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Characterization of Proteins from Continuous Ambulatory Peritoneal Dialysis (CAPD) Fluids

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ABSTRACT

Peritoneal dialysis fluids originally devoid of protein were collected from Continuous Ambulatory Peritoneal Dialysis (CAPD) volunteers. Dwell samples were collected in early morning, mid-day, mid-afternoon and evening. These fluids were found to contain substantial amounts of proteins (90 mg/100 ml for n = 5 patients). Following SDS-polyacrylamide gel electrophoresis, some 16 to 44 different proteins could be detected in the CAPD fluid. The number of protein bands was significantly higher in the morning dwell compared to the last dwell collected in the evening. Albumin was identified as the protein found in highest amounts for all patients. These data suggest that peritoneal dialysis places additional metabolic stresses on patients due to the movement of proteins into the peritoneal cavity from the blood.

INTRODUCTION

Kidney diseases are often life threatening and are increasingly being treated by peritoneal dialysis. However, this type of dialysis may have subsequent detrimental effects on patients and the mesothelial cells that line the peritoneal cavity may be stressed and damaged during long term peritoneal dialysis. This stress could then allow increased leakage of materials into and out of the peritoneal cavity. Peritonitis, a severe bacterial infection, is also a common consequence of peritoneal dialysis treatment. Gram negative bacteria that release lipopolysaccharide (LPS) into the peritoneal cavity as part of the infective process may have some role in further damage to the kidney. The kidney seems especially sensitive to LPS insult. However, the biochemical mechanisms by which the LPS reaches the kidney are not well understood. Mesothelial cells, which line the peritoneal cavity, are thought to form a barrier between the peritoneal cavity and the blood stream. These cells, if damaged during long term dialysis treatments, could allow increased movement of materials such as LPS from the cavity into the blood. Since LPS has such potent effects on body temperature and membrane permeability as well as a

number of other physiological responses, movement of LPS from the peritoneal cavity to the blood can lead to wide spread effects.

Proteins, such as albumin, IgG, C3 and transferrin, have been reported in peritoneal dialysis fluid [1,2,3]. Concentrations of these proteins range from 1 to 50 mg/100 ml of fluid [1,3]. DeVecchi et al. (1990) reported the mean dialysate concentration of IgG was 6.9 $\pm$ 4.2 mg/100 ml with no difference between men and women and no correlation with time from the last peritonitis episode[4]. Lee et al. (1990) reported that the amount of drained protein ranged from 20 to 50 mg/100 ml fluid[5]. Kagen et al. (1990) reported that all lipoproteins (VLDL, IDL, LDL, and HDL) were present in peritoneal effluent. They also reported that HDL loss into the effluent may account for the lower HDL levels in CAPD patients[6]. However, little work has been done evaluating time of day of fluid collection. We have extended these previous studies in this pilot study using Continuous Ambulatory Peritoneal Dialysis (CAPD) fluids collected from five volunteers. The amounts of total protein and the numbers of types of proteins in each fluid are reported and compared within the same patient at different times during a day and between patients for fluids collected at the same time of day.

METHODS

Unless otherwise stated, all reagents were obtained from Sigma Chemical Co., St. Louis, MO. The peritoneal dialysis fluid used (Dianeal PD-2) was obtained from Baxter Healthcare Corp., Il., Round Lake, Il. The Dianeal PD-2 contains mineral salts and glucose but is devoid of protein. The CAPD was collected from the peritoneal cavity of volunteers after 4-8 hours in vivo and frozen in aliquots at -80ºC in sterile polypropylene tubes. For each patient, four dwells were collected (morning, mid-day, late afternoon, and evening). The volume of each dwell was determined using a 2 L graduate cylinder. Protein was determined by the Bio-Rad Micro Assay[7]. Five patients were evaluated whose age and sex are as follows: 22 year old female, 31 year old female, 35 year old male, 81 year old male, and 83 year old female. For each patient a CAPD volume containing 450 ug of protein was lyophilized. The resulting pellets were resuspended in 6 mM Tris-phosphate, 7% sodium dodecyl sulfate (SDS) in a final volume of 50 ul. The entire samples were loaded onto SDS polyacrylamide gels for electrophoresis. Samples were evaluated by one dimensional slab gels (9%) using the method of Laemmli[8] or by a gradient gel using the method of Anderson et al.[9] The gradient gel consisted of 3 cm of 15% acrylamide at the bottom of the gel, overlayered by 5 cm of 12%, 4 cm of 9% and 3.5 cm of 4.5% acrylamide. Preliminary work indicated better protein separation using the gradient system. Following electrophoresis, the gels were stained with 0.03% Coomassie Blue[10]. Bio-Rad Low Molecular Weight Standards were used for determination of apparent molecular weights of CAPD proteins and standard MW proteins migrated as a linear function with a correlation coefficient of 0.993. An image analyzer (Visage 100, BioImage, Ann Arbor, MI) was used to spectrophotometrically scan the gels and determine relative concentrations of proteins. For some samples, antiserum which recognizes human albumin (Sigma Chemical Company, St. Louis, MO) was used to immunoprecipitate a protein found in large amounts in the CAPD. Following incubation (overnight at 4ºC) of CAPD fluid with the antiserum, the immunoprecipate was collected using glass fiber filters. After filtration, the filters were incubated for 4 hours at room temperature in 1 ml of 30% SDS to solubilize the proteins. Both the filtrate and the

