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The influence of solution composition on protein loss during peritoneal dialysis

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A number of recent studies in man [1–3] and rats [1, 4, 5] has investigated the influence of the composition of commercial peritoneal dialysis solutions on the microcirculation and on the removal of solutes during peritoneal dialysis. One of those studies [3] demonstrated that dialysis with a normal osmolality Krebs solution greatly enhanced the concentration of protein in the drainage solution in comparison to that obtained with commercial, 1.5% dextrose solutions. We proposed that these results represented an effect of osmolality on interstitial movement of protein [6]. Since protein movement in the interstitium probably occurs through water channels in the gel-like matrix of the interstitium [7, 8], a change to a high osmolality dialysis solution could dehydrate the interstitium. This would increase the resistance to protein movement and thus decrease the loss of protein in the dialysate. During the first few exchanges, the high osmolality of commercial dialysis solutions could also pull residual protein out of the interstitium because it produced a generalized dehydration of the interstitial tissues. This would lead to large concentrations of protein in the drainage solution during the first few exchanges and then a progressive fall in dialysate protein with subsequent exchanges. An alternative hypothesis to explain such results would be that there is simply a washout of residual protein from the peritoneal cavity or from tissue spaces within the cavity.

The following studies were designed to test which of these two hypotheses, an increased resistance to protein movement through dehydration of the interstitial tissue or a washout of residual protein already present in the peritoneal cavity, could explain our previous findings [3].

Methods

Clinical studies. The patient studies were performed on sixteen patients who manifested stable cardiovascular systems as reflected by their heart rate, blood pressure, and an electrocardiogram. Patients with cardiac arrhythmias, fluctuating blood pressures, severe congestive heart failure, or peritonitis were not accepted for this study. The patients used in the study had chronic renal failure and were scheduled to be treated with peritoneal dialysis. The patients did not experience any infections in these studies or any other major complications. All studies were performed in the Clinical Research Center at the University of Missouri Medical School. All patients gave informed consent.

At least 2 days before the start of an experiment a chronic indwelling Tenckhoff catheter was placed in the abdomen under local anesthesia and peritoneal dialysis was begun. For all experiments each exchange period consisted of the instillation by gravity of 2 liters of a dialysis solution over a 10-min period, a 30-min dwell period for the solution in the peritoneal cavity, and a 20-min period for draining the solution. This provided a total exchange time of 60 min. In each study, a series of six or more exchanges with one type of solution were alternated with a series of six to seven exchanges with another type. Each is described in detail with the results.

During the dialysis studies, fluid intake by the patient approximated net insensible and urine losses. Any negative water balance during any dialysis exchange was replaced by a 0.5% sodium chloride solution during the next dialysis exchange period to prevent any significant volume depletion. For patients who needed to lose sodium and water, a negative balance not to exceed 10 ml/hr was permitted.

For clearance measurements, the total drainage volume per exchange was measured and each dialysate was analyzed for urea, creatinine, inulin, and total protein concentrations. Concentrations of urea, creatinine, and inulin in plasma were measured periodically throughout an experiment, and extrapolated values were used to calculate clearance. A standard mean clearance rate (c ml/min) during the exchange period was calculated for urea (C_{urea}), creatinine (C_{Cr}), and inulin (C_{In}) by the formula: (Drainage volume/time) × (dialysate concentration/serum concentration). Creatinine and urea were measured by Technicon autoanalyzer techniques. Inulin concentrations were measured by the spectrophotometric technique of Brown and Nolph [9] and proteins by the method of Henry, Sobel, and Segalone [10].

The experiments consisted of three studies. Each study compared two of three dialysis solutions: 1.5% Dianeal® and two Krebs solutions. Both Krebs solutions contained 120 mM NaCl, 20 mM $NaHCO_3$, 3.1 mM KCl, 1.7 mM $CaCl_2 \cdot 2\,H_2O$ and 1 mM $MgCl_2 \cdot 6\,H_2O$. The first Krebs had 11.6 mM dextrose and the 1.5% Krebs contained 83.3 mM dextrose. Table 1 lists the three studies along with the major factors by which the solution used in the studies differ.

