ORIGINAL ARTICLES

ERYTHROCYTES AS VOLUME MARKERS IN EXPERIMENTAL PD SHOW THAT ALBUMIN TRANSPORT IN THE EXTRACELLULAR SPACE DEPENDS ON PD FLUID OSMOLARITY

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◆ **Background:** Macromolecules, when used as intraperitoneal volume markers, have the disadvantage of leaking into the surrounding tissue. Therefore, ⁵¹Cr-labeled erythrocytes were evaluated as markers of intraperitoneal volume and used in combination with ¹²⁵I-labeled bovine serum albumin to study albumin transport into peritoneal tissues in a rat model of peritoneal dialysis (PD).

◆ **Methods:** Single dwells of 20 mL of lactate-buffered filter-sterilized PD fluid at glucose concentrations of 0.5%, 2.5%, and 3.9% were performed for 1 or 4 hours. Tissue biopsies from abdominal muscle, diaphragm, liver, and intestine, and blood and dialysate samples, were analyzed for radioactivity.

◆ **Results:** The dialysate distribution volume of labeled erythrocytes, measured after correction for lymphatic clearance to blood, was strongly correlated with, but constantly 3.3 mL larger than, drained volumes. Erythrocyte activity of rinsed peritoneal tissue biopsies corresponded to only 1 mL of dialysate, supporting our utilization of erythrocytes as markers of intraperitoneal volume. The difference between the distribution volumes of albumin and erythrocytes was analyzed to represent the albumin loss into the peritoneal tissues, which increased rapidly during the first few minutes of the dwell and then leveled out at 2.5 mL. It resumed when osmotic ultrafiltration turned into reabsorption and, at the end of the dwell, it was significantly lower for the highest osmolality PD fluid (3.9% glucose). Biopsy data showed the lowest albumin accumulation and edema formation in abdominal muscle for the 3.9% fluid.

◆ **Conclusion:** Labeled erythrocytes are acceptable markers of intraperitoneal volume and, combined with labeled albumin, provided novel kinetic data on albumin transport in peritoneal tissues.

Ultrafiltration volume is a main variable in the characterization of peritoneal dialysis (PD) efficiency. The traditional way of measuring net ultrafiltration volume (net UF) is based on measurements of drained volume, which is hard to perform in an accurate and reproducible way. In the clinical setting, the reproducibility of drained volume measurements may be improved by performing repeated measurements in each patient. In animal models, like the rat, draining of the peritoneal cavity in a reproducible way requires wiping the peritoneal cavity after emptying its content. This procedure for practical reasons necessitates sacrificing the animal. Consequently, kinetic studies of net UF development during PD dwells require a separate group of animals for each time point.

In order to circumvent this problem, marker dilution techniques have been used extensively in PD studies in animals. Labeled albumin, besides macromolecular dextran, is a standard volume marker (1) and, in addition to animal studies (2,3), it has also been used in clinical studies (4–6). It has been recognized that distribution volumes of albumin, when added to PD fluid, are considerably larger than the corresponding drained volumes. This finding suggests that albumin accesses part of the peritoneal extracellular tissue space in addition to the fluid volume in the peritoneal cavity in animals (7,8) and in humans (4,9). Albumin is cleared by the lymphatics, and it is possible to characterize this process by blood sample measurements (4,7–9). Conversely, the amount of albumin absorbed to the peritoneal tissue space is hard to estimate, and direct measurements require invasive techniques (7,10).

The tissue interstitium has been involved in the modeling of peritoneal transport during recent years, and spatially distributed models (11) add the effects of the interstitium to the established 3-pore model of the peritoneal transport barrier (12,13). There is experimental evidence of albumin transport in both directions between blood and the peritoneal cavity during a PD dwell (14,15), but the peritoneal tissue in between may act as an albumin “sink” and retain a large

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fraction of the albumin that enters (7,16). Theoretically, the transport of macromolecules like albumin is considered to be mainly convectional and directionally asymmetric (10,17). Consequently, it should depend on the prevailing direction of solvent flow during the different phases of the UF process, although there is evidence suggesting the presence of simultaneous bidirectional volume flow (18–20). In theory, multi-pore models may support the existence of spatially separated pathways for flow in different directions, a phenomenon called “volume recirculation” (21). Osmotically driven flow is well characterized in the various theoretical models of PD but the effects of hydrostatic and oncotic pressure gradients are less well understood (20).

A volume marker that is strictly confined to the intraperitoneal cavity would offer repeated measurements of net UF (intraperitoneal volume) in the same animal from measurements on dialysate samples. The difference in distribution volumes between a truly intraperitoneal volume marker and labeled albumin could provide information on albumin transport between the peritoneal cavity and the peritoneal extracellular tissue space during different phases of a PD dwell after correction for direct absorption of markers from the peritoneal cavity by lymphatics. An obvious candidate for a particulate volume marker would be an inert cell like the erythrocyte. The transperitoneal transport of erythrocytes in rats has been studied by Flessner *et al.* (22) who showed a slow lymphatic clearance. Erythrocyte distribution volumes were not measured in this study.

The present study was performed in order to evaluate the use of labeled erythrocytes as volume markers of the peritoneal cavity and to compare the distribution volumes of erythrocytes and albumin in relation to those of the peritoneal cavity and tissue compartments at different PD fluid osmolarities. An established model for experimental PD in the rat was used to compare distribution volumes of albumin and erythrocytes in PD fluid samples, drained volumes and hydration, and clearance of volume markers into tissue biopsies.

