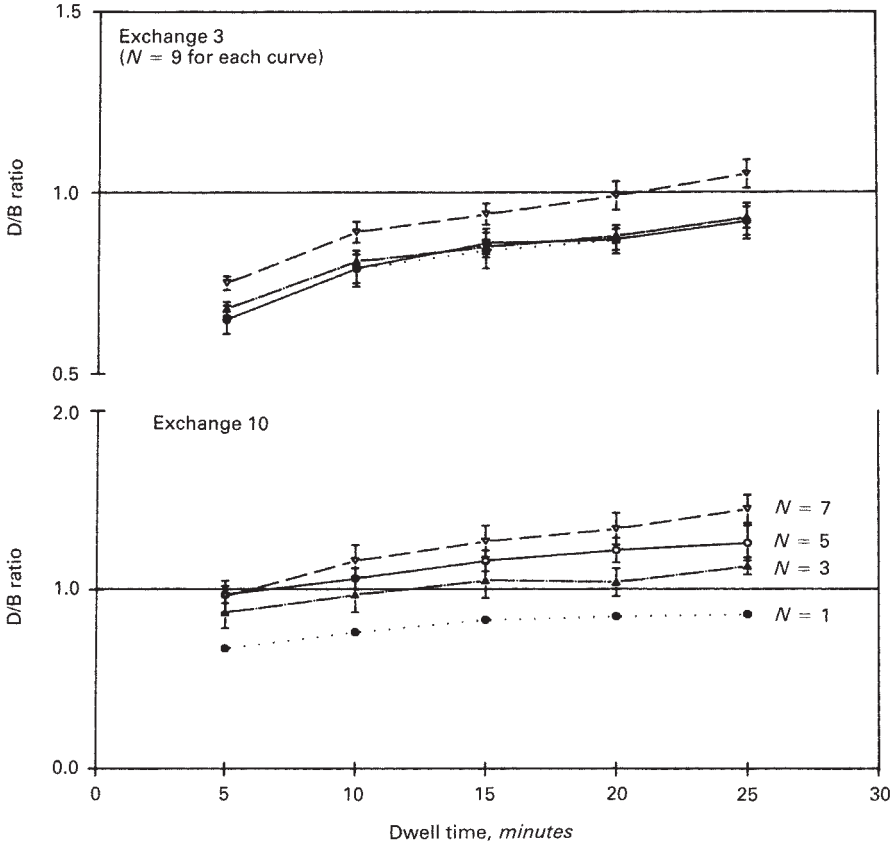

Fig. 5. *Dialysate-to-blood (D/B) ratios for CO_2 gas in exchanges 3 and 10 of the isosmotic-hyperosmotic and the hyperosmotic-isosmotic dialysis protocols.* Symbols are: (○——○) arterial isosmotic; (● · · · ●) venous isosmotic; (▽——▽) arterial hyperosmotic; and (▲) venous hyperosmotic.

Table 1. Diffusive mass transport coefficients ($K_{BD}s$) for CO_2 gas obtained after the first minutes of dwell in rats of the group with simple CO_2 transport ($N = 9$)

	$K_{BD}s$ (ml/min) calculated according to the equation of					
	Garred et al [15]				Lindholm et al [16] simplified	
	Full		Simplified			
	Arterial	Venous	Arterial	Venous	Arterial	Venous
Near isosmotic conditions						
Mean	1.94	1.94	1.95	1.95	1.98	1.98
SEM	0.13	0.16	0.13	0.16	0.13	0.15
Hyperosmotic conditions						
Mean	2.86	2.50	2.69	2.32	3.26	2.83
SEM	0.08	0.16	0.10	0.14	0.10	0.17

Venous $K_{BD}s$ were significantly lower than arterial. In the group with probable local CO_2 generation ($N = 5$), $K_{BD}s$ calculated according to the simplified equation of Garred et al [15] reached a mean value of 3.48 ± 0.48 ml/min.

$K_{BD}s$ for urea showed the same pattern of changes during both protocols as did urea clearances (Fig. 6).

Monitored rat parameters

Temperature, hct values, arterial blood pressure and heart rate were stable during both dialysis protocols. Blood glucose concentrations at the beginning of exchanges and a mean respiration rate are presented in Table 2. In two cases glucose levels exceeded 400 mg/dl at the end of third hyperosmotic exchange of the isosmotic-hyperosmotic protocol. In one of these cases the respiration rate increased as shown in Fig. 7.