retentate were lyophilized and prepared for electrophoresis as above. Data are reported as mean ± SD or SEM and were compared using analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) post test. Differences were considered significant at $p < 0.05$. In some cases, the coefficient of variation was also calculated.

RESULTS AND DISCUSSION

Following peritoneal dialysis, the CAPD fluid contained substantial amounts of protein, approximately 90 ± 50 mg/100 ml fluid (n = 20 dwells). This value is approximately 2-3 fold higher than the values reported by Lee et al.[5] The average total volumes of each dwell was 2235 ± 328 ml (mean ± SD for 20 dwells). Therefore the average dwell contained a total of approximately 2 g of protein. This value is similar to that of Steinhauer et al. (1992) who reported total dialysate protein of 2.62 g using dialysis fluid containing glucose. They also reported that addition of amino acids (2.6%) in the dialysis fluid increased the amount of protein (about 30%) found in the dialysate[14]. Figure 1 shows the average amount of total protein per dwell relative to the sequence in which samples were collected. The dwells were in sequence with # 1 being the first dwell collected for the day and dwell #4 being the last dwell sample of the day. There was no significant difference in total amount of protein per dwell at any time of the day. This differs from the report by Kagen et al. (1990) who concluded that long dwell times (6-8 hours) result in continuous loss of protein throughout the dwell time so that an apparent equilibrium is not reached[11].

Table I shows the range of times for dwell collections for the 5 patients and average length in hours of dwell residence. Dwell sequences 1 through 4 correspond with those in Figure 1. Three of the dwell resident times were constant with an average value of 5 hours. Dwell 1 was about 38% longer since the dwell remained in the peritoneal cavity overnight.

Table I: Time of dwell collection and dwell residence

Dwell Sequence	Dwell Length (hours)*	Time of Collection (range)
#1: morning	8.2 ± 1.0[a]	5:30am - 8:15am
#2: mid-day	5.2 ± 0.3[b]	11:00am - 2:25pm
#3: late afternoon	4.8 ± 0.3[b]	3:30pm - 7:00pm
#4: night	5.1 ± 0.6[b]	8:30pm - 11:15pm

* mean ± SEM for 5 patients; values with the same letter are not significantly different at $P < 0.05$.

Figure 2 shows the average daily protein for all 4 dwells for each individual patient. Between patients there is approximately a two fold variation in the amount of protein removed during the peritoneal dialysis procedure with the values ranging from about 1.5 to 3.0 g of protein per dwell. There was no statistical difference between any of the 5 patients, between males and females or between the different age groups. The coefficient of variation (Cv) for each patient was calculated at 0.5, 0.2, 0.4, 0.3, and 0.4 for patients # 1,2,3,4, and 5 respectively. This value is very similar for 4 of the 5 patients and the average Cv is 0.3. The source of this large variation within and between individuals is

not obvious but is interesting. The data suggest that the average CAPD patient loses about 8 grams of protein per day during peritoneal dialysis.

Using SDS polyacrylamide gel electrophoresis which separates proteins by apparent MW, a number of different proteins in the CAPD were observed. The number and relative concentration of the proteins depended on the patient and time of day collected as shown in Figure 3a and b. For Figure 3a, the same volume of sample (0.5 ml) for dwell # 1 of each patient was lyopholized and then prepared for electrophoresis. Lane 1 contains the molecular weight standards. Figure 3b is the same dwell from the same patients; however, each lane contains the same amount of protein (450 ug). Lane 1 again is molecular weight markers. A comparison of these two gels shows the same relative patterns of protein bands between patients whether using a consistent amount of protein per lane or volume of CAPD fluid per lane. However, all subsequence work was done using the same amount of protein per lane so that the proportion of each protein relative to total protein applied could be estimated. Most of the same proteins as determined by relative migration distance (Rf) are seen in all 5 patients as shown in Figures 3a and b. However, some bands appear to be more prevalent than others and this observation was more evident than when the same volume of sample was applied to the gel.