MATERIALS AND METHODS

EXPERIMENTAL PROTOCOL

A total of 60 male Sprague-Dawley rats, having been equipped with implanted intraperitoneal catheters (see below) were subjected to a single dwell of 20 mL of PD fluid for either 1 or 4 hours dwell time. Three different fluids, based on either 0.5%, 2.5%, or 3.9% glucose, were each assigned to a group of rats.

Volume markers ^{125}I bovine serum albumin and ^{51}Cr erythrocytes were added to the PD fluid before the dwell. Dialysate samples were taken immediately after the start of the dwell and after each hour of dwell time. At the end of the dwell time, a blood sample was taken from the heart before weighing and sacrificing the animal.

In some animals, the dialysate was drained and wiped out of the peritoneal cavity for volume determination and in

other animals, biopsies were taken from the diaphragm, the liver, the anterior abdominal wall and the small intestine at the end of the experiment. The biopsies were used for dry/wet weight determinations and for measurements of volume marker concentrations in relation to peritoneal surface area.

The blood and dialysate samples and the biopsies were analyzed for ^{125}I and ^{51}Cr activity. The osmolality of the dialysate samples was determined by freezing point depression measurements.

Measured radioactivities were used to calculate the distribution volumes for erythrocytes and albumin at each sampling time and the average clearance into the blood (lymphatic), and into the tissue biopsies over the entire dwell time. The distribution volumes and clearances were compared with drained dialysate volumes and biopsy surface areas, weights, and weight gains.

ANIMALS, SURGICAL PROCEDURES AND ANESTHESIA

The study protocol was approved by the Gothenburg Animal Ethics Committee and the experiments adhered to the NIH Guide for the Care and Use of Laboratory Animals. Sprague-Dawley rats (male) weighing between 300 and 400 grams were used in the experiments. A 7 French silicone catheter (Renasil SILO80; Braintree Scientific Inc., Braintree, MA, USA) was implanted under sterile conditions and general anesthesia 1 week prior to the experiment. The tip of the catheter was heparinized using the Corline Lab site Heparin Coating kit (Corline Systems AB, Uppsala, Sweden).

General anesthesia was induced and maintained by inhalation of Isofluran, Baxter 2.8 – 3.6% in room air. In the experiments, the animals were anesthetized during the intravenous injection of drugs and the PD fluid infusion and initial PD fluid sampling. They were thereafter allowed to recover from anesthesia and were then intermittently subjected to short durations of anesthesia during PD fluid sampling. The termination of the experiment at the end of the 1 or 4 hours of dwell time was performed under general anesthesia and the animals were finally sacrificed by opening the thorax and the heart.

DETERMINATION OF LYMPHATIC CLEARANCE AND DISTRIBUTION VOLUMES OF TRACERS

The relative concentrations of volume markers were determined by gamma counting of biopsies and plasma and dialysate samples in a triple window Beckman Gamma Counter with energy windows adapted for ^{51}Cr and ^{125}I . ^{51}Cr caused 7.7% interference in the ^{125}I window and cross-counting was compensated for. The effect of intra-abdominal erythrocyte sedimentation was measured in 3 animals by comparing erythrocyte concentrations in a sample drawn through the catheter, from the lowest part of the peritoneal cavity, and a sample from a thoroughly mixed drained volume at 4 hours of dwell time.

In the following representation of the applied calculations, samples are indexed from $n = 0$ at the beginning of the

experiment to $n = N$ at the end of the experiment. The sampling times are indexed accordingly from t_0 to t_N .

Lymphatic clearances, Q_b , of tracers from the peritoneal cavity to the circulating blood were calculated from total tracer activity in blood at the end of the experiment, Mb_N , and time-averaged tracer concentrations, $\mu_{0..N}(Cd)$, in dialysate during the dwell. The tracer activity in blood was assumed to be 0 at t_0 .

$$Q_b = \frac{Mb_N}{\mu_{0..N}(Cd) (t_N - t_0)}$$

$$Mb_n = Cb_n Vb$$

$$\mu_{0..N}(Cd) = \frac{\sum_{n=1}^N ((Cd_{n-1} + Cd_n) (t_n - t_{n-1}))}{2(t_N - t_0)}$$

where Cb_n and Cd_n are the radioactivity concentration (Bq/mL) of blood and dialysate, respectively, at time t_n , and Vb , the blood volume of the animal, assumed to correspond to the blood mass of 6% of the body weight (23). $\mu_{0..N}(Cd)$ is the average radioactivity concentration of the dialysate during the time interval $t_0..t_N$.

The distribution volume, Vd_k , of a radioactive tracer at a given time, t_k , was calculated from the total dialysate radioactivity Md_k and the radioactivity concentration of the dialysate, Cd_k . Cd_k was determined directly from the sample taken at t_k . Md_k was calculated from the initially supplied radioactivity, Md_0 , minus the radioactivity lost by sampling and lymphatic clearance to blood for $k > 0$.

$$Vd_k = \frac{Md_k}{Cd_k}$$

$$Md_k = Md_0 - \sum_{n=0}^{k-1} Msd_n - Q_b \mu_{0..k}(Cd) (t_k - t_0)$$

where Msd_n is the radioactivity of sample n .

The difference between the albumin and erythrocyte distribution volumes was used as a measurement of the dialysate albumin distribution volume in the peritoneal tissue: the "tissue albumin space." When analyzing albumin mass transfer from the peritoneal cavity into the tissue, the term "albumin loss" was used to express the accumulated clearance of fresh PD fluid.

