THE ROLE OF LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS

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"Ascitic, thy lymph runneth over"

Witte MH,

PREFACE

I have been fortunate to spend a two year Fellowship in Nephrology with Professor Karl D. Nolph at the University of Missouri Health Sciences Centre, Harry S. Truman Veterans Administration Hospital and Dalton Research Centre, Columbia, Missouri. Professor Nolph and his colleagues, Dr. Ramesh Khanna and Dr. Zbylut J. Twardowski, are internationally renowned for their clinical expertise and research in peritoneal dialysis and have innovated many improvements in peritoneal dialysis therapy during the past ten years. Their guidance and continued support were invaluable in helping me conduct these studies during my Fellowship.

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or Ringer's lactate solution with added albumin

performed in the preceding four days.

ACKNOWLEDGEMENTS

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SUMMARY

Studies of peritoneal dialysis kinetics have focused on fluid
and solute exchange between the peritoneal microcirculation and the
hypertonic dialysis solution instilled into the peritoneal cavity.
The intraperitoneal fluid, however, is also absorbed continuously by
convective flow into the peritoneal cavity lymphatics. Thus,
measured net ultrafiltration at the end of each exchange (drain
volume minus infusion volume) represents the difference between total
net transcapillary fluid transport into and lymphatic drainage out of
the peritoneal cavity during the dwell time.

Lymphatic absorption from the peritoneal cavity occurs mainly
via stomata on the undersurface of the diaphragm and exceeds 50
ml/hour in patients with ascites unless the subdiaphragmatic or
mediastinal lymphatics are obstructed by tumour or fibrosis.
Lymphatic absorption in the iatrogenic "ascites" of peritoneal
dialysis may also be significant and thus merits investigation.

The role of lymphatic absorption during peritoneal dialysis was
evaluated in a rat model and in adults and children on continuous
ambulatory peritoneal dialysis (CAPD). Lymphatic absorption was
calculated from the rate of removal of albumin added to the infused
dialysis solution, and net transcapillary ultrafiltration was
estimated from the dilution of the initial dialysate albumin
concentration.

Intraperitoneal volume and lymphatic absorption were determined
serially during six hour exchanges in rats using 15% dextrose

dialysis solution. The net transcapillary ultrafiltration rate decreased exponentially to zero after 330 minutes, whereas lymphatic absorption proceeded at an almost linear rate throughout the exchanges, averaging 4.7 ± 0.9 (SEM) ml/hour. Peak ultrafiltration volume was observed before osmolar equilibrium between serum and dialysate was reached and occurred when the net transcapillary ultrafiltration rate had decreased to equal the lymphatic absorption rate. Thereafter, the net fluid absorption rate represented lymphatic absorption in excess of concurrent net transcapillary ultrafiltration. Measured net ultrafiltration at the end of the exchanges averaged 24 ± 2 ml and represented only 46 ± 5% of total net transcapillary ultrafiltration during the dwell time.

Standardised four hour exchanges using 2 litres of 2.5% dextrose dialysis solution were performed in 18 adult CAPD patients. Cumulative lymphatic absorption averaged 343 ± 39 ml and reduced potential net ultrafiltration at the end of the exchanges by 56 ± 6%. Extrapolated to four x six hour exchanges per day, lymphatic absorption reduced potential daily net ultrafiltration by 82 ± 9%, daily drain volumes by 18 ± 2%, daily urea clearances by 14 ± 1% and daily creatinine clearances by 13 ± 1%. These findings indicate that net ultrafiltration and solute clearances are reduced significantly by lymphatic absorption in all CAPD patients.

Eight of the patients had transperitoneal solute transport rates indicative of high peritoneal permeability x area. Absolute lymphatic absorption did not differ between patients with average and high peritoneal permeability x area, but caused a proportionately

greater reduction in net ultrafiltration in patients with high peritoneal permeability x area ($p < 0.005$) since these patients had more rapid glucose absorption from the dialysate ($p < 0.001$) and lower cumulative net transcapillary ultrafiltration ($p < 0.05$). Patients with high peritoneal permeability x area had daily net fluid absorption from the dialysis solution even though daily net transcapillary ultrafiltration averaged 2.1 ± 0.4 litres. Failure of peritoneal ultrafiltration in CAPD, in the absence of a dialysate leak, occurs when daily lymphatic absorption exceeds daily net transcapillary ultrafiltration.

Four hour exchanges using 40 ml/Kg of 2.5% dextrose dialysis solution were performed in six children on peritoneal dialysis. Cumulative lymphatic absorption averaged 10.4 ± 1.6 ml/Kg and reduced total net transcapillary ultrafiltration by 73 ± 10%. Extrapolated to four x six hour exchanges per day, lymphatic absorption reduced potential daily drain volumes by 27 ± 5%, daily urea clearances by 24 ± 4% and daily creatinine clearances by 22 ± 5%. The infusion volumes of dialysis solution, corrected for body surface area, were similar in children and adults. Net ultra-filtration, scaled for body surface area, was lower in children than in adults with average peritoneal permeability x area ($p < 0.05$) due to a combination of relatively higher lymphatic absorption and lower net transcapillary ultrafiltration. Thus, lymphatic absorption caused a proportionately greater reduction in net ultrafiltration and solute clearances in children than in adults.

Intraperitoneal glucose and insulin absorption were evaluated during two hour exchanges using 15 ml of 2.5% dextrose dialysis solution in diabetic rats with and without peritonitis. Greater than 50% of insulin absorption in both groups was via the lymphatics. Dialysate insulin and glucose were absorbed more rapidly during exchanges in the rats with peritonitis (p < 0.05). However, the relative absorption rates of insulin and glucose and the hypoglycaemic response to absorbed insulin were similar in the two groups. These data suggest that intraperitoneal insulin requirements in diabetic CAPD patients are not increased during peritonitis provided that the dialysate glucose load and oral carbohydrate intake remain unchanged.

Intraperitoneal cholinergic drugs constrict the subdiaphragmatic stomata and so may decrease lymphatic drainage. Two hour exchanges were performed in ambulant rats using 20 ml of 2.5% dextrose dialysis solution with and without the addition of the anticholinesterase agent, neostigmine. Net ultrafiltration (p < 0.01) and solute clearances (p < 0.05) were enhanced in the neostigmine group by a reduction in lymphatic absorption (p < 0.01) and without an increase in transperitoneal transport of water and solutes into the peritoneal cavity. These results indicate that the efficiency of peritoneal dialysis can be increased when lymphatic absorption is reduced.

Phosphatidylcholine is a normal component of fluid within the serous cavities and is removed in the dialysate effluent during CAPD. Four hour exchanges were performed in ambulant rats using 20 ml of 4.25% dextrose dialysis solution with and without added

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phosphatidylcholine. Net ultrafiltration ($p < 0.05$) and solute clearances ($p < 0.05$) were enhanced after the exchanges with phosphatidylcholine due to a reduction in lymphatic absorption ($p < 0.01$). Transperitoneal transport of water and solutes did not differ between the treated and control rats. Phosphatidylcholine may have clinical application in alleviating poor ultrafiltration after long-dwell peritoneal dialysis exchanges.

These studies emphasise the important contribution of lymphatic absorption to loss of ultrafiltration and solute clearances after long-dwell peritoneal dialysis exchanges. Thus, reappraisal of current understanding of peritoneal dialysis kinetics is required to incorporate the role of lymphatics. Pharmacological reduction of lymphatic absorption may provide an alternative means of improving the efficiency of peritoneal dialysis without altering transperitoneal transport of water and solutes into the peritoneal cavity.