Choice of parameters for EPBF estimation

According to the previous theoretical background and experimental evidence [6, 9, 10], we have decided to estimate EPBF

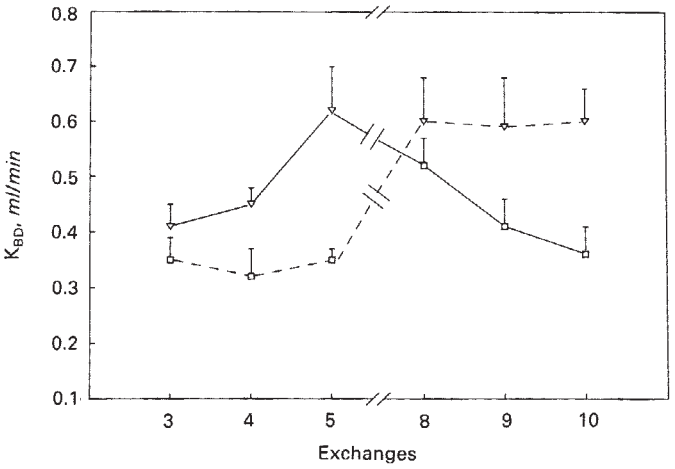

Fig. 6. *Values of diffusive mass transport coefficient (K_{BD}) for urea during the isosmotic-hyperosmotic (– – –) and the hyperosmotic-isosmotic (——) protocols (N = 3 for each curve point).* K_{BD} values are: (□) isosmotic; (▽) hyperosmotic.

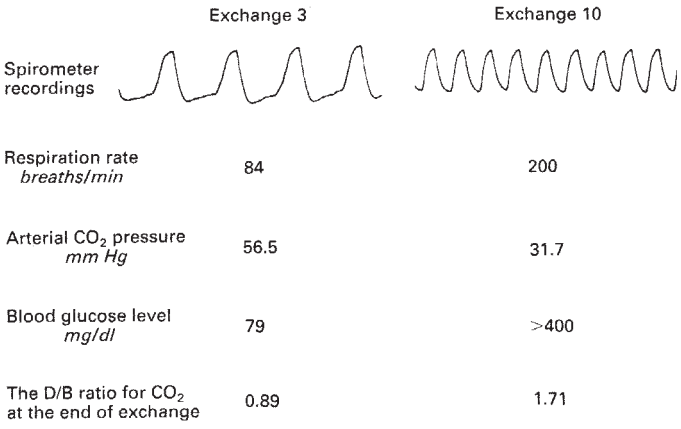

Fig. 7. *Changes in respiration rate during the isosmotic-hyperosmotic protocol in the rat 13 with a very high D/B ratio for CO_2 gas.*

Table 2. Values of blood glucose concentration at the beginning of exchanges and a mean respiration rate during the isosmotic-hyperosmotic and the hyperosmotic-isosmotic protocols

	Exchange					
	3	4	5	8	9	10
The isosmotic-hyperosmotic protocol						
Blood glucose concentration *mg/dl*						
mean	65	70	63	225	225	258
SEM	9	10	7	32	17	31
N	9	9	9	9	7	7
Respiration rate *breaths/min*						
mean	67	69	68	89	118	140
SEM	3	3	3	2	7	11
N	9	9	9	9	7	7
The hyperosmotic-isosmotic protocol						
Blood glucose concentration *mg/dl*						
mean	133	154	242	220	170	149
SEM	22	19	29	30	39	29
N	9	9	9	9	7	5
Respiration rate *breaths/min*						
mean	87	90	93	93	86	82
SEM	4	5	5	5	5	5
N	9	9	9	9	7	5

using maximum nUFR, short dwell time C_pCO_2 and K_{BD} for CO_2.

Levin et al [12] found that 15% dextrose dialysis solution yields maximum nUFR during short cycle peritoneal dialysis in well hydrated rats and that further increases in dextrose concentration or the use of vasoactive drugs are not able to increase ultrafiltration. Using 15% dextrose dialysis solution we have obtained results comparable to those of Levin et al [12]. Because there was no significant differences between values of nUFR obtained in all hyperosmotic exchanges (Fig. 1), a mean maximun nUFR was calculated using values found in all these exchanges (N = 48). Thus, with a transmembrane osmotic gradient over 500 mOsm/kg, exerting a potential osmotic pressure able to support a 9,500 mm column of mercury [18], maximum nUFR (0.43 ± 0.01 ml/min, N = 48) for the EPBF calculation was achieved in our studies.