BIOPSY HANDLING AND QUANTIFICATION OF EDEMA AND OF TRACER ACCUMULATION

After excision, the biopsies were stored between 2 sheets of plastic film in order to prevent evaporation during the registration of biopsy peritoneal surface area, followed by weighing of

the specimens. After counting the radioactivity, the biopsies were dried for at least 48 hours and repeatedly weighed until no further weight loss could be registered. Radioactivities were used to calculate the accumulation of albumin and erythrocytes per unit peritoneal surface area in the peritoneal tissues, expressed in mL of fresh PD fluid.

Biopsy thickness, D , was calculated from biopsy surface area, A , and biopsy wet weight, Ww , assuming 1.06 kg/L tissue specific weight.

$$D = \frac{Ww}{1.06 A}$$

The dry weight, Wd , and wet weight, Ww , determinations were combined to calculate the water fraction, Fw , of the biopsies.

$$Fw = \frac{Ww - Wd}{Ww}$$

Control values for water fraction were calculated from biopsies obtained from untreated animals and the average water fraction for each organ of this control group was used as a reference value. The dry and wet weights (Wd and Ww , respectively) of the sample biopsy and the reference values from the corresponding average biopsy from untreated animals (Wd_0 and Ww_0 , respectively) were used to calculate the increment of tissue water, Δw , compared with an average normal tissue biopsy of the corresponding dry tissue weight.

$$\Delta w = Ww - \frac{Ww_0 Wd}{Wd_0}$$

The water accumulation (edema) was expressed per unit peritoneal surface area, $\Delta w/A$, of the biopsy in $\mu\text{L}/\text{cm}^2$.

PD FLUIDS AND ADDITIVES

The PD fluid composition was 5.4 g/L NaCl, 0.051 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.198 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ buffered with 40 mM lactate (7.5 g/L 60% sodium lactate syrup), which corresponds to a standard lactate-buffered PD solution. Glucose was added as osmotic agent at 3 different concentrations, 5 g/L, 25 g/L, or 39 g/L, yielding osmolalities of 302 ± 2 , 409 ± 2 , or 483 ± 2 mOsmoles/L, respectively. The solutions were filter-sterilized (0.2 μm) into sterile bottles. The PD fluid was warmed to 37°C prior to infusion.

Volume tracers were added to obtain measured activities in the gamma counter in the order of 10^4 CPM/mL for each isotope, requiring approximately 5 μL of ^{125}I albumin solution and 50 μL of ^{51}Cr erythrocyte suspension per 20 mL of PD fluid. A quantity of 200 μg of unlabeled bovine serum albumin (being approximately 20 times more than the amount of labeled albumin) was added in order to reduce surface adsorption of labeled albumin.

LABELING OF VOLUME TRACERS

The intraperitoneal tracer ^{125}I -labeled human serum albumin was prepared by iodination of bovine serum albumin (BSA) (Sigma, #A9647-100G). A combination of 750 μL phosphate-buffered saline (PBS), 50 μL 20 mg/mL BSA and 3 – 5 μL ^{125}I sodium iodide solution (15 MBq) was mixed in IODO-GEN Pre-coated Iodination Tubes (Pierce, Rockford, IL, USA # 28601). The mixture was incubated at room temperature for 15 minutes and occasionally swirled. The ^{125}I conjugated albumin was then removed from the reaction mixture by a single passage through a PD-10 column Sephadex G-25 M (GE Healthcare Bio-Sciences AB, Iselin, NJ, USA # 17-0851-01) and further purified by UF twice in an Amicon Ultra - 4 Ultracel 10k centrifugal filter device (Millipore Corporation, Billerica, MA, USA). The final fraction of unbound ^{125}I activity was below 1:1,000.

Erythrocytes were obtained from a donor rat by heart puncture. The sampled blood was anticoagulated in ethylene diamine tetra acetic acid (EDTA) and erythrocytes were separated and resuspended in PBS at normal hematocrit; 0.5 mL of erythrocyte suspension was incubated for 30 minutes with 20 – 50 μL sodium ^{51}Cr chromate solution (1 MBq) and then washed 4 times. The final fraction of unbound ^{51}Cr activity was below 1:100.

STATISTICS

Student's *t*-test was used to evaluate differences between treatments. The chosen level of significance was $p < 0.05$. Multiple comparisons were compensated for by a sequentially rejective Bonferroni procedure. Data are presented as mean $\pm$ standard error of the mean (SEM).

RESULTS

The effects of erythrocyte sedimentation were evaluated by comparing ^{51}Cr activities from samples drawn through the PD catheter with samples from mixed drained volumes. No significant difference between these 2 modes of sampling was found (data not shown).

Both tracers were cleared from the peritoneal cavity to blood during the dwell, at an approximately 4-times higher

average rate for albumin (0.95 mL/h) than for erythrocytes (0.21 mL/h), as estimated by the appearance rate of the marker in blood. The clearance rates in mL/h were similar at 1 and 4 hours of dwell time and were not significantly affected by increasing the glucose concentration from 0.5% to 3.9% (Table 1).

Distribution volumes of labeled erythrocytes and albumin showed a slight difference in time course. At all time points, the distribution volume of albumin was significantly larger than that of erythrocytes (Figure 1). Time zero, when the first sample was taken, actually corresponded to a time range of 1 to 2 minutes of dwell time due to the duration of the infusion and sampling procedures. At this time, however, most of the difference between albumin and erythrocyte distribution volumes was already established. In some earlier studies, the initial rapid loss of approximately 4% of the albumin has been attributed to adhesion to the peritoneal lining (8). This possibility was evaluated by increasing the addition of unlabelled ("cold") albumin to the PD fluid by a factor of 10 in 5 animals, which did not observably affect the initial distribution volume of albumin.