PART I   <u>REVIEW AND RATIONALE</u>

Chapter 1. ANATOMY AND PHYSIOLOGY OF PERITONEAL DIALYSIS

1.1 HISTORICAL PERSPECTIVE

The clinical potential of using the peritoneal membrane as a dialysing membrane was recognised over sixty years ago (1,2). Long-term intermittent peritoneal dialysis, however, did not become a viable alternative to haemodialysis until a permanent indwelling peritoneal catheter was developed in 1964 by Palmer and colleagues (3) and later modified by Tenckhoff (4). Nevertheless, due to inadequate dialysis and repeated episodes of peritonitis, the cumulative technique survival of intermittent peritoneal dialysis was relatively low (5) and haemodialysis remained the predominant dialysis therapy.

The concept of portable/wearable equilibrium peritoneal dialysis, using several long-dwell exchanges each day, was introduced by Popovich and Moncrief in 1976 (6) and heralded a new era for peritoneal dialysis. Clinical studies demonstrated that adequate steady state control of uraemia, sodium and water balance, acidosis and hyperkalaemia could be achieved in patients with end-stage renal failure using four or five exchanges per day, and the technique was renamed Continuous Ambulatory Peritoneal Dialysis (CAPD) (7). Oreopoulos replaced the use of glass bottles containing dialysis solution with a polyvinylchloride bag which could be rolled up when empty and carried under the clothing without being disconnected from the transfer set (8). After equilibrating for the selected dwell

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time, the dialysate was drained into the polyvinylchloride bag and the connection-disconnection procedure repeated using an aseptic technique. This development markedly reduced the peritonitis rate and, apart from modifications in the design of the administration set and peritoneal catheter, this basic CAPD system remains unchanged today.

The number of patients on peritoneal dialysis has increased annually during the past decade (9,10) and CAPD now has an established role in renal replacement therapy (11). At the end of 1986 more than 30,000 patients were on peritoneal dialysis therapy worldwide and in some countries, including the United Kingdom, more than half of all patients beginning dialysis are now started on CAPD (12).

1.2 THE PERITONEAL DIALYSIS SYSTEM

During peritoneal dialysis fluid and solutes exchange across the peritoneal membrane between the peritoneal microcirculation and hypertonic solutions infused into the peritoneal cavity.

THE PERITONEUM

The peritoneum is a continuous membrane which covers the inner surface of the abdominal wall, invests the visceral organs and forms the mesentery that connects loops of bowel. The total surface area of the peritoneum approximates the patient's body surface area (13,14). The inner surface of both the parietal and visceral

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peritoneum is lined by a monolayer of flattened cells with numerous microvilli (mesothelium), supported by its basement membrane and the interstitium (15,16). The blood vessels and lymphatics within the peritoneal interstitium are interspersed between extracellular fluid and a gel-like matrix containing collagen and elastin fibres, fibroblasts and fat (15,16). The morphology of peritoneum from normal and non-dialysed uraemic patients is similar, but after beginning peritoneal dialysis alterations in peritoneal ultrastructure are often observed (15-17). The mesothelial layer may exhibit absence of the microvilli, widening of the intercellular junctions or even loss of mesothelial cells, whereas the interstitium may become thickened due to submesothelial fibrosis and stromal oedema. These changes in peritoneal morphology tend to be more pronounced in patients with previous episodes of peritonitis (15-17).

PERITONEAL BLOOD FLOW

The absolute peritoneal blood flow rate in man is not known but gas diffusion studies suggest that the effective blood flow rate is at least 70 ml/min (18). The peritoneal capillaries are located mainly in the deeper layers of the interstitium and are usually greater than 20 μm from the mesothelial surface (17). Under basal conditions only 20% of the peritoneal capillaries are perfused and perfused capillaries may underlie less than 0.5% of the total peritoneal surface area (19).

DIALYSATE FLOW RATE

The peritoneal cavity normally contains only a small volume of isosmotic fluid (less than 100 ml), yet most adult patients can tolerate rapid instillation of at least 2 litres of dialysis solution without abdominal discomfort. The dialysate flow rate can be adjusted by changing the infusion volume or total cycle time. In intermittent peritoneal dialysis dialysate flow rates of 40 ml/min are achieved by rapid cycling while in CAPD flow rates usually average 7 ml/min.

THE DIALYSING MEMBRANE

Solutes and fluid may transfer across the peritoneal membrane by intercellular, vesicular or transcytoplasmic pathways. At least six major anatomical resistance sites to transperitoneal transport have been identified (20): the fluid films within the capillary lumen, the endothelium, the endothelial basement membrane, the interstitium, the mesothelium and the fluid films and stagnant pools of dialysis solution within the peritoneal cavity. The permeability of the peritoneal membrane is related inversely to the sum of these resistances and the area of the peritoneum effectively involved in solute and fluid transport is always much lower than total peritoneal surface area because of perfusion : dialysate flow mismatching.

Thus the peritoneum in peritoneal dialysis may be regarded as a composite, reusable, biological, dialysing membrane which is almost continuously exposed to non-physiological dialysis solutions.

1.3 KINETICS OF PERITONEAL DIALYSIS

In peritoneal dialysis net removal of water and sodium is
dependent on the net ultrafiltration volume at the end of the
exchange and the net removal of solutes is determined by the solute
mass transfer rate.   The net ultrafiltration volume is equal to the
drain volume minus the infusion volume of dialysis solution, assuming
that the intraperitoneal residual volume remains constant.   The mass
transfer rate of each solute is calculated as:

$$\frac{(V_t \times C_t) - (V_o \times C_o)}{t} \qquad \dots\dots\text{equation 1.}$$

where V_o and V_t = infusion volume and drain volume, respectively

C_o and C_t = solute concentration in the infusion and drain

volume

t = total cycle time

The solute clearance rate is calculated by dividing the solute
mass transfer rate by the mean serum solute concentration. Solute
clearances in peritoneal dialysis are determined primarily by the
lowest of the effective peritoneal blood flow rate, peritoneal
permeability x effective membrane transfer area for the solute and
the dialysate flow rate (18).   The relatively low efficiency of
peritoneal dialysis is compensated for in CAPD by dialysis being
performed almost continuously (20).

Ultrafiltration and solute mass transfer in peritoneal dialysis are dependent on:

a) the dwell time, osmolality and volume of exchanges.

b) interindividual and intraindividual variation in the dialysing membrane.

1.3.1 ULTRAFILTRATION

DWELL TIME OF DIALYSIS SOLUTION

In peritoneal dialysis ultrafiltration is induced by osmotic pressure, and the transperitoneal osmotic pressure gradient is generated primarily by the glucose concentration of the dialysis solution (21). The water flux rate across the peritoneum (Jw) is equal to the product of hydraulic permeability (Lp), effective membrane area (A), and the sum of osmotic ($\Delta\pi$) and hydrostatic (ΔP) transmembrane pressure gradients. That is:

$$J_w = L_p \, A \, (\Delta\pi + \Delta P) \qquad \ldots\ldots \text{equation 2.}$$

Consequently the net transcapillary ultrafiltration rate (J_w) is maximum at the beginning of the exchange and decreases exponentially as the transmembrane glucose concentration gradient is dissipated by a combination of transperitoneal glucose absorption and dilution of the dialysis solution by the ultrafiltrate. The intraperitoneal volume increases until a maximum is reached at or near crystalloid osmolar equilibrium (22). Thereafter, the dialysis solution remains

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isosmotic with serum and net absorption of fluid proceeds almost linearly at an average rate of 40 ml per hour (23-26).