One protein (Rf = 0.49) was found in such substantial concentrations that it could have masked other proteins. Using the standard curve, an apparent MW was calculated at 64,000 to 68,000 suggesting that this is serum albumin. The width of this band makes Rf determinations difficult. Immunoprecipitation with human albumin antiserum tentatively identified this protein but was not completely effective since about 30% of the human albumin was still detectable in the filtrate and 70% in the precipitate. The immunoprecipitates were not used to obtain quantitative data since the antiserum itself contributed a substantial amount of protein which was visible on gels and thereby obscured the data which we wanted to obtain. Future work might use a highly purified monoclonal antibody for the immunoprecipitation procedure.

There were five major proteins which were found in the highest proportions on the gradient gels as judged from the Image Analyzer data (Figure 3b). An overlay plot of authentic standards tentatively identified protein #1 as IgG, protein #3 as transferrin, and protein #4 as serum albumin. Proteins #2 and #5 (with apparent molecular weights of 106 and 24 kD respectively) were not identified.

To compare the number of proteins found in the CAPD fluids within and between patients, gradient gels were run using the same total amount of protein (450 ug) for each lane. The gels were scanned with the image analyzer to determine the number of bands per patient per dwell and data are show in Table II. The average number of proteins was fairly constant between patients and within a single patient. The mean number of protein bands for dwell #1 (37.6 ± 5.0) was significantly higher than dwell # 4 (28.2 ± 4.3). In dwell #4 there was an apparent decrease of about 25% in the number of detectable proteins on the gel. The large error bar found in dwell #4 in Figure 1 could be partly due to this lower detectability. Other techniques have resulted in detection by electrophoresis of at least 30 serum proteins[12].

Table II: Number Of Proteins Detected In CAPD

Patient #	Dwell #1	Dwell #2	Dwell #3	Dwell #4	Patient mean±sd
1	30	40	37	33	35 ± 4
2	38	37	38	32	36 ± 3
3	38	34	23	28	31 ± 7
4	44	24	32	25	31 ± 9
5	38	16	31	23	27 ± 10
Dwell mean± sd	38 ± 5	30 ± 10	32 ± 6	28 ± 4	

Table III represents the 5 specific proteins labeled on the gel in Figure 3b. The detectable range is from approximately 2% of the total (protein #5) to 26% of total (protein #4) protein of the lane. Of the 450 ug protein loaded, 113 ug was estimated to be protein #4 which is tentatively identified as serum albumin.

Table III: Specific proteins (1-5) as mean ± sd percent of total detected/lane/patient for 4 dwells

Patient #	Protein 1	Protein 2	Protein 3	Protein 4	Protein 5
1	4.2±0.7	8.2±1.8	2.9±0.8	23.0±4.4	1.2
2	4.7±1.8	6.2±2.0	3.0	19.7±5.3	2.1±0.1
3	4.3±1.1	6.3±4.6	4.2±2.6	29.7±9.5	1.8±0.1
4	3.2±1.3	5.1±1.5	3.6±3.3	29.9±6.9	1.7
5	4.6±1.1	6.4±1.2	3.5	26.5±13.5	2.5±0.4
Total Group mean±sd	4.2±0.6	6.4±1.1	3.4±0.5	25.8±4.4	1.9±0.5
Cv	0.14	0.17	0.12	0.12	0.26

Since the quantitative technique used here is able to detect relative amounts, this value of 26% is reasonable. Albumin accounts for some 47-60% of the total serum protein[12]. Due to the width of the gel band, we may have exceeded the linear response region of the Image Analyzer for this protein. The value of 26% is therefore likely to be an underestimate of actual amount of this protein. Also since the protein band found in this region of the gel is so large, this protein could have masked other proteins with a similar Rf but present in smaller amounts. Proteins such as HLA class I and II antigens (with MW of 50,000 and 60,000 respectively) have been reported by Gelder et al. (1990)[13].