Because of a significant decrease in C_pCO_2 during dwell (Fig. 2), values obtained in the tenth or later minutes of dwell cannot be used instead of or simultaneously with the values from five minutes of dwell as can be done with no great error in the case of urea peritoneal transfer parameters [19]. Thus, the following five-minute dwell-C_pCO_2 values were taken for further analysis: arterial and venous from isosmotic exchange 3 (1.20 ± 0.08 and 1.19 ± 0.08 ml/min, respectively) as well as arterial and venous from hyperosmotic exchange 3 (1.84 ± 0.04 and 1.72 ± 0.04 ml/min, respectively). All chosen results of C_pCO_2 were achieved in rats with simple CO_2 transport.

K_{BD}s for CO_2 gas obtained in rats of the group with simple CO_2 transport of both protocols chosen as estimates of EPBF for isosmotic and hyperosmotic conditions are presented in Table 1.

Comparison of EPBF estimates

K_{BD}s for CO_2 achieved under isosmotic conditions were significantly smaller than K_{BD}s chosen as estimates of EPBF for hyperosmotic conditions when values obtained using the same source of blood and the same equation for calculation were compared. Also C_pCO_2 values were higher during hyperosmotic exchanges then during isosmotic ones. C_pCO_2 results were smaller compared to K_{BD} values obtained under the same conditions.

EPBF, calculated according to the equation presented in the **Methods** using maximum nUFR and assuming that filtration fraction is 50%, was 1.63 ± 0.06 ml/min (N = 48), which was significantly lower than all K_{BD} values chosen as estimates of EPBF under very hyperosmotic conditions.

The peritoneal filtration fraction calculated as nUFR/[EPBF × (1 − hct)], where EPBF = K_{BD} for CO_2 gas, yielded values of 32 ± 2% for the hyperosmotic conditions and 4 ± 1% for the isosmotic ones.

Discussion

Based on the previous gas analysis results [9, 10], we have assumed that peritoneal transfer parameters of CO_2 gas can represent EPBF. However, in our rat model of peritoneal dialysis arterial D/B ratios for CO_2 significantly exceeding unity were found. Such values indicate CO_2 appearance in dialysate in greater amounts than can be accounted for only by simple transport from arterial blood and/or intensive CO_2 removal from blood by ways other than peritoneal dialysis (such as, hyperventilation) [20]. According to Nolph et al [10], pH adjustment of dialysis solutions to values exceeding 7.0 should have prevented CO_2 generation from bicarbonate in dialysate. However, local peritoneal capillary CO_2 generation from bicarbonate and/or local peritoneal membrane or visceral CO_2 production could be involved. The D/B ratios for CO_2 exceeding unity to a greater degree in the final than in the beginning exchanges, as well as higher values of C_pCO_2 during the isosmotic exchanges of the hyperosmotic-isosmotic than the isosmotic-hyperosmotic protocol, suggest changes occurring with the prolongation of dialysis. On the other hand, because urea is a solute which is not locally generated, the fall of peritoneal transfer parameters of urea in the final isosmotic exchanges suggests that peritoneal permeability was transiently enhanced, probably due to the previous use of very hyperosmotic dialysis solution, with values decreasing towards those usually observed under isosmotic conditions. Increased peritoneal transfer parameters during and after series of hyperosmotic exchanges (when near isosmotic exchanges are resumed) have been previously described [21]. In cultured human proximal tubule cells, it has been demonstrated that glucose concentrations occurring in diabetic urine alter the paracellular, and possibly also transcellular routes, of transport regulation [22]. Although a like influence could be still more pronounced when 15% dextrose dialysis solution acted on mesothelial cells, Levin et al [12] demonstrated that mesothelial boundaries were intact and no mesothelial denudation or injury was apparent after few exchanges with 15% dextrose solution. Moreover, a typical sieving of sodium occurred with maximum nUFR, indicating a functional integrity of the peritoneal membrane [12].