OSMOLALITY OF DIALYSIS SOLUTION

For any given patient the net transcapillary ultrafiltration rate (J_w) correlates linearly with the glucose concentration of the dialysis solution and the transperitoneal osmotic gradient (27). Thus increases in the osmolality of the dialysis solution result in a higher initial transcapillary ultrafiltration rate, an increase in the peak intraperitoneal volume, prolongation of the dwell time until peak ultrafiltration and osmolar equilibrium are observed, and delay until net fluid absorption begins (28,29). After peak ultrafiltration the rate of intraperitoneal fluid absorption is similar, irrespective of the tonicity of the infused dialysis solution (25,26,28). However, the absorbed glucose load also increases proportionately with higher initial dialysate glucose concentrations (27,30). The absorbed glucose makes a significant contribution to total daily calorie intake and promotes anabolism by stimulating endogenous insulin secretion (30), but has the disadvantage of predisposing patients to obesity and hyperlipidaemia (31). Despite active interest in alternative, less absorbable osmotic agents, glucose remains the only agent used in current commercial dialysis solutions (28,32).

VOLUME OF DIALYSIS SOLUTION

In exchanges using dialysis solutions of equal initial glucose concentration, drainage volumes are proportional to the instillation volume (23,33). Although the initial net transcapillary ultrafiltration rate is unaltered, the increased dialysate glucose load with higher infusion volumes augments the peak ultrafiltration volume and prolongs the dwell time until osmolar equilibrium is reached (23,28). Nevertheless the clinical application of instilled volumes of dialysis solution greater than 2.5 litres is limited by patient tolerance and by the increased risk of complications related to raised intra-abdominal pressure (34-36).

INTERINDIVIDUAL VARIATION IN ULTRAFILTRATION

CAPD patients exhibit a wide interindividual variation in net ultrafiltration even if the dwell time, osmolality and volume of exchanges are standardised (37-39). Transcapillary ultrafiltration during exchanges using the same dialysis solution is primarily dependent on interpatient differences in peritoneal permeability x area (equation 2). Transperitoneal osmotic pressure is equal to the sum of the products of the osmolar gradient and the peritoneal reflection coefficient of each solute. Therefore high peritoneal permeability x area reduces cumulative net transcapillary ultrafiltration by two related mechanisms:

a)   rapid absorption of glucose from the dialysate dissipates the

transperitoneal osmotic gradient more quickly during the dwell time.

b) at any given glucose concentration gradient the lower peritoneal reflection coefficient for glucose generates reduced osmotic pressure ($\triangle\pi$) and decreases water flux (J_w).

Representative differences in ultrafiltration kinetics between patients with normal and high peritoneal permeability x area are depicted in Figure 1.

INTRAINDIVIDUAL VARIATION IN ULTRAFILTRATION

Irreversible loss of peritoneal ultrafiltration capacity in peritoneal dialysis patients has been observed most frequently in Europe (40-42) and usually results from a persistent increase in peritoneal permeability x area (Type I membrane failure). Although an aetiological role is as yet unproven, alterations in the mesothelial layer (43) and the use of dialysis solutions containing acetate (41,42) have both been associated with increased peritoneal permeability x area. Similar but temporary changes in peritoneal morphology and physiology occur during episodes of peritonitis (43,44). However, peritoneal ultrafiltration capacity in stable CAPD patients (37,45) and in patients with ultrafiltration failure (41) appears to be unrelated to the number of prior episodes of peritonitis.
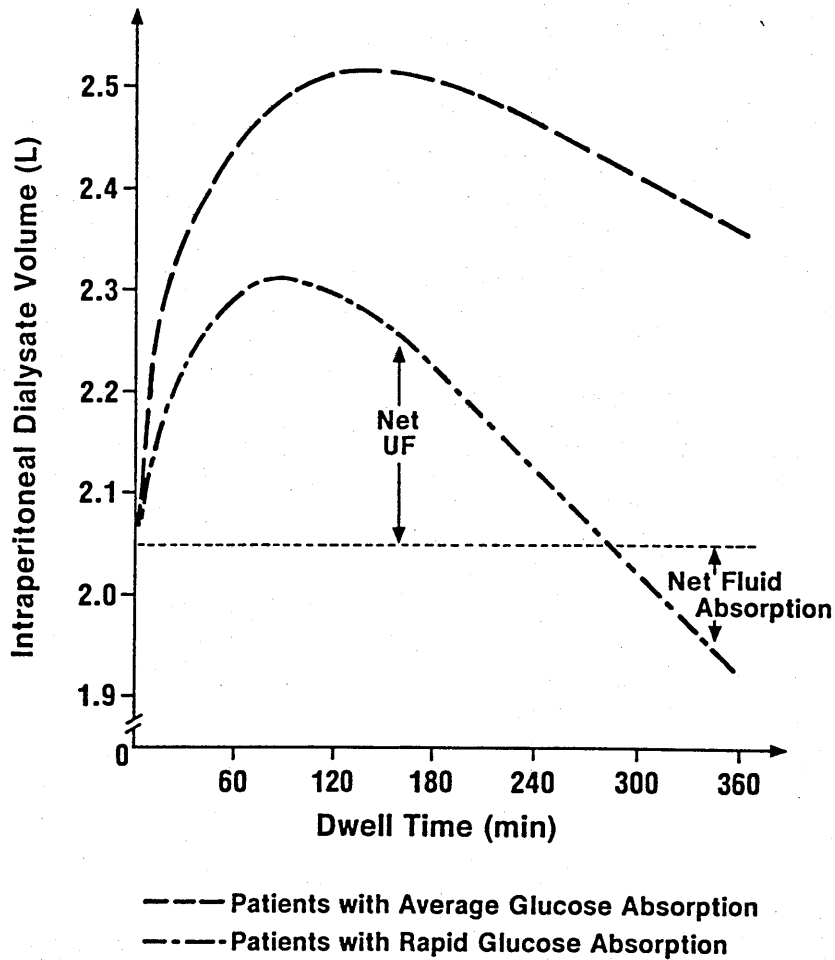
Figure 1: Representative intraperitoneal fluid volumes during exchanges using 2 litres of 2.5% dextrose dialysis solution in CAPD patients with average and high transperitoneal absorption of glucose from the dialysate.

DWELL TIME OF DIALYSIS SOLUTION

In peritoneal dialysis the rate of equilibration of dialysate solutes with plasma depends on the solute molecular weight, configuration and charge (46). Thus in most patients small molecular weight solutes, such as urea, almost completely equilibrate with the plasma within six hours (dialysate/plasma concentration ratios approach unity) whereas large solutes continue to equilibrate at an almost linear rate until the end of the dwell time.

Solutes diffuse across the peritoneum in a direction to discharge their concentration gradients. Other than glucose, ionised calcium and buffer anion, the direction of solute movement is from the peritoneal microcirculation to the dialysis solution. The diffusive mass transfer of solutes (J_s) is equal to the product of membrane permeability (P_s), effective peritoneal surface area (A), and solute concentration gradient ($\triangle C$). That is:

$$J_s = P_s \ A \ \triangle C \qquad \ldots\ldots\text{equation 3.}$$

The concentration gradient for diffusion is maximum near completion of infusion of the dialysis solution and for small solutes decreases exponentially until equilibrium with extracellular fluid is approached.