Erythrocyte and albumin distribution volumes at the end of the dwell both correlated significantly ($p < 0.0001$) with drained volumes. Linear fits of the values at 4 hours of dwell time yielded slopes of 1.03 and 0.99, and intercepts at 3.3 mL and 7.8 mL, respectively (Figure 2). The experiments performed for a dwell time of 1 hour showed a similar correlation between drained volumes and the distribution volumes of the 2 markers (Figure 2). Thus, the marker distribution volumes accurately reflected the drained volumes except for a positive offset that was larger for albumin than for erythrocytes.

The difference between the erythrocyte and albumin distribution volumes, corresponding to the tissue albumin space, was calculated over dwell time for each of the PD fluids studied (Figure 3). The time history of this variable seemed to depend on the osmolarity of the PD fluid, showing a decreasing trend for the 3.9% PD fluid after 2 hours and significant increases for the other 2 fluids during the last hour. At 4 hours, it was significantly smaller for the most hypertonic solution (3.9% glucose) as compared to the other 2 (0.5% and 2.5% glucose).

The accumulation of radioactive markers in the tissue biopsies during the 4-hour dwells was consistently higher for

TABLE 1
The Clearance of Volume Markers from Dialysate to Blood*

Dwell time	Erythrocyte clearance mL/h			Albumin clearance mL/h		
	0.5% PDF	2.5% PDF	3.9% PDF	0.5% PDF	2.5% PDF	3.9% PDF
1 hour		0.19 $\pm$ 0.05			0.89 $\pm$ 0.07	
4 hours	0.22 $\pm$ 0.06	0.23 $\pm$ 0.03	0.16 $\pm$ 0.02	0.86 $\pm$ 0.14	1.00 $\pm$ 0.07	1.06 $\pm$ 0.07

PDF = peritoneal dialysis fluid; SEM = standard error of the mean.

* Determined from dialysate samples drawn at the beginning of the dwell and at each hour of dwell time and from a blood sample drawn at the end of the dwell. Clearance values (mean $\pm$ SEM) from 1-hour and 4-hour dwells were similar, suggesting that clearance rates were linear over time. No significant differences were detected between PD fluids of different glucose concentrations (0.5%, 2.5%, and 3.9%). The clearance of erythrocytes was, however, significantly slower than that of albumin.

Figure 1 — Distribution volumes for erythrocytes (Ery) and albumin (Alb) over 4 hours' dwell time. Erythrocyte distribution volumes were significantly smaller than albumin distribution volumes throughout the dwell. The hypertonic PD solutions (2.5% glucose and 3.9% glucose) produced a net volume gain and the isotonic solution (0.5% glucose) suffered a net volume loss over dwell time. PD = peritoneal dialysis.

Figure 2 — Correlations between drained volumes and erythrocyte (Ery) and albumin (Alb) distribution volumes at the end of the 4-hour dwells (left panel) and 1-hour dwells (right panel). Correlations were significant and positive for both volume markers at both dwell times. Linear regression analysis (lines shown) yielded slopes of 1.03 and 0.99 and intercepts at 3.3 and 7.8 mL respectively at 4 hours and slopes of 0.95 and 0.92 and intercepts of 3.3 mL and 6.4 mL respectively at 1 hour.

albumin than for erythrocytes (Table 2). The 3.9% glucose PD fluid generally tended to cause less albumin accumulation in the biopsies than did the 0.5% and 2.5% glucose PD fluids (Table 2). Differences between mean values were however statistically significant only in abdominal muscle between 3.9% and 2.5% PD fluid. In 5 animals, gentle rinsing with saline of biopsies from abdominal muscle and intestine was performed immediately after harvesting. This procedure removed radioactivity corresponding to 1.5 μL dialysate/ cm^2 surface area (the same clearance volume for erythrocytes and albumin) compared with paired replicates that were not

Figure 3 — The difference between albumin (Alb) and erythrocyte (Ery) distribution volumes was calculated for each sampling time. Assuming that the Ery distribution volume represents the volume of the peritoneal cavity, the Alb-Ery difference corresponds to the distribution volume of albumin in the surrounding tissue, the "tissue albumin space." The time histories differed during the last part of the dwell and the 3.9% glucose PD fluid produced a significantly smaller tissue albumin space than each of the other 2 fluids at 4 hours (**). PD = peritoneal dialysis.

rinsed. The erythrocyte activity was, in this way, reduced by approximately 60% by rinsing. Any effects of further rinsing were not evaluated.

The abdominal muscle biopsies were significantly thinner in the control group of untreated animals. However, biopsies from other organs did not differ significantly in thickness between the groups (Table 3). The biopsies from untreated animals also tended to have smaller water fractions than those from PD-treated animals, but statistically significant differences were found only for abdominal muscle (0.5% and 2.5% glucose solutions) and diaphragm (2.5% glucose solution). The water fraction increase in relation to average values from untreated animals was recalculated to express fluid accumulation (edema) per unit peritoneal surface area ($\mu\text{L}/\text{cm}^2$; Table 3). When comparing edema ($\mu\text{L}/\text{cm}^2$) between the biopsies, there was a significant difference between the 3.9% and 0.5% glucose PD fluids in abdominal muscle and between the 3.9% and 2.5% glucose PD fluids in diaphragmal muscle (Table 3).