The first study of five patients involves changes in osmolality, pH, P_{CO_2} (which is near 40 mm Hg in the bicarbonate solutions and less than 10 mm Hg in commercial solutions), and buffer anion. The second study, also of five patients, involves changes in all of the above, except osmolality. The third study involves six patients and examines changes in osmolality without

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Table 1. Clinical studies comparing the use of different peritoneal dialysis solutions

Group	Osmolality	pH	Buffer anion
1 - 1.5% Dianeal	~355	5.6	Lactate
Krebs	~290	7.4	Bicarbonate
2 - 1.5% Dianeal	~355	5.6	Lactate
1.5% Krebs	~355	7.4	Bicarbonate
3 - 1.5% Krebs	~355	7.4	Bicarbonate
Krebs	~290	7.4	Bicarbonate

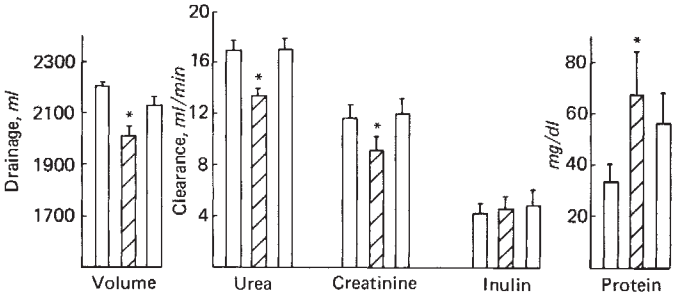

Fig. 1. *A comparison of solutions during peritoneal dialysis differing by osmolality, pH, and buffer anion (see Table 1, group 1).* Seven exchanges with a commercial solution were followed by seven exchanges with a Krebs solution and then another seven exchanges with the commercial solution. The data (mean value ± SEM) are given for each seven exchange sequence. An *asterisk* (*) indicates a significant difference from the first and third *bars* in each triad and was determined by a paired *t* test. Symbols: □, commercial peritoneal dialysis solution (355 mOsm); ▨, Krebs dialysis solution (290 mOsm).

changes in pH, P_{CO_2}, or buffer anion. We have reported previously that change in pH alone with a sodium hydroxide addition to commercial solutions does not alter solute clearances [2] and that changes in pH and P_{CO_2} do not alter solution vasoactivity [1, 4, 6].

Animal studies. In the animal experiments Sprague-Dawley rats (230 to 300 g) were anesthetized with a subcutaneous injection of pentobarbital (50 mg/kg) and prepared for peritoneal dialysis. The animals were placed on a heating pad and rectal temperature was monitored and maintained at 37°C. Back heat was kept below 40°C to avoid painful stimuli which could result from overheating the animal. The left femoral artery was cannulated for measurement and recording of arterial blood pressure was done with a Statham pressure transducer and a Brush recorder. A midline incision approximately 5-mm long was made in the abdominal wall. Tygon tubing (3 mm OD, ~ 20 cm long) was inserted through this incision and the end of the tubing placed near the left, rear wall of the peritoneal cavity. The opening in the abdominal wall, around the Tygon tubing cannula, was closed with a purse-string suture.

A modified Krebs solution (290 mOsm) was used as the dialysis solution and contained 113 mM NaCl, 11.6 mM Dextrose, 4.7 mM KCl, 1.2 mM $MgSO_4 \cdot 7\ H_2O$, 1.2 mM $KH_2\ PO_4$, 2.6 mM $CaCl_2 \cdot 2\ H_2O$, and 25 mM $NaHCO_3$. It was kept heated to 37°C and maintained at a pH = 7.4 and a P_{CO_2} of 40 mm Hg by bubbling carbon dioxide into the solution.

There were nine dialysis exchanges in each experiment. The protocol for each exchange was similar to that used by Brown et al [11]. The dialysis solution was instilled into the peritoneal cavity (1 ml/10 g rat) in a 15-sec period. There was a dwell-time in the peritoneal cavity of 12 min, and then 3 min were allowed for gravity flow drainage of the dialysis solution into a 50-ml graduated cylinder. The drainage volume was measured and saved for analysis. After the first exchange, rat serum albumin which had been conjugated with fluorescein isothiocynate (FITC-RSA) by the method of Chadwick and Fothergill was injected slowly through the femoral artery cannula [12]. Before and after each series of exchanges, blood was collected in a capillary tube via a small incision in the tail for hematocrit and plasma FITC-RSA determinations.

Dialysate from each exchange was analyzed for FITC-RSA concentration, urea concentration, total protein concentration, and osmolality. FITC-RSA concentration was determined using an Aminco Bowman Spectrophotofluorometer. Dialysate and blood was analyzed for urea and protein by the same techniques used in the clinical studies [9, 10].