Solute transport across the peritoneal membrane also occurs with ultrafiltration due to solvent drag. The peritoneum offers

greater resistance to accompanying solutes than to water so that the concentration of solutes in the ultrafiltrate is less than in plasma water (solute sieving). Hence convective solute transport (J_s) is the product of water flux (J_w), sieving coefficient of the peritoneal membrane (S) and mean serum solute concentration (C_s). That is:

$$J_s = J_w \; S \; C_s \qquad \ldots\ldots\text{equation 4.}$$

Consequently the contribution of convective transport to solute mass transfer diminishes as water flux decreases during the dwell time. Thus the dialysate sodium concentration initially decreases due to solute sieving with ultrafiltration and increases later in the dwell time due to diffusion (47). For this reason hypernatraemia may result if excessive ultrafiltration is sustained by consecutive, short-dwell hypertonic exchanges. Solute sieving also leads to osmolar equilibrium being reached before glucose equilibrium.

Convective and diffusive solute transport are interrelated. Ultrafiltration enhances diffusive mass transfer of solutes other than glucose, ionised calcium and buffer anion by maintaining the concentration gradient with solute sieving. In contrast diffusion limits ultrafiltration because of glucose absorption.

Since the dialysate concentration of urea approaches equilibrium with plasma after six hours dwell time, net removal of urea in CAPD is determined primarily by the dialysate drain volume (dialysate flow rate) (18). In contrast mass transfer of large solutes, such as

proteins, is influenced predominantly by the patient's peritoneal permeability x area. If the exchange dwell time is reduced below four hours, small solute mass transfer is also influenced by peritoneal permeability x area. The effective membrane area (A) for each solute is unknown and so peritoneal permeability x area have to be considered together in studies of peritoneal transport (48).

OSMOLALITY OF DIALYSIS SOLUTION

The rate of equilibration of small solutes with plasma is only marginally reduced with hypertonic solutions despite solute sieving with ultrafiltration (25,46). Accordingly dialysate/plasma solute ratios show a minor decrease during the initial hours of hypertonic exchanges. Hyperosmolar glucose and buffer anion dialysis solution have an additive vasodilator effect on the peritoneal microcirculation (49). Increased peritoneal permeability x area is observed both during and after exchanges using hypertonic glucose solutions (50,51) and will tend to counteract the delay in solute equilibration induced by the concurrent increase in hypotonic ultrafiltrate. Consequently in most patients urea approaches equilibrium after six hours during hypertonic exchanges and small solute mass transfer will depend predominantly on the dialysate drain volume.

Adverse effects of hypertonic exchanges on peritoneal transport of solutes other than glucose are relatively minor. Protein losses in the dialysate are unrelated to the daily ultrafiltration volume (33,52) and, in the absence of peritonitis, for a given patient

remain almost constant each day (53,54). A small net mass transfer of calcium from the dialysate during 1.5% hydrated dextrose exchanges is usually reversed during 4.25% exchanges due to the increased ultrafiltration volume (55).

VOLUME OF DIALYSIS SOLUTION

The rate of equilibration of dialysate solutes with their plasma concentration is slowed during exchanges using higher volumes of dialysis solution (33,56). The delay in solute equilibration during exchanges with higher instilled volumes is most likely secondary to reduced peritoneal surface area/dialysate volume relationships since indices of peritoneal permeability (solute dialysance ratios and protein losses) are unchanged (33,56). With long-dwell exchanges small solutes approach equilibration with plasma, and solute mass transfer is again dependent mainly on the drain volume.

INTERINDIVIDUAL VARIATION IN SOLUTE TRANSPORT

Interindividual variation in peritoneal solute transport rates can be demonstrated mathematically by calculating the mass transfer area coefficient (24,57-59) or clinically by comparing solute equilibration rates during standardised exchanges (Figure 2) (60,61).

Interpatient differences in solute equilibration rates during standardised exchanges are dependent primarily on peritoneal permeability x area (equations 3 and 4). In patients with high peritoneal permeability x area the mass transfer of small molecular

UREA

CREATININE

GLUCOSE

PROTEIN
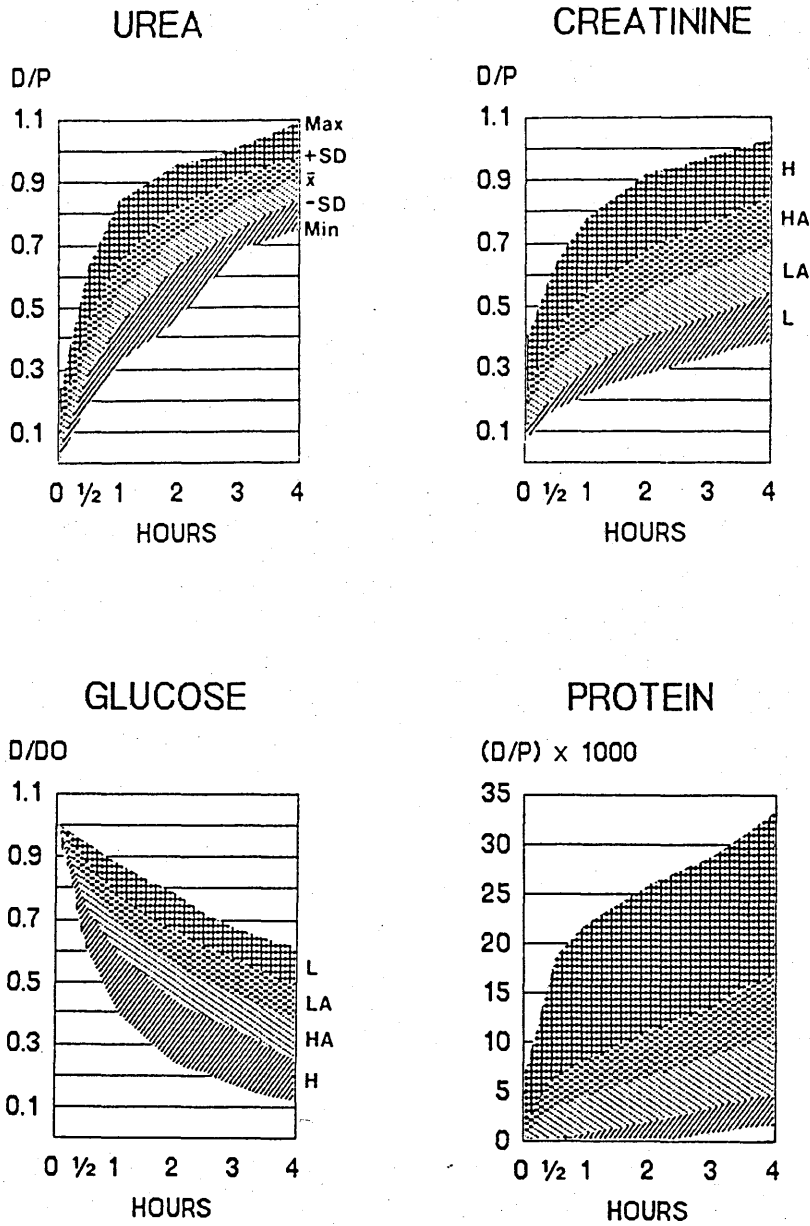
Figure 2: Equilibration curves during standardised four hour exchanges using 2 litres of 2.5% dextrose dialysis solution. The mean ± 1 SD solute ratios categorise CAPD patients with high (H), high average (HA), low average (LA) and low (L) peritoneal transport. Reproduced with permission of Dr. Z.J. Twardowski, University of Missouri-Columbia.

weight solutes in long-dwell exchanges decreases proportionately with the reduction in drain volume (61). Reducing the dwell time in these patients captures maximum ultrafiltration while maintaining near complete equilibration of small molecular weight solutes and so increases net solute removal. Incomplete equilibration of small solutes after long-dwell exchanges is observed in patients with markedly reduced peritoneal permeability x area. No apparent cause for reduced peritoneal transport is evident in the majority of these patients (61), although some have systemic vascular disease or extensive peritoneal fibrosis (62,63). These patients require high dialysate flow rates to achieve adequate daily peritoneal clearance rates.