In order to compare albumin accumulation in peritoneal tissues with albumin loss from the peritoneal cavity, the albumin activities per cm^2 biopsy area were recalculated for 600 cm^2 , corresponding to the total peritoneal surface area (24,25) and presented as "albumin accumulation" (Figure 4). In addition, the volumes of the tissue albumin space at the end of the dwell (Figure 3) were recalculated as clearance of fresh PD fluid and presented as "albumin loss" (Figure 4). Values for albumin loss and albumin accumulation were in the same order of magnitude, indicating that the largest part of the peritoneal membrane was involved in albumin exchange with the dialysate. Water accumulation (edema) in the biopsies,

TABLE 2
The Accumulation of Erythrocytes and Albumin in Tissue Biopsies*

Biopsy origin	Erythrocyte accumulation $\mu\text{L}/\text{cm}^2$			Albumin accumulation $\mu\text{L}/\text{cm}^2$		
	0.5% PDF	2.5% PDF	3.9% PDF	0.5% PDF	2.5% PDF	3.9% PDF
Abd. muscle	1.87 $\pm$ 0.32	1.62 $\pm$ 0.33	2.54 $\pm$ 0.43	5.14 $\pm$ 0.63	5.67 $\pm$ 0.70	3.79 $\pm$ 0.43 ^a
Intestine	3.09 $\pm$ 0.50	3.09 $\pm$ 0.44	2.53 $\pm$ 0.39	5.24 $\pm$ 0.52	5.30 $\pm$ 0.58	4.88 $\pm$ 0.46
Liver	1.11 $\pm$ 0.19	1.08 $\pm$ 0.24	1.00 $\pm$ 0.23	3.20 $\pm$ 0.28	2.48 $\pm$ 0.23	2.49 $\pm$ 0.19
Diaphr. muscle	2.91 $\pm$ 0.48	2.61 $\pm$ 0.70	2.70 $\pm$ 0.32	6.53 $\pm$ 0.60	6.34 $\pm$ 0.50	5.42 $\pm$ 0.51

PDF = peritoneal dialysis fluid; Abd. = abdominal; Diaphr. = diaphragmal; SEM = standard error of the mean; PD = peritoneal dialysis.

* Determined from the radioactivities per unit peritoneal surface area after 4 hours of dwell time and expressed as $\mu\text{L}/\text{cm}^2$ of fresh PD fluid (mean $\pm$ SEM). For all 3 PD fluids, the accumulation of erythrocytes was significantly smaller than the accumulation of albumin.

^a $p < 0.05$ vs 2.5% PDF.

TABLE 3
Water Accumulation in Tissue Biopsies*

Biopsy origin	No PD	Average thickness (mm)			Water fraction (%) No PD	Edema ($\mu\text{L}/\text{cm}^2$)		
		0.5% PDF	2.5% PDF	3.9% PDF		0.5% PDF	2.5% PDF	3.9% PDF
Abd. muscle	1.40 $\pm$ 0.02	1.81 $\pm$ 0.09 ^a	1.87 $\pm$ 0.09 ^a	1.74 $\pm$ 0.09 ^a	72.14 $\pm$ 0.35	20,01 $\pm$ 2,96 ^b	17,79 $\pm$ 1,58 ^b	8,56 $\pm$ 4,33 ^c
Intestine	0.97 $\pm$ 0.09	0.96 $\pm$ 0.07	0.96 $\pm$ 0.09	0.88 $\pm$ 0.04	76.36 $\pm$ 1.42	5,42 $\pm$ 1,49	2,54 $\pm$ 4,67	3,66 $\pm$ 3,53
Liver	0.66 $\pm$ 0.03	0.82 $\pm$ 0.05	0.80 $\pm$ 0.05	0.79 $\pm$ 0.06	69.87 $\pm$ 0.65	1,14 $\pm$ 0,62	0,12 $\pm$ 0,95	2,48 $\pm$ 1,23
Diaphr. muscle	0.81 $\pm$ 0.08	0.73 $\pm$ 0.03	0.88 $\pm$ 0.07	0.70 $\pm$ 0.03	74.84 $\pm$ 0.78	2,55 $\pm$ 1,45	6,03 $\pm$ 1,48 ^b	1,90 $\pm$ 0,86 ^d

PD = peritoneal dialysis; PDF = PD fluid; Abd. = abdominal; Diaphr. = diaphragmal.

* The average thickness of tissue biopsies was determined from the measured peritoneal surface area and the weight of each biopsy after 4-hour dwell. The water fractions were calculated as (wet weight–dry weight)/wet weight, and the values from control animals, neither exposed to catheter implantation nor to PD, were used as reference values for each organ. The increase in water fractions in the biopsies PD-treated animals after 4-hour dwell, in relation to the reference values, were recalculated as water accumulation (edema) in $\mu\text{L}/\text{cm}^2$ peritoneal area in relation to a constant dry weight of the tissue.

^a $p < 0.05$ vs intact tissue “No PD”

^b $p < 0.05$ increase over the water fraction in the intact tissue “No PD”

^c $p < 0.05$ vs 0.5% PDF

^d $p < 0.05$ vs 2.5% PDF

expressed as mL of edema/600 cm^2 peritoneal surface area, was in the same order of magnitude as the corresponding albumin accumulation, except for abdominal muscle, which showed unproportionately large water accumulation (Figure 4).