In both human and rat studies for comparisons of three or more groups, Duncan's new multiple range test with correction for differences in group size was used when group differences were found by analysis of variance [13]. The Student's *t* test was used for paired comparisons. Differences were considered statistically different at $P < 0.05$.

Results

Clinical studies. In the first set of clinical peritoneal dialysis experiments (Table 1, group 1) the effects of commercial peritoneal dialysis solution (lactate-buffered) at a high osmolality (355 mOsm) were compared to the effects of a Krebs dialysis solution (bicarbonate buffer) at a normal osmolality (290 mOsm). The protocol consisted of seven exchanges of the commercial solution followed by seven exchanges of the Krebs solution and then another seven exchanges with the commercial solution. The mean data for the five patients in this study are shown in Figure 1. As expected, there was a decrease in drainage volume during dialysis with the normal osmolality Krebs solution. In addition, small solute clearances (urea and creatinine) were decreased significantly with the normal osmolality Krebs solution while there was a very large increase in dialysate protein concentration (proportionately much higher than can be attributed to the volume decrease).

In the second set of clinical experiments, the effects of the commercial dialysis solution were compared to that of a high osmolality (355 mOsm) Krebs solution in five patients. A group of seven exchanges with the commercial solution (355 mOsm) preceded and followed a group of seven exchanges with a high osmolality Krebs solution (355 mOsm). Figure 2 gives the mean data for this experiment. There were no significant changes in dialysate volume, urea, creatinine, or inulin clearances, or dialysate protein concentrations.

The third clinical group was used to compare the effects of a high osmolality Krebs dialysis solution (355 mOsm) to a normal osmolality Krebs dialysis solution (290 mOsm). In this study, each patient underwent 12 exchanges on 2 different days. On day 1, six exchanges with one of the solutions was followed by six exchanges with the other solution. On study day 2, the order of the solutions was reversed. The composite data in Figure 3 demonstrates that this difference in solution osmolality pro-

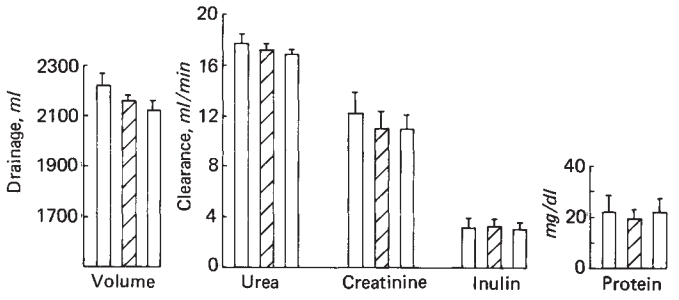

Fig. 2. *A comparison of solutions during peritoneal dialysis differing by pH and buffer anion (group 2).* The same protocol was used as in Figure 1. There were no significant differences for any of the measured parameters. Symbols: □, commercial peritoneal dialysis solution (355 mOsm); ▨, hyperosmolar Krebs dialysis solution (355 mOsm).

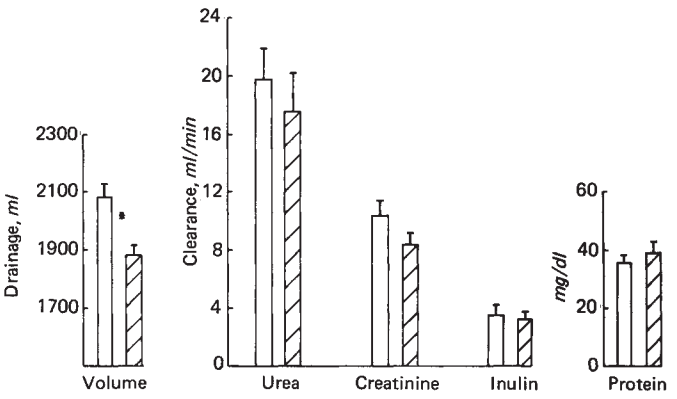

Fig. 3. *A series of 12 peritoneal dialysis exchanges (six with one solution followed by six exchanges with a second solution) in each of six patients on 2 different days (group 3).* Data are presented as the mean value ± SEM for all patients with each solution regardless of the day or order. The only significant difference (paired *t* test) was in the drainage volume. Symbols: □, hyperosmolar Krebs dialysis solution (355 mOsm); ▨, normal osmolar Krebs dialysis solution (290 mOsm).

duced the expected decrease in drainage volume with the normal osmolality solution but no changes in solute clearances.