INTRAINDIVIDUAL VARIATION IN SOLUTE TRANSPORT

Significant sequential changes in peritoneal solute mass transfer are infrequent in CAPD patients (64-68) and, other than during peritonitis episodes, the diffusive mass transfer properties of the peritoneum remain relatively constant in most patients (64-66). Even if membrane resistances to small solute transport are altered (57), dialysate/plasma ratios still approach equilibrium by the end of long-dwell exchanges in CAPD. A minority of CAPD patients develop reduced peritoneal mass transfer of solutes without concomitant loss of ultrafiltration (Type II membrane failure) (60). Decreased permeability x area in these patients is often due to massive intraperitoneal adhesion formation or sclerosing peritonitis and usually necessitates transfer from CAPD (69,70).

Chapter 2   ANATOMY AND PHYSIOLOGY OF THE PERITONEAL CAVITY LYMPHATICS

2.1   HISTORICAL PERSPECTIVE

Von Recklinghausen in 1863 was the first to propose that carbon
particles, red blood cells, proteins and fluid move from the
peritoneal cavity into the diaphragmatic lymphatics via openings in
the subdiaphragmatic peritoneum, which he called stomata (71). Other
investigators subsequently claimed that the stomata were artifacts
(72,73), but the presence of these specialised terminal lymphatics in
animals and man has since been confirmed by light and electron
microscopy (74-78). The considerable absorptive capacity of the
peritoneal cavity lymphatics was recognised by physiologists earlier
this century (79-82) and has been utilised to perform
intraperitoneal blood transfusions in the foetus and infant (83,84).

The recent expansion of peritoneal dialysis therapy has
promoted renewed interest in the morphology of the peritoneum (14-19)
and the kinetics of transperitoneal ultrafiltration and solute mass
transfer (20-68). Review of the anatomy and physiology of the
peritoneal cavity lymphatics indicates that the role of lymphatic
absorption in the kinetics of peritoneal dialysis also merits
investigation.

2.2 ANATOMY OF THE PERITONEAL CAVITY LYMPHATICS

2.2.1   THE LYMPHATIC PATHWAYS

Lymphatic drainage from the peritoneal cavity occurs mainly through specialised end lymphatic openings (stomata) located on the undersurface of the diaphragm (85-88). Moreover, absorption is greatest from the right side overlying the liver (87). Absorption by the lymphatic capillaries within the interstitium of the mesentery, parietal peritoneum and omentum contributes a minor proportion of the total peritoneal lymphatic drainage (88-90).

The lymphatic capillaries coalesce to form a rich plexus of collecting lymphatics within the muscular portion of the diaphragm, which anastomoses with the lymphatics from the pleural surface. From the diaphragmatic lymph nodes, most of the lymphatic trunks travel retrosternally with the internal mammary vessels to the anterior mediastinal lymph nodes around the thymus and return almost 80% of the peritoneal lymphatic drainage to the venous circulation via the right lymph duct (89,91). Efferent lymphatics from the anterior mediastinal nodes may, however, occasionally drain to the central veins on the left side in association with or separate from the thoracic duct. Lymphatic drainage from the remainder of the peritoneum, including part of the diaphragm, returns to the systemic circulation via the thoracic duct (89). Cannulation of the thoracic duct during peritoneal dialysis in the rat collected less than 30% of the total estimated lymphatic drainage (92).

The major substernal and other minor lymphatic pathways from the peritoneal cavity are summarised in Figure 3.

```
                    ┌─────────────────────────┐
                    │   Internal Jugular and  │
                    │  Subclavian Vein Junction│
                    └─────────────────────────┘
                         ↗              ↖
                 (Right)                    (Left)
                 70-80 %                    20-30 %
         ┌──────────────────┐        ┌──────────────────┐
         │ Right Lymph Duct │        │   Thoracic Duct  │
         └──────────────────┘        └──────────────────┘
                 ↑                            ↑
         ┌──────────────────┐        ┌──────────────────┐
         │     Anterior     │        │   Cisterni Chyli │
         │ Mediastinal Nodes│        └──────────────────┘
         │ and Lymphatic Trunks│          ↗        ↘
         └──────────────────┘                        < 5% Small
                 ↖              ↗              →      Lymphatico-
         ┌──────────────────────────┐               venous
         │ Prenodal Lymphatic Trunks│  ──────→      Connections
         └──────────────────────────┘
                 ↑    ↗      ↑
         ┌──────────────────────────┐
         │   Collecting Lymphatics  │  ──────→
         └──────────────────────────┘
                 ↑    ↗      ↑
         ┌──────────────────────────┐
         │   Lymphatic Capillaries  │
         └──────────────────────────┘
                 ↑              ↑
    ┌──────────────────┐  ┌──────────────────────┐
    │   Diaphragmatic  │  │  Omental, Mesenteric │
    │     Stomata      │  │    End Lymphatics    │
    └──────────────────┘  └──────────────────────┘
              ↖              ↗
              Intraperitoneal Fluid
```

Figure 3: Anatomical pathways of lymphatic absorption of intraperitoneal fluid.

2.2.2 THE SUBDIAPHRAGMATIC STOMATA

The lacunae of the end lymphatics in the subdiaphragmatic peritoneum are only separated from the peritoneal cavity by mesothelium, a loose meshwork of connective tissue and lymphatic endothelium (75,76). Transmission and scanning electron microscopy show that the stomata permit absorption of intraperitoneal fluid, cells, particles and colloids into the underlying lymphatic lacunae via extracellular pathways (75,93).

The mesothelial cells that overlie the lymphatic lacunae are smaller and separate from each other more readily than the cells in the surrounding mesothelium (75,94). The stomata are formed by the separation of adjacent mesothelial cells and, in the rat, can accommodate spherical particles up to 22.5 μm in diameter (95). The submesothelial basement membrane and lattice of connective tissue become fenestrated at the stomata (96) and the mesothelial cells adjoin the lymphatic endothelial cells to form a channel from the peritoneal cavity to the lumen of the underlying lacuna (97,98). Actin filaments are abundant in the cytoplasm of the mesothelial and endothelial cells around the stomata and may help maintain the patency of the stomata during relaxation of the diaphragm (99,100).