The dialysate osmolalities fell during the dwell (Figure 5) and reached 295 mOsm/L as the intraperitoneal volumes peaked for the three PD fluids (Figure 1). The osmolalities leveled out and did not change significantly during the last hour of the 4-hour dwells. At the end of the dwells, however, the osmolality of the 0.5% PD fluid (288.5 $\pm$ 1.67 mOsm/L) was still significantly lower than the osmolality of the 2.5% (291.9 $\pm$ 0.69 mOsm/L), and the 3.9% (293.4 $\pm$ 0.68 mOsm/L) glucose PD fluids.

DISCUSSION

In concert with data from earlier studies (8), the clearance of albumin and erythrocytes to plasma, conceivably by the lymphatics, occurred at a constant rate over time since the

values obtained at 1 hour and 4 hours were similar (Table 1). The rate of albumin and erythrocyte clearance to plasma, respectively, was similar to that reported in earlier studies (22,26). The much different values of albumin vs erythrocyte clearance poses a question as to which one of them, if any, can be considered as an estimator of direct lymphatic flow from the peritoneal cavity. Typically, clearance of macromolecules was considered as such an estimator in earlier clinical and experimental studies (7,9,10). It is well established that cells and particles can enter the lymphatics of the muscular diaphragm and there is limited evidence that this may occur also in other regions of the peritoneum (27), although the diaphragmal lymphatics probably contribute the major part. The transport of erythrocytes is delayed by the regional lymph nodes, but the cells eventually reach the blood circulation through central lymphatic pathways (22). Fluid uptake, on the other hand, is not regionally restricted. The current results support the presence of sieving effects that slow down the uptake of cells in relation to that of fluid in the peritoneum as

Figure 4 — The upper panels show comparisons between the accumulation of albumin in the tissue biopsies (upper left) and the accumulated loss of albumin from the peritoneal cavity into the tissues (upper right) at the end of the dwell. Tissue accumulation (Table 2) was recalculated for 600 cm² (the known total surface area of rat peritoneum). Both albumin loss and albumin accumulation are expressed as clearance of fresh PD fluid in order to allow direct comparisons. The lower panels present the accumulation of water (edema) in the tissue biopsies (Table 3), recalculated for 600 cm² peritoneal surface area. The volume of the tissue albumin space is included to allow comparisons. Albumin accumulation in tissues was generally larger than the corresponding albumin loss from the cavity whereas water accumulation in the tissues was small in relation to albumin accumulation in most tissues. In some cases, the 3.9% glucose fluid produced significantly lower values than other fluids. ** 3.9% PD fluid < 2.5% and 0.5% PD fluids. * 3.9% PD fluid < 2.5% or 0.5% PD fluid. Abd = abdominal; PD = peritoneal dialysis.

a whole. Lymphatic elimination at a known stable rate should not, however, constitute an obstacle for the application of erythrocytes as a volume marker (28). Hypothetically, direct uptake of labeled albumin from the tissue to blood may increase its concentration in blood, mimicking lymphatic uptake. This should yield a difference in the albumin clearance to blood at 1-h vs 4-h values; this was not observed in our data (Table 1).

Erythrocyte distribution volumes were constantly smaller than those for albumin and hence were in closer agreement with drained volumes. The difference in distribution volume between the 2 tracers was established during the first 2 minutes of the dwell and was thereupon approximately doubled over the following 4 hours of dwell time except for the 3.9% glucose solution (Figure 3). Although erythrocyte distribution volumes correlated significantly with drained volumes, they showed systematically higher values. Data indicated a constant offset suggesting that approximately 3 mL of the erythrocyte

Figure 5 — Dialysate osmolalities over 4 hours' dwell time. During the last hour of the dwell, the average osmolality was below 295 mOsm for all fluids.

distribution volume could not be recovered as drained volume at the end of the experiment (Figure 2). The complex geometry of the peritoneal cavity makes it difficult to drain all fluid. Therefore measurements of drained volume probably underestimate the true intraperitoneal volume. Adding and draining new fluid after finalizing the dwell will mobilize labeled erythrocytes and albumin corresponding to approximately 1 mL of dialysate (unpublished data), leaving at most 2 mL to be accounted for (repeated rinsing would probably recover some more erythrocytes). Draining of rats immediately after filling with PD fluid will usually retrieve 1 – 2 mL less than the filling volume (unpublished data). A reasonable estimate of the maximal erythrocyte loss from the intraperitoneal fluid volume should be the erythrocyte activity remaining in the biopsies after rinsing, approximately 1 – 1.5 $\mu\text{L}/\text{cm}^2$. This activity, probably reflecting surface adhesion and/or local lymphatic uptake of erythrocytes, corresponds to a volume of 0.6 – 0.9 mL/600 cm² peritoneal surface area, which should be the maximal difference between erythrocyte distribution volume and intraperitoneal fluid volume in a 300 – 400 g rat. The data scatter (standard deviation) of measured erythrocyte distribution volumes seems to be similar to that of drained volumes. In the current analyses, the erythrocyte distribution volume was used as a measurement of the fluid volume in the peritoneal cavity, accepting a possible overestimation by not more than 1 mL (such overestimation is however not seen, for example, at 0 min for glucose 0.5% and 2.5% solutions).