Group 3 was also used to examine the change in dialysate protein concentrations with progressive exchanges. The curves shown in Figure 4 demonstrate that regardless of the osmolality of the first dialysis solution used in the study there is a high protein concentration in the first exchange which declines with subsequent exchanges until a plateau is reached. Changing the dialysis solution (at exchange 7) from a high to a normal or a normal to a high osmolality solution did not effect this plateau concentration of protein.

Animal studies. Data for the rat dialysis experiments is depicted graphically in Figure 5. Total protein in the dialysate (given in grams per milliliters per minute) is very high in the first exchange but declines in the second, third, and fourth exchanges and then plateaus by the fifth exchange. In contrast, the fluorescent-labeled albumin (FITC-RSA), which is injected after the first exchange, has a slightly lower dialysate concentration (given in nanograms per milliliters per minute) in the first few exchanges and plateaus at about exchange seven. Blood hematocrit, drainage volumes, and urea clearances were not altered during these series of exchanges.

Discussion

The initial observation in these studies is shown in Figure 1. There is a higher concentration of dialysate protein when Krebs solution is used as the dialysis solution in comparison to that obtained with 1.5% commercial dialysis solution. These data imply that pH, buffer anion, osmolality or a combination of these factors (Table 1) may be involved in the regulation of the transfer of protein from the vasculature to the peritoneal cavity.

Others have shown that hyperosmolar solutions may increase small solute clearances but the effects on dialysate protein concentration were not examined in these studies [11, 14]. It does appear that with the large solutes, osmolality may alter solute clearances [3, 15, 16]. In comparisons of commercial solutions that used either lactate or acetate buffering systems, no differences were found for large or small solute clearances [2]. It is also unlikely that changes in pH alone could account for these data (Fig. 1) since pH adjustment does not effect clinical clearances or vasodilatory effects of commercial solutions [1, 5, 17]. Thus, of the major differences in solution composition for group 1, osmolality seemed most likely to be involved in the altered protein concentrations found in the dialysate.

In the second set of clinical experiments (group 2), the effects of a commercial dialysis solution were compared to those with a hyperosmolar Krebs solution. In this comparison, no difference was found in protein dialysate concentrations. Thus, a difference in pH and/or buffer anion, both of which were present in this comparison (Table 1) could not account for the increased protein dialysate concentrations seen in group 1.

The third set of data (group 3) compared a normal Krebs solution to a hyperosmolar Krebs solution. pH and buffer anion were the same in the two solutions. Since again no difference in dialysate protein concentrations were found, it does not appear that osmolality changes alone could explain the differences found in group 1. It is of interest that others have found that going from commercial 1.5% dextrose solutions to 4.5% dextrose solutions did increase dialysate protein concentrations [16].

Data for group 3 (Fig. 4) demonstrates that with both high and normal osmolality dialysis solutions, protein concentrations in the dialysate are very high in the first exchange. There is then a progressive decrease in protein concentration with successive exchanges, and a change from a normal to a high osmolality solution or the converse, does not alter steady state levels. A fall in dialysate protein concentration with successive exchanges has also been reported in intermittent peritoneal dialysis studies which used one hyperosmolar commercial solution [16]. The rat studies (Fig. 5) also demonstrate an initially high protein concentration in the dialysate and a progressive fall with successive exchanges. FITC-RSA concentrations, however, started at a low value and then rose slightly with successive exchanges. The FITC-RSA did not have pre-experiment time to equilibrate in the interstitium or accumulate in the peritoneal cavity. Therefore, these data suggest that with FITC-RSA in the first few exchanges, there is an interstitial equilibration, and then with subsequent exchanges there is a constant rate of transfer of protein to the peritoneal cavity.

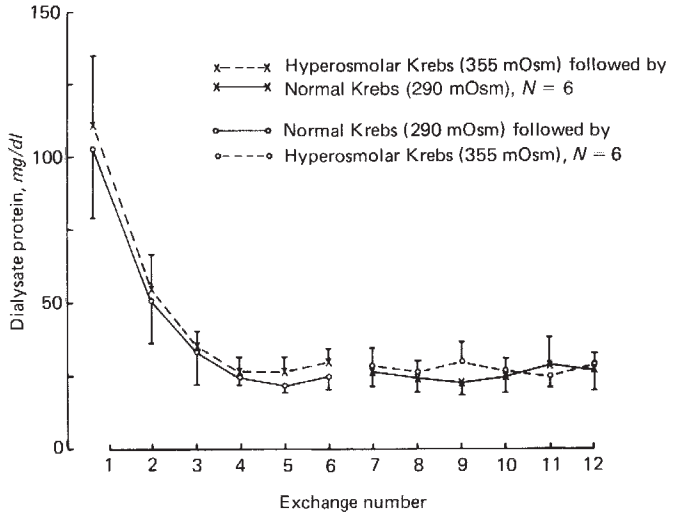

Fig. 4. *The data* (mean value ± SEM) *for each exchange and for each of the two orders of solutions used.* The protocol (group 3) is given in Figure 3.