2.2.3 MECHANISM OF LYMPHATIC ABSORPTION

The rate of lymphatic absorption from the peritoneal cavity depends on the excursions of the diaphragm during respiration (99-

101). During expiration the diaphragm relaxes, adjacent mesothelial and endothelial cells in the roofs of the lacunae separate from each other to form open junctions, suction is created by the distension of the lacunae and intraperitoneal fluid is absorbed. In inspiration the contraction of the diaphragm closes the gaps between the overlying mesothelial and endothelial cells and empties the lacunae into the efferent lymphatics. Backflow into the peritoneal cavity during inspiration is prevented by the overlapping of the endothelial cells in the roofs of the lacunae (76), and the valves in the efferent lymphatics maintain forward flow induced by lymphatic contractility and by changes in intrathoracic pressure (76,101). The increased lymphatic absorption rate from the right hemidiaphragm is most likely due to compression of the liver against the subdiaphragmatic stomata during respiration (94).

2.3 PHYSIOLOGY OF THE PERITONEAL CAVITY LYMPHATICS

2.3.1   FUNCTION

The lymphatics draining the peritoneal cavity act as a one-way system, returning excess intraperitoneal fluid and protein to the systemic circulation. The sum of hydrostatic and osmotic pressure gradients across the peritoneum normally favours a minor net inflow of fluid into the peritoneal cavity (28). Bidirectional transfer of small solutes occurs by diffusion and by solvent drag. Macromolecules (molecular weight greater than 20,000), however,

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exhibit minimal direct reabsorption into the peritoneal capillaries (102) and, after unidirectional transport from the peritoneal microcirculation, are transported back from the peritoneal cavity to the venous circulation by convective flow in the lymphatics. Peritoneal lymphatic drainage of serous fluid normally equals its rate of formation and only a small volume of isosmotic fluid is maintained in the peritoneal cavity.

The lymphatics carrying fluid and solutes from the intestinal mucosa traverse the mesentery before draining into the cisterna chyli and the thoracic duct but are not significantly involved in absorption of isosmotic fluid from the peritoneal cavity per se (88,89).

Absorption by the peritoneal cavity lymphatics and phagocytosis by resident intraperitoneal macrophages are the major first lines of defence after a bacterial inoculum enters the peritoneal cavity (103,104). Bacteria have been observed in the thoracic duct lymph of dogs within 10 minutes of intraperitoneal injection (105) and have been recovered from the mediastinal lymph nodes of patients who have died of peritonitis (106). The macrophages in the omentum provide an efficient defence against bacteria, but the omental lymphatics only play a minor role in absorption of fluid from the peritoneal cavity (90).

2.3.2   ABSORPTIVE CAPACITY OF THE PERITONEAL LYMPHATICS

The lymphatics draining the peritoneal cavity are virtually the sole pathway for absorption of intraperitoneal isosmotic fluid, biologically inert particles, colloids and cells (89,94,101,107). The absorptive capacity of the peritoneal cavity lymphatics can be evaluated in normal animals from the rate of uptake of isosmotic fluid (plasma, whole blood, crystalloid solutions) instilled into the peritoneal cavity (79-82,86-88,108-114). Representative values of the considerable lymphatic absorption rates in animals and man are shown in Table 1. Obliteration of the subdiaphragmatic peritoneum or ligation of the parasternal lymphatic trunks markedly reduces the rate of intraperitoneal fluid absorption (88,108).

TABLE 1: RATES OF LYMPHATIC ABSORPTION FROM THE PERITONEAL CAVITY IN DIFFERENT SPECIES

SPECIES	INFUSION VOLUMES (BODY WEIGHT)	INFUSED SOLUTION	ABSORPTION RATE	REF.
Rat	20 ml (per Kg)	Homologous Plasma	6 ml/Kg/hour	108
Rabbit	20 ml (per Kg)	Homologous Plasma	3.5 ml/Kg/hour	108
Cat	50 ml (2.4-2.7 Kg)	Homologous Serum	4.2-6.0 ml/hour	88
Dog	1000 ml (13-18 Kg)	0.9% Saline	16.8 ml/hour*	109
Man	1000 ml (45-86 Kg)	0.9% Saline	33 ml/hour*	110

* After serum and intraperitoneal fluid reached osmolar equilibrium (references 109 and 110).

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2.3.3   FACTORS CONTROLLING LYMPHATIC ABSORPTION

Physiological factors influencing the rate of lymphatic absorption include the intraperitoneal fluid volume and pressure, posture and the rate and depth of respiration.

Lymphatic and peritoneal transcapillary absorption are both increased by raised intraperitoneal hydrostatic pressure (112) and decreased after paracentesis (115).   Hyperventilation, induced by breathing carbon dioxide, increased whereas anaesthesia and phrenic neurectomy reduced lymphatic absorption (113,114).   Upright posture with small intraperitoneal volumes reduces the rate of lymphatic flow, although absorption still occurs due to propulsion of intraperitoneal fluid towards the diaphragm by intestinal peristalsis (108).   The circulation of intraperitoneal fluid towards the diaphragm also explains the relative frequency of abscess formation in the right subphrenic space following entry of bacteria into the peritoneal cavity (116). Fowler successfully localised infection in the pelvis of patients with diffuse peritonitis by elevating the head of their beds by twelve to fifteen inches (117).   Although obstruction of the subdiaphragmatic stomata by fibrosis or fibrin may reduce lymphatic absorption after infectious peritonitis, chemical peritonitis induced by sodium hypochlorite increased the rate of lymphatic drainage in the recovery period (118).   This rise in lymphatic absorption may be related to rapid regeneration of end lymphatics after injury (119).

Factors known to influence peritoneal lymphatic drainage are summarised in Table 2.

TABLE 2: FACTORS INFLUENCING LYMPHATIC ABSORPTION FROM THE PERITONEAL CAVITY

1. Intraperitoneal fluid volume

2. Intraperitoneal hydrostatic pressure

3. Rate and depth of respiration

4. Posture

5. Intestinal peristalsis

6. Patency of the diaphragmatic and mediastinal lymphatics

Chapter 3.   ROLE OF LYMPHATIC ABSORPTION IN ASCITES

3.1 PATHOPHYSIOLOGY OF ASCITES

The role of peritoneal lymphatic absorption in the pathogenesis
of ascites is well established (120-123). Ascites does not develop
until net transperitoneal inflow of fluid into the peritoneal cavity
exceeds the rate of fluid efflux via the peritoneal cavity
lymphatics.

Net influx of fluid into the peritoneal cavity may be induced by
several pathological alterations in transcapillary forces (equation
2), including:

a)   A rise in hepatic sinusoidal and portal hydrostatic pressure

b)   A reduction in serum oncotic pressure

c)   An increase in peritoneal hydraulic permeability

The rate of peritoneal lymphatic absorption of this inflow of
fluid   is   influenced   by   the   intraperitoneal   fluid volume,
intraperitoneal pressure and the patency of the lymphatic pathways.
During accumulation of ascites, the resultant fluid shifts into the
peritoneal cavity cause a relative reduction in the intravascular
volume and stimulate sodium and water retention by the kidneys. As
the ascitic fluid volume increases, the concurrent rise in

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intraperitoneal pressure will reduce transcapillary forces for further ascites formation. Accordingly a steady state will be reached eventually when the volume of ascites and body weight remain constant, and the rate of ascites formation is balanced by its rate of absorption.

The continuous bidirectional exchange of fluid in ascites precludes estimation of lymphatic drainage directly from the rate of absorption of isosmotic fluid. Consequently lymphatic absorption in ascites must be calculated indirectly.