In some cases, all protein bands were not found in every patient. For example, protein #3 was not detected in all dwells of all patients. Therefore, there is individual variation which is yet not easily correlated with other physiological or biochemical factors. These 5 proteins account for approximately 42% of the total number of individual bands on the gels. The coefficient of variations in Table III for proteins 1-4 were fairly consistent indicating a relatively small range of variability in the population. The Cv for protein #5

is larger suggesting a less consistent biological response to the stress of peritoneal dialysis. This small MW protein may represent a breakdown product.

Table IV represents the 5 specific proteins labeled on the gel in Figure 3b. There was no significant difference in the percent of each protein as a function of the time of day. However, protein #3 was only detected in one patient in dwell #4, the last dwell of the day collected. Also protein #5 was not detected in any of the patients in the last dwell. In other dwells, the average percent value of the sum of proteins #3 and #5 constitutes about 5% of the total protein loaded onto the gel. From this it is evident that even though the dwells have approximately the same total amount of protein, not all types of proteins are found in each dwell. Kagent et al. (1990) reported that higher molecular weight proteins, such as albumin and immunoglobulins, were lost into the dialysate faster than smaller molecular weight species[11].

Table IV: Specific proteins (1-5) as percent of total protein detected/lane as a mean ±sd for group/dwell

Dwell Time	Protein 1	Protein 2	Protein 3	Protein 4	Protein 5	Total
Morning	5.0±1.0	5.1±2.1	3.7±2.4	22.4±11.3	2.1±0.5	38.3
Noon	4.7±1.3	6.6±1.6	3.0±0.9	26.8±8.4	1.7±0.5	42.8
Afternoon	3.1±0.8	9.0±1.7	4.7±1.7	28.2±11.7	2.0±0.4	47.0
Night	3.9±0.9	5.2±2.5	1.9	25.6±6.2	0	36.6
Mean±sd	4.2±0.8	6.5±1.8	3.3±1.2	25.8±2.5	1.9±0.2	41.7

Peritoneal dialysis has been shown to be an important treatment for kidney failure yet it is not without long term consequences. How much of these consequences is due to the loss of protein in the dialysis fluid as well as stress of the mesothelial cells lining the peritoneal cavity is not well understood at this time. Since a substantial amount of the protein found in the peritoneal dialysis fluid is serum albumin, there clearly is a transport of protein from serum to peritoneal cavity during dialysis. Serum exudate likely accounts for some or most of the other proteins found in the CAPD fluid. The mechanism of this migration may be passive or active. The results from this study suggest that at least some of the proteins found in the CAPD are in apparent equilibrium with blood since there was no significant difference in the amount of protein per dwell with shorter (about 5 hours) or longer dwell times (about 8 hours). With longer dwell time, the coefficient of variation was smaller however. There also was no statistical difference relative to age or gender of the patients suggesting that the mechanism of translocation is common and that an apparent equilibrium between the blood and peritoneal cavity can easily be established. The average 70 kg person contains about 2.8 L of blood which is very similar to the average volume of dwell fluid (2.2 L) recovered from the 5 patients. If the movement of protein from blood to peritoneal cavity is only diffusion controlled, a larger amount of protein is predicted for the CAPD fluid at equilibrium since average serum levels of total protein range from 65-80g per L[12] and we find approximately 1.0g per L in the fluid. We calculate that about 3-4% of the total serum protein is lost per day by peritoneal dialysis patients. This may add a substantial metabolic stress to the system. Clearly, other forces

are involved which specifically decrease the flux of protein from blood to CAPD or increased reabsorption through the lymphatics. This is emphasized by the observation that the last dwell of the day (#4) contained little detectable amounts of some specific proteins (# 3 and #5) as judged by electrophoresis. It is apparent that there are substantially different amounts of these two proteins later in the day relative to early dwells. Also, there was approximately 25% fewer protein bands evident by electrophoresis in the last dwell relative to the first dwell. This pilot study should now be extended to evaluate a larger population of CAPD patients to substantiate our results. A future study to determine the effects of diurnal rhythms on these two proteins or a possible transport system specific for these two proteins would be of interest. Clearly more work remains to determine the source of all of the proteins found in CAPD as well as determining the rate of specific protein influx into the CAPD. The presence of serum proteins in CAPD may stress the mesothelial cells which could result in movement of solutes from the peritoneal cavity to the blood. Further work using mesothelial cells in culture should be considered.

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Figure 1. Average mg protein/dwell for 5 patients as a function of time. Error bars are the SEM values.