The tissue biopsies were taken from organs that contribute large fractions of the peritoneal surface area in order to provide relevant data on the peritoneal tissue compartment. According to a comprehensive study of the peritoneal surface anatomy of the rat (24), the parietal peritoneal walls and the liver contribute 17% each and the abdominal viscera, excluding the liver, contribute 60% to the total peritoneal surface area of 595 cm². Comparable results were obtained in a later study (25). Thus, the biopsies represent organs that contribute over 90% of the

peritoneal surface. We have not found any published values for albumin transport into liver and intestine, but our values for abdominal muscle and diaphragm (Table 2) are in accordance with data in the literature when recalculated as mL/100 g (7). The relationship between albumin loss from the peritoneal cavity and accumulation of this marker in the tissue biopsies suggested most of the peritoneal surface area participated in an exchange with the dialysate (Figure 4). Studies in anesthetized rats indicate that PD fluid has an efficient exchange only with a small fraction (25 – 30%) of the total peritoneal surface area (25). Movements of the abdominal organs that occur in conscious animals, as in the present study, most likely improve the contact between the PD fluid and the peritoneal membrane (29) mainly for the visceral part of the peritoneal membrane (26). In the study by Zakaria *et al.*, the clearance of albumin out of the peritoneal cavity of anesthetized rats increased by vibrating the animals and reached levels compatible with the albumin transport measured in the present investigation (26).

The accumulation of albumin per unit peritoneal area was not greater in the abdominal muscle than in the diaphragm despite the fact that the abdominal muscle biopsies were generally twice as thick as those from the diaphragm. This may suggest that the depth of penetration into the tissue for albumin did not exceed the thickness of the diaphragm, approximately 0.8 mm (Table 3). The biopsy data show a trend toward more accumulation of erythrocytes and albumin in diaphragmal muscle and intestine than in abdominal muscle and liver, possibly reflecting local lymphatic uptake (Table 2).

Water accumulation in the biopsies was interpreted as edema formation although it cannot be excluded that changes in intracellular water contributed to this finding for the 2.5% and 3.9% glucose solutions (30). However, after 4 hours of dwell time, the osmolarities of the hypertonic PD fluids had normalized (Figure 5) and the intracellular water content should be very close to normal. Previous studies have demonstrated that hydrostatic pressure, above a given threshold level, is the main driving force behind peritoneal edema formation (7,30). In the study by Zakaria *et al.* (30), the osmolarity of the PD fluid had no significant effect on edema formation. However, that study was based on short dwells where the osmolarity was maintained at a high level, preventing the establishment of a reabsorption phase. In the present study, the difference in tissue albumin space between the different fluids developed during the reabsorption phase (Figure 3) and corresponded to the difference in edema development in abdominal muscle (Figure 4; Table 3). Interestingly, the intraperitoneal volume and thus the intraperitoneal pressure, in all likelihood, was higher with the 3.9% fluid than with the 2.5% and 0.5% fluids, but still might not have reached the threshold for promoting edema (30). According to a reported relationship between intraperitoneal volume and pressure in the rat, pressures in the present experiments should have been below 1 mmHg since intraperitoneal volumes were below 30 mL (31). However, the present study differs from most previous ones by using awake animals in the prone position, and this might have

resulted in a somewhat higher intraperitoneal pressure level in the anterior part of the peritoneal cavity. The prone position of the animals was also a likely reason for the increased thickness and edema of the abdominal muscle in contrast to the internal abdominal organs (Table 3). In contrast to the other organs, the water accumulation in abdominal muscle was larger than the corresponding albumin accumulation (Figure 4). This suggests that a large fraction of the accumulated fluid here originated from sources other than the dialysate.

The use of double-volume markers offered a new way to study the kinetics of the volume of the tissue albumin space (Figure 3). The obtained time histories were dependent on PD fluid osmolarity and differed from the time histories of tissue albumin uptake as described in earlier studies. The importance of osmolarity is controversial since it is supported by some previous reports (2,8) while being refuted by some others (18,19). In the present study, the significant differences between PD fluids in albumin loss from the cavity are matched by differences in albumin and water accumulation in abdominal muscle, strongly supporting the finding that the tissue absorption of dialysate is smaller at higher osmolarity (Figure 4). For other organs and tissues, some trends point in the same direction but there are no significant differences between different PD fluid osmolarities.

Conceptually, the time history of the tissue albumin space (Figure 3) corresponds to integration over time of the clearance of albumin from the peritoneal cavity into the tissue. Approximately half of the albumin transfer from the peritoneal cavity into the tissue took place during the first 2 minutes of the dwell, when the albumin concentration was virtually the same for all 3 fluids. Consequently, albumin loss from the cavity was highly nonlinear over time. In order to facilitate a presentation in terms of transport mechanisms, the time histories of the tissue albumin space (Figure 3) have been recalculated as “albumin loss” which equals the net mass transfer of albumin from the cavity into the tissue (Figure 6). The albumin concentrations in the peritoneal cavity are also displayed and both variables are presented in relation to the albumin concentration of fresh PD fluid.