3.2 CALCULATION OF LYMPHATIC ABSORPTION IN ASCITES

The peritoneal lymphatic absorption rate in ascites has been estimated from the rate of mass transfer of labelled colloids from the peritoneal cavity to the systemic circulation. This formulation is dependent on the following observations:

a)   Intraperitoneal macromolecules of molecular weight greater than 20,000, such as albumin, are returned to the venous circulation almost exclusively by the peritoneal lymphatics (89,102,124, 125).

b)   Isosmotic intraperitoneal fluid is drained by the peritoneal lymphatics without change in the concentration of index macromolecules (111,112,126-128).

Thus lymphatic absorption during the time of study (F_L) may be calculated as:

$$F_L = \frac{V_D \times \Delta C_D}{C_P} \qquad \ldots\ldots\text{equation 5.}$$

where V_D = volume of distribution of the tracer colloid (plasma volume)

ΔC_D = rise in plasma concentration of the tracer colloid

C_P = time averaged mean intraperitoneal concentration of the marker colloid

The stability of radio-labelled colloids in tissue fluids, the adequate mixing of the tracer colloid in the intraperitoneal fluid, and the safety and reproducibility of this technique have been established (129-131).

3.3 RATES OF LYMPHATIC ABSORPTION IN ASCITES

The mass transfer rates of radio-iodinated serum albumin (128, 130,131) and other radio-colloids (131-134) from the intraperitoneal fluid to the blood have been utilised to compare lymphatic flow rates from the peritoneal cavity in patients with hepatic (128,130,131), malignant (131-134) and nephrogenic ascites (130).

Lymphatic drainage in hepatic ascites is increased via two major pathways (135-137).   Prior to the onset of ascites in hepatic cirrhosis, increments in hepatic lymph formation, induced by increased intrasinusoidal hydrostatic pressure, are initially matched by rises in hepatic regional lymph drainage via the thoracic duct (136).   After the capacity of hepatic lymph drainage is exceeded, interstitial fluid leaks from the surface of the liver into the peritoneal cavity.   This rise in regional lymphatic absorption in hepatic cirrhosis is represented pathologically as an increase in both the number and size of the lymphatics draining through the porta hepatis (138).   The upper limit to hepatic lymphatic drainage via the thoracic duct is at least 10 litres per day (122,139). Hepatic lymphatic drainage is most likely limited since, although the thoracic duct becomes dilated with increasing flow (140), its ostium at the junction of the left internal jugular and subclavian veins remains fixed in diameter (139).   Consequently cannulation or surgical bypass of the ostium of the thoracic duct increases regional lymphatic absorption, reduces fluid overflow into the peritoneal cavity and improves ascites (140-142).

Lymphatic absorption from the peritoneal cavity, however, is relatively independent of the above pathway, since most of the absorbed fluid drains via the right lymph duct rather than the thoracic duct.   Mediastinal lymphoscintigraphy confirms that the substernal lymphatic pathways are the major route for absorption of

intraperitoneal fluid and macromolecules in hepatic ascites (131, 132). Estimations of peritoneal lymphatic absorption in 10 patients with hepatic ascites ranged from 24 to 223 ml per hour and averaged 80 ml per hour (128,130,131). The ascitic volume per se probably produces the high lymphatic flow rate. The large intraperitoneal fluid volume will ensure constant contact of fluid with the undersurface of the diaphragm and the concurrent rise in intraperitoneal pressure will enhance movement of fluid into the diaphragmatic lymphatics. Hepatic ascites has been controlled successfully by insertion of a peritoneo-venous shunt, which in effect further increases lymphatic absorption from the peritoneal cavity (143).

3.3.2 MALIGNANT ASCITES

Metastatic invasion of the subdiaphragmatic peritoneum is not uncommon in patients with intra-abdominal malignancy (144,145) and may at least partially obstruct lymphatic drainage from the peritoneal cavity (146,147). Subdiaphragmatic lymphatic capillaries filled with malignant cells are shown in Figure 4. Mediastinal lymphoscintigraphy in patients with malignant ascites often fails to demonstrate the patency of the diaphragmatic lymphatics or identify the mediastinal lymph nodes (131-134). Peritoneal lymphatic absorption in 22 patients with malignant ascites ranged from 1 to 63 ml per hour and averaged only 11 ml per hour (131). Moreover, the calculated lymphatic flow rate correlated with the concurrently
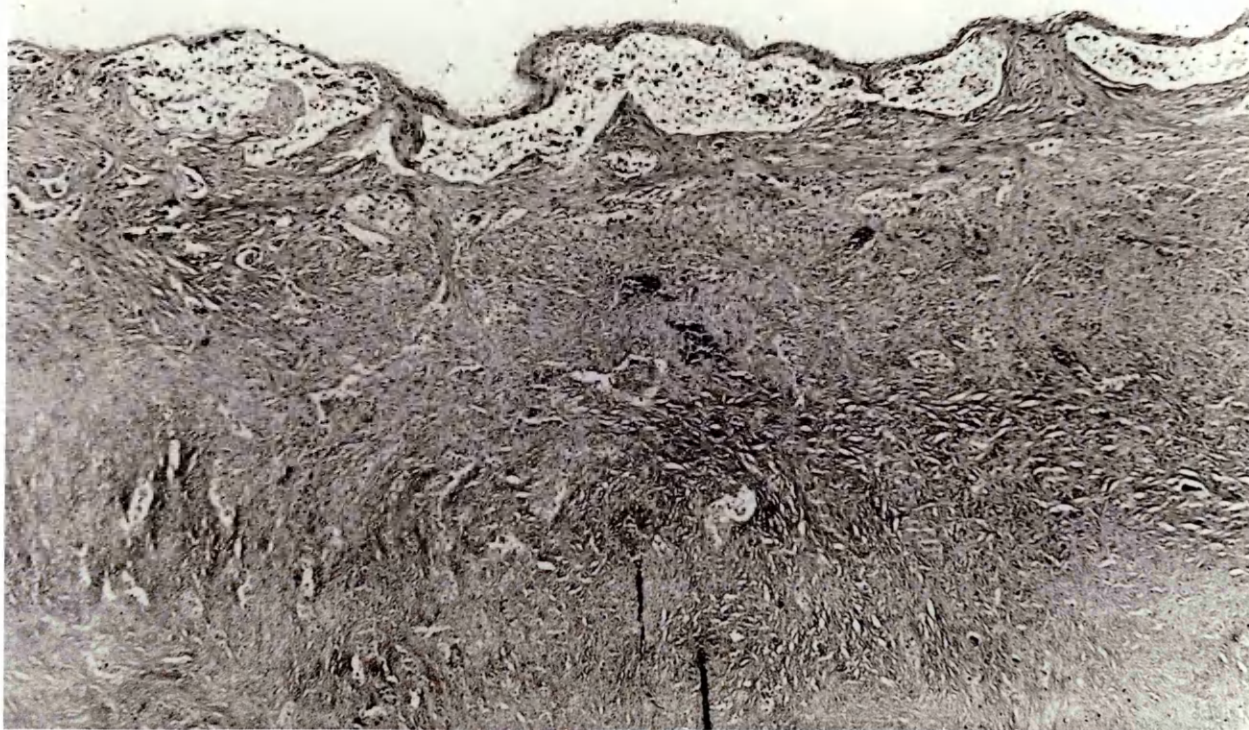
Figure 4.a: Autopsy specimen of the diaphragm and subdiaphragmatic peritoneum from a patient with malignant ascites secondary to adenocarcinoma of the body of the pancreas. The subdiaphragmatic lymphatic capillaries are filled with malignant cells (original magnification x 40).