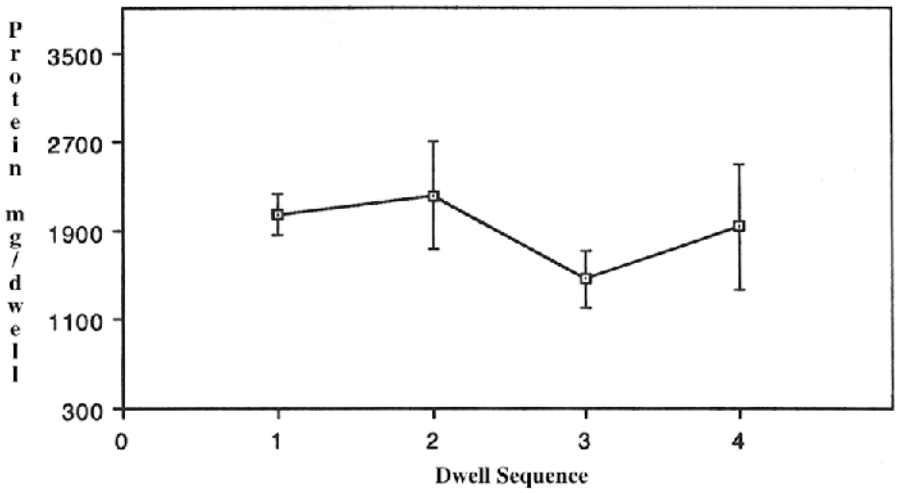


Figure 2. Average daily protein for all 4 dwells for each individual patient is shown. Error bars are the SEM values.

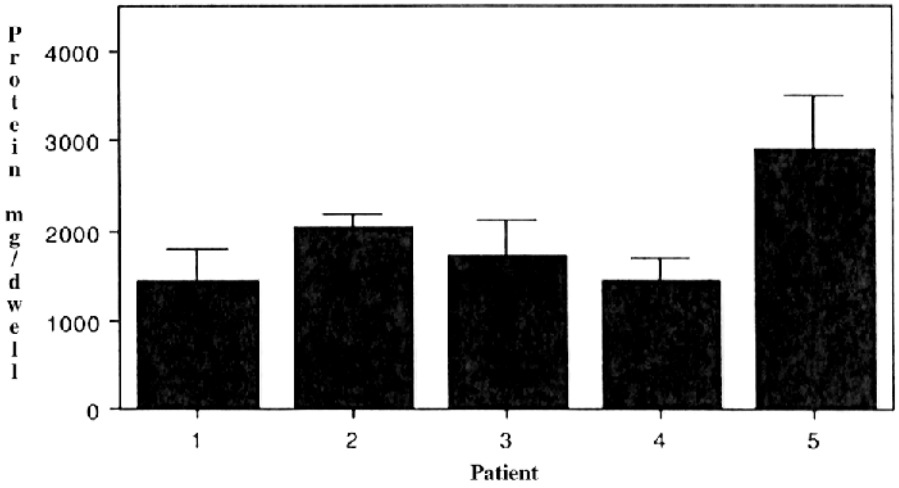
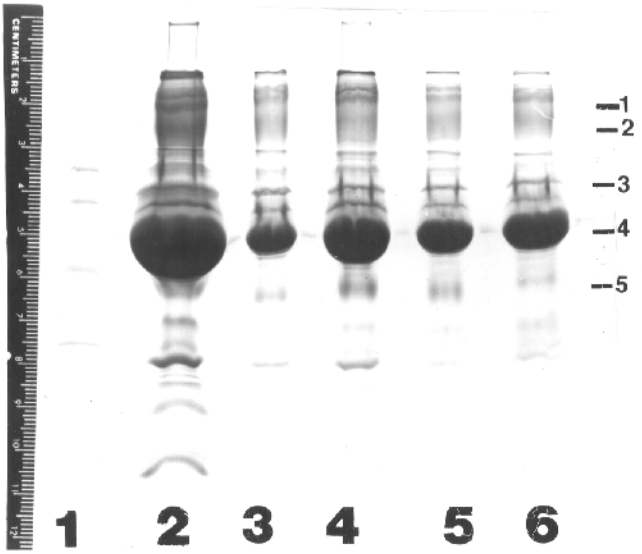


Figure 3. a) A representative gel showing protein profiles from all 5 patients using the same dwell and using the same volume of CAPD fluid per lane. Lane 1 is MW standards. Lanes 2-6 are from patient number 1,2,3,4,and 5 respectively



b) A representative gel showing protein profiles from all 5 patients using the same dwell and using the same amount of CAPD protein per lane. Lane 1 is MW standards. Lanes 2-6 are from patient number 1,2,3,4,and 5 respectively