In some cases hyperventilation leading to a rapid decrease of CO_2 pressure in blood could contribute to the development of extremely high D/B ratios for CO_2 gas (Fig. 7), as the change in blood CO_2 pressure during the exchange would not be linear and the interpolated values would be too low if an abrupt decrease in blood CO_2 pressure occurred late in the exchange.

Appearance of processes influencing diffusive peritoneal transport of CO_2 makes C_pCO_2 and K_{BD} for CO_2 gas obtained under such conditions less reliable for an estimation of EPBF. Our results indicate that such influences should be excluded even with isosmotic solution. In order to keep animals under more physiological conditions (without hyperglycemia and its consequences), a poorly absorbed osmotic agent could be estimated in further investigations of maximum nUFR.

K_{BD} values for CO_2 obtained in rats with simple CO_2 transport exceeded C_pCO_2 about 1.5 times when measured after the first five minutes of dwell. Thus, five minutes is too long a dwell time to equalize C_p and K_{BD} for CO_2. We attempted to measure clearances immediately after dialysis solution instillation, but probably because of differences in the instillation rate during the 15 second inflow which are difficult to avoid with manual technique, the coefficient of variation was unacceptably high. Because of significant difference between C_p and K_{BD} for CO_2, the former cannot be used as an estimate of EPBF.

K_{BD}s for CO_2 calculated according to the simplified equation of Garred et al [15] using arterial CO_2 pressure yield mean EPBF of 1.9 ± 0.1 ml/min under isosmotic and 2.7 ± 0.1 ml/min under hyperosmotic ones, that is 4.9 ± 0.3 (range 3.9 to 6.5) and 8.1 ± 0.5 (range 5.8 to 11.6) ml/min/kg body weight, respectively. Our results representative for near isosmotic exchanges are close to those reported by other investigators in anesthetized animals under similar conditions of dialysis solution osmolality. Aune [9] using peritoneal clearance of hydrogen gas in rabbits obtained values between 2.5 and 6.2 ml/min/kg body weight. Granger et al [23] estimated EPBF in cats with a radioactive microsphere technique and obtained a mean value of 4.0 ml/min/kg body weight; however, they recognized it was underestimated because peritoneal blood flow to the surfaces of liver, pancreas and spleen was not included in the calculation.

There is no published data for a comparison of EPBF obtained in our studies under very hyperosmotic conditions with findings of other investigators. However, Granger et al [23] found that replacement of 1.5% dextrose dialysis solution with 4.25% dextrose causes a 45 to 51% increase in EPBF through mesentery, parietal peritoneum and omentum; EPBF through intestinal serosa was enhanced nearly three times under these conditions. Our results indicate a mean increase in EPBF of 42% (or 65% when recalculated per kg body weight) under very hyperosmotic conditions compared to isosmotic ones.

Does an increment in K_{BD}s for CO_2 represent an increase in EPBF? The K_{BD} parameters, which characterize the peritoneal membrane diffusion resistence, are usually measured in the absence of ultrafiltration or when very little ultrafiltration occurs in order to eliminate the influence of convective transport [15, 24]. Thus, one may question how much the K_{BD} obtained during hyperosmotic conditions is influenced by ultrafiltration and how much by blood flow. However, Popovich et al [25] have shown that convective transfer mechanisms become increasingly important as the molecular weights of solutes increase. CO_2 gas a has low molecular weight (44 daltons) and diffuses very easily across the peritoneal membrane [26], probably also directly through endothelial and mesothelial cells [10, 27]. Thus, the ultrafiltration effects on K_{BD}s for CO_2 gas are expected to be relatively small. The use of the equations of Garred et al [15] for K_{BD} calculation should eliminate to some degree this influence as is indicated by the comparison of K_{BD} values obtained using the equation of Lindholm et al [16] without correction for dialysate volume.

When high ultrafiltration occurs in short cycle peritoneal dialysis with the use of a very hyperosmotic dialysis solution, periods of dwell in which the dialysate volume does not change significantly (the so-called "dialysate isovolemia" [16]), practically do not exist. Thus, the equations of Garred et al [15] for K_{BD} calculation are preferred under such conditions. When the near isosmotic solution is used and little ultrafiltration is seen, K_{BD} values for CO_2 gas obtained according to the equations of Garred et al [15] and Lindholm et al [16] differ much less but also significantly. Both the full and simplified equations of Garred et al [15] yield similar results; therefore, for the routine use a simplified equation is more convenient.