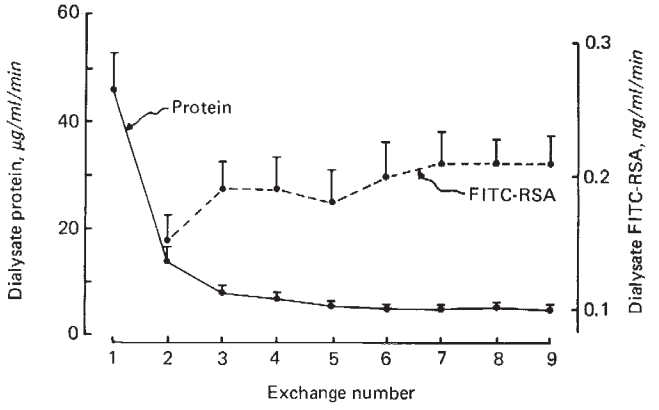

Fig. 5. *Seventeen dialyzed rats using a normal osmolality Krebs solution.* FITC-RSA (rat serum albumin tagged with fluorescein isothiocynate) was injected (i.a.) after the first exchange. Data (mean value ± SEM) are given for protein and FITC-RSA concentrations for each exchange.

The data from group 1 and the work of other investigators on the role of the interstitial space for the passage of proteins was the basis for the hypothesis tested in this paper. Under conditions of normal hydration (normal osmolality) interstitial resistance to protein movement is low and protein transfer would occur easily. In the presence of the high osmolality dialysis solutions, the interstitial space would become dehydrated. Pathways for protein transport would become compressed resulting in an increase in the resistance to protein movement and a decrease in dialysate protein. This hypothesis was not supported by the data from group 3 (Fig. 3). A difference in osmolality did not produce a difference in steady state dialysate protein concentrations.

If the decrease in dialysate protein with successive exchanges (Fig. 4) is due to a dehydration of the interstitium as our hypothesis would also predict, then we would expect in this plot to get the greatest decreasing slope for the highest osmolality solutions and the least slope for the lowest osmolality solution. On the other hand, if the decrease in dialysate protein with successive exchanges is due to a washout of residual protein, then the slopes of the plots of dialysate protein versus the exchange number should be the same with different osmolality solutions. Group 3 data (Fig. 4) gave the same slopes for different osmolality solutions suggesting that this is a washout phenomena.

This conclusion is substantiated by the dialysis studies in the rat (Fig. 5). In these experiments, there was a fall in the dialysate protein concentration which had had time to accumulate in the peritoneum (the total protein measurement, solid line) but not in the protein which was acutely injected (FITC-RSA, dashed line). Thus, both the human and rat experiments, tend to refute the hypothesis that interstitial dehydration leads to diminished protein transfer. But these data do not explain the results obtained in group 1 which show a higher concentration of protein when a normal osmolality Krebs solution is used for dialysis. Such a difference cannot be substantiated by differences in the vasoactivity of the various solutions [1, 4, 5, 17]. It may be that the combination of a different pH and osmolality in group 1 could have influenced protein binding and the availability of appropriate water channels in the interstitium resulting in an altered protein concentration in the dialysate. This alternative has not yet been tested.

Summary. Peritoneal dialysis studies were performed in humans and rats to investigate the hypothesis that the high osmolality of peritoneal dialysis solutions dehydrates the interstitial tissue in the peritoneal cavity resulting in a decrease in dialysate protein concentration. Dialysis with solutions which differed in osmolality, pH, and/or buffer anion indicated that protein dialysate concentration is influenced by changes in two or more of these factors but that changes in one variable only (osmolality, pH, or buffer anion alone) cannot produce these effects. These studies also demonstrated that protein dialysate concentration is very high during the first exchange of the day and then progressively falls with subsequent exchanges. Our data suggest that this phenomenon is due to a washout of residual protein in the peritoneal cavity.

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