The time histories of albumin loss generally started with a steep increase followed by a plateau phase that transited into a phase of gradual increase, except for the highest osmolarity fluid where the plateau phase persisted (Figure 6). The first phase of rapid increase has earlier been attributed to adhesion of albumin to the peritoneal lining and has been treated as an artifact (8). In our evaluation, the initial loss of albumin was interpreted as a rapid initial albumin transport into the tissue interstitium, since it was not affected by changing the fraction of “cold” albumin in the PD fluid. Unpublished data from our model show that this initial rapid transport actually continued until the plateau level (Figure 6) was reached, within less than half an hour of dwell time. According to our concept, this phase is due to convection, since albumin diffusion in the interstitium is a slow process (17). A high rate of tracer disappearance during the first 30 minutes of the dwell was also observed in earlier studies for albumin (8) and IgG (10). During the initial

Figure 6 — Albumin mass transfer, presented as albumin loss from the peritoneal cavity into the tissue in mL of fresh PD fluid. The data are recalculated from the volume of the tissue albumin space (Figure 3). The albumin concentration of the peritoneal cavity is included to allow estimations of the conditions for diffusive and convective transport during different phases of the time history. An initial step increase of albumin loss is followed by a plateau phase that, in the case of 0.5% and 2.5% glucose transits into a phase of gradual increase. Conc. = concentration; PD = peritoneal dialysis.

part of the dwell there is a concentration gradient of labeled albumin as well as a rapid increase of the hydrostatic pressure gradient (7) that supports a flow of dialysate into the tissue. The fast inflow of water and albumin into the intact subperitoneal tissue was also predicted in theoretical studies (16).

The second, plateau, phase persists while the albumin concentration in the peritoneal cavity falls to a level that depends on the UF volume (Figure 6, lower panel). Here, the absence of significant net albumin transport from the tissue back into the cavity despite the UF-driven solvent flow in this direction is somewhat puzzling. Possible explanations include binding of albumin to the tissue and recirculation of albumin between the peritoneal cavity and the tissue interstitium (21). For immunoglobulins, tissue binding has been demonstrated (32), but albumin may circulate through the interstitium. We suggest that recirculation of albumin between the peritoneal cavity and the tissue interstitial compartment could maintain the equilibrium between the tissue and the intraperitoneal pool despite the net inward fluid flow.

The plateau phase ended after 120 minutes at 0.5% glucose, at 180 minutes at 2.5% glucose, and persisted throughout the dwell at 3.9% glucose. Thus, the final incremental phase of albumin transport out of the peritoneal cavity seemed to be

delayed in proportion to the osmolarity of the PD fluid and thus in proportion to the duration of positive UF. Hypothetically, the osmotic water flow from the tissue into the peritoneal cavity, counteracted or balanced any convective albumin transport in the opposite direction during the plateau phase but did not wash out the total amount of albumin that had entered the tissue during the initial rapid hydration of the tissue (Figure 6, upper panel). During the third incremental phase, the tracer albumin transport followed the direction of the water flow which is then dominated by reabsorption of dialysate, except for 3.9% glucose (17).

Thus, the experimental model presented here allows us to follow the kinetics of absorption of macromolecules (labeled albumin, dextran) from the PD fluid to the tissue. Typically, it is assumed that the rate of this absorption is constant and proportional to the protein concentration in dialysis fluid and the coefficient of proportionality is a measure of fluid absorption from the peritoneal cavity (20). The absorption is usually estimated as the total absorption that includes the absorption to the lymphatic vessels open to the peritoneal cavity and to the tissue together and is calculated by the disappearance rate of the macromolecule from the peritoneal cavity. Our results challenge this approach and show that the amount of labeled albumin, or albumin space, lost from the peritoneal cavity to the tissue changes with dwell time and has 3 phases with increased absorption after 2–3 hours during dwells with 0.5% and 2.5% glucose solutions. However, the current study differs in a few respects from the clinical conditions: firstly the tissue is in its physiological equilibrium before infusion of dialysis fluid, in contrast to the tissue status during repeated infusions of PD (11), secondly the hydrostatic pressure in the peritoneal cavity filled with fluid is low (probably lower than 1–2 mmHg), in contrast to the typical pressure of 7–10 mmHg in patients in recumbent position (31). Studies in animals at higher intraperitoneal pressures indicate that albumin transport into the tissue may take place during active UF (10). These differences may be responsible for a possible disagreement between absorption patterns in patients on PD and the current study in rats. Like the 3-pore model of peritoneal transport that predicts the occurrence of net fluid absorption from the peritoneal cavity only when osmotic pressure of dialysis fluid is dissipated (13,17), our data actually show net transport of albumin. In both cases, net transport may be the result of concomitant bidirectional flow between the tissue and the cavity.

The clinical relevance of kinetic studies of macromolecular markers remains to be established. Although the transport of labeled albumin from the peritoneal cavity to the tissue is not important *per se*, the “albumin space” may have relevance for the dialysis process since it is related to the extracellular tissue volume engaged by the osmotic gradient during PD. Hypothetically, measurements of albumin exchange may be used to study some aspects of how the PD fluid interacts with the peritoneal tissue. For example, edema formation during PD may be a factor behind the development of peritoneal fibrosis based on the notion that such a relationship has been shown for other tissues (33).

To summarize, the present results show that labeled erythrocytes are reliable markers of the intraperitoneal volume during PD using PD fluids over the normal clinical range of osmolarities. Experimental models based on this concept offer new possibilities for kinetic studies of transperitoneal transport. In the present study, combining erythrocytes with labeled albumin allowed for a characterization of the albumin exchange between the peritoneal cavity and the surrounding tissues during the PD dwell, which provided novel information on the kinetics of this process and its dependence on PD fluid osmolarity. The most hypertonic, 3.9% glucose, PD fluid induced significantly less albumin transport into the peritoneal tissues than did the less hypertonic (2.5% glucose) or the isotonic (0.5% glucose) fluids. This finding was supported by parallel measurements of water and albumin accumulation in tissue biopsies indicating the same type of relationships. The model will be used for further studies of how PD interacts with the peritoneal tissue.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

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