For clinical practice the choice of a blood source is very important, thus, we have compared arterial as well as venous peritoneal transfer parameters of CO_2. In our model, arterial and venous C_pCO_2 (and K_{BD} for CO_2) did not differ under isosmotic conditions, but when very hyperosmotic dialysis solution was used greater differences in CO_2 pressure between arterial and venous blood resulted in smaller values of the venous parameters.

Using maximum nUFR for EPBF calculation, we could not obtain agreement with the value of EPBF estimated from the K_{BD} for CO_2 gas when plasma filtration fraction was assumed to be 50% as according to Ronco et al [6]. In rats under normal hydropenic conditions and hct values, filtration fraction appears to be far below 50%. Using the EPBF estimated by K_{BD} for CO_2 and nUFR, we have calculated the plasma filtration fraction as nUFR/[EPBF × (1 − hct)] and obtained mean values of 32 ± 2% and 4 ± 1% under hyperosmotic and isosmotic conditions, respectively. The value obtained during very hyperosmotic exchanges presumably represents the maximum peritoneal filtration fraction in rats. It is worthwhile to notice that our value of maximum peritoneal filtration fraction approximates the glomerular filtration fraction which is predicted to be 30% in rats [28]. Thus, our data indicate the following relationship between EPBF and maximum nUFR, which may be useful at least in the rat model of peritoneal dialysis:

$$\text{EPBF} = (\approx 3 \times \text{maximum nUFR})/(1 - \text{hct}).$$

When dialysis solution has an osmolality which is not able to create the maximum nUFR, filtration fraction was smaller and in rats under near isosmotic conditions EPBF exceeded nUFR about 57 times. When maximum nUFR was achieved, EPBF was about six times greater than maximum nUFR.

The measurements of EPBF using K_{BD} for CO_2 can be helpful in an estimation of the potential maximum nUFR, assuming peritoneal filtration fraction equals glomerular filtration fraction. In our rat model, the potential maximum nUFR under isosmotic conditions is predicted to be 0.32 ml/min. This value is only slightly lower than maximum nUFR obtained with very hyperosmotic solution. Since our results indicate a great difference between EPBF and nUFR, it is unlikely that normal EPBF significantly limits nUFR during peritoneal dialysis as previously suggested [6, 12]. Thus, the major limiting factor for ultrafiltration remains speculative. The very high osmotic pressure driving force applied in our studies was unable to overcome forces which oppose ultrafiltration. Using the polysulphone hollow-fiber hemofilter, Ronco et al [6] could obtain a filtration fraction of 50% with the low blood flow and high transmembrane pressure under conditions similar to those observed during peritoneal dialysis. Thus, resistence exerted by the peritoneal membrane may limit water movement from peritoneal capillaries to dialysate. Enhanced lymphatic absorption may diminish the maximum nUFR. These two factors in our rat model of peritoneal dialysis may be the major differences between the in vitro model of Ronco et al [6]. Sclerotic changes in the peritoneal membrane and enhanced lymphatic absorption are well known reasons of impaired ultrafiltration during clinical peritoneal dialysis [3].

Mean EPBF in humans, calculated by Ronco et al [6] assuming a filtration fraction of 50%, is 22.4 ml/min. This value is significantly lower than those reported by other investigators: 68 to 82 ml/min by Nolph et al [10] and over 100 ml/min by Granger et al [22]. Peritoneal clearances of some small solutes, as for example $C_p$urea [10], can exceed EPBF calculated by Ronco et al [6]. However, even if it is assumed according to our rat studies that in humans the peritoneal filtration fraction also approximates the glomerular filtration fraction, an estimation of EPBF yields a value as small as 60 ml/min when calculated using the plateau in nUFR and hct value found by Ronco et al [6] with 2.5% dextrose dialysis solution. This result most likely is lower than those mentioned above because the plateau in nUFR obtained with 2.5% dextrose dialysis solution probably does not represent the maximum nUFR which can be achieved in humans. Thus, previous estimates of EPBF near 68 to 100 ml/min in humans during peritoneal dialysis may be more reasonable.

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