Photomicrograph courtesy of Dr. Mark Hurt, Department of Pathology, University of Missouri-Columbia.
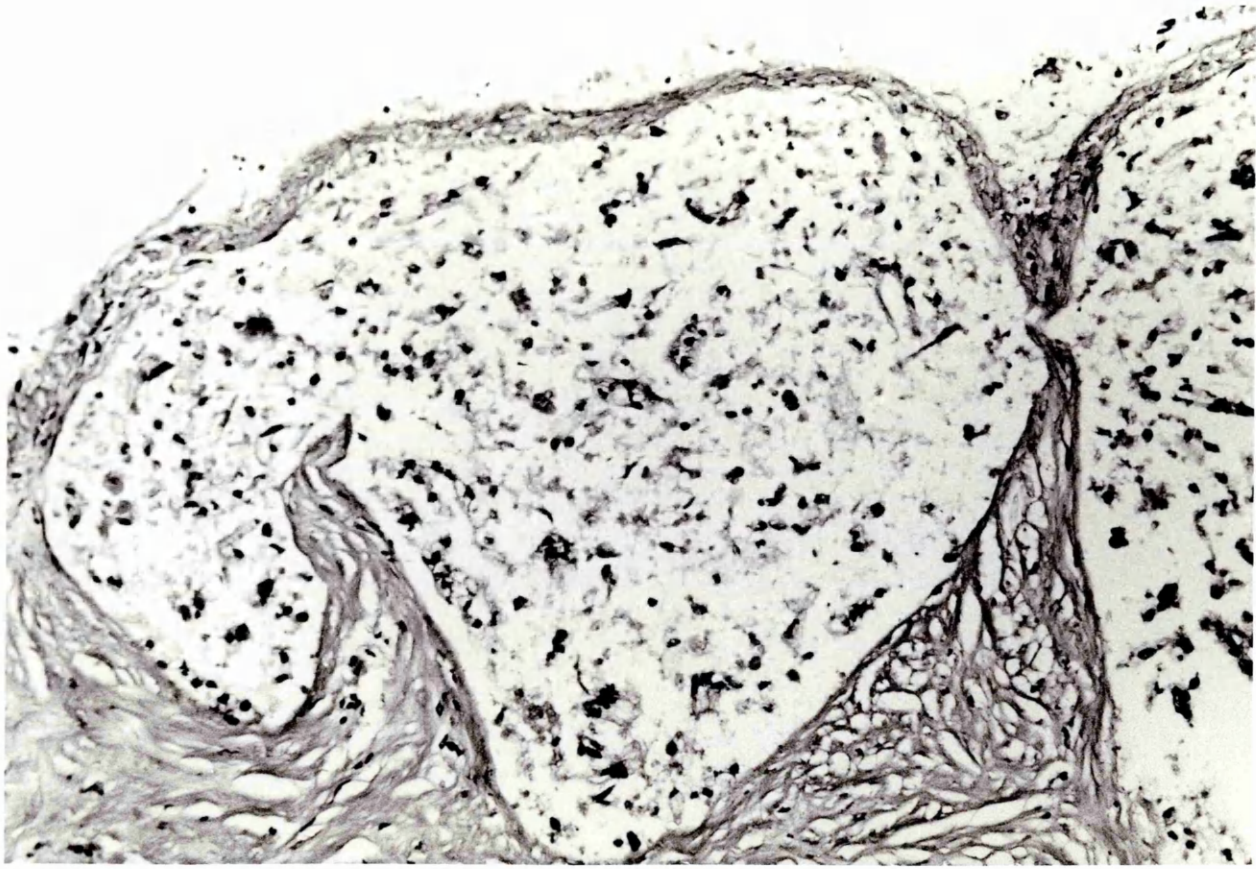
Figure 4.b: High power photomicrograph of the subdiaphragmatic lymphatics of the same patient, showing a subdiaphragmatic lymphatic capillary partially occluded by malignant cells (original magnification x 200).

performed lymphoscintigram (131). Thus, lymphatic absorption in malignant ascites may be markedly reduced by tumour invasion of the diaphragmatic and/or mediastinal lymphatics.

Likewise patients with schistosomal hepatic fibrosis and ascites have significant fibrous thickening of the subdiaphragmatic peritoneum, which limits flow into the diaphragmatic lymphatics (148). Obliteration of the undersurface of the diaphragm with fibrous tissue significantly reduces the rate of absorption of instilled isosmotic fluid in experimental animals (88, 124) and increases the incidence and severity of ascites in animals with infrahepatic portal hypertension (123). Neither portal hypertension or obstruction of the diaphragmatic lymphatics alone induced ascites in most of the study animals (123).

3.3.3   NEPHROGENIC ASCITES

Nephrogenic ascites is defined as dialysis associated ascites for which no primary cause can be identified (130). Most of the patients who have developed this form of ascites have been treated with intermittent haemodialysis and have a preceding history of cardiac failure or refractory fluid overload (149). Lymphatic absorption rates in 3 of these patients averaged 15 ml per hour, which is comparable to results in patients with malignant ascites (130). Increased central venous pressure secondary to right heart failure or fluid overload reduces the pressure gradient for lymphatic flow into the innominate veins (150) and may explain the relatively

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low lymphatic absorption rates observed in these patients. Alternatively reduced lymphatic drainage may be due to obstruction to lymphatic flow elsewhere in the substernal and diaphragmatic pathways.

The role of lymphatic absorption from the peritoneal cavity in the pathophysiology of these different forms of ascites is summarised in Table 3.

TABLE 3:   TRANSCAPILLARY FLUID INFLUX AND LYMPHATIC ABSORPTION RATES IN ASCITES

ASCITES	NET TRANSCAPILLARY FLUID INFLUX RATE	LYMPHATIC ABSORPTION RATE
Hepatic	++++	++
Malignant	+	-
Nephrogenic	+	-
Peritoneal Dialysis	+++	+ ?

Abbreviations: + increase
                - decrease

Chapter 4. ROLE OF LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS

4.1 RATIONALE

Despite the importance of peritoneal cavity lymphatic absorption
in the pathophysiology of ascites, the role of lymphatic drainage in
the kinetics of peritoneal dialysis "ascites" has been disregarded
(Table 3). Several theoretical and clinical considerations, however,
suggest that lymphatic absorption may significantly reduce net
ultrafiltration and solute mass transfer in peritoneal dialysis.

4.1.1   ANALOGY WITH ASCITES

Previous studies in ascitic patients indicate that lymphatic
drainage exceeds 50 ml per hour unless the diaphragmatic or
mediastinal lymphatics are obstructed by tumour or fibrosis (131).
Intraperitoneal fluid volumes during peritoneal dialysis in adults
routinely exceed 2 litres and should also ensure continuous contact
of fluid with the undersurface of the diaphragm. Moreover, the
patency of the peritoneal cavity lymphatics should be preserved if
the subdiaphragmatic parietal peritoneum only undergoes the same
minor histological changes following long-term peritoneal dialysis as
the parietal peritoneum lining the anterior abdominal wall (15-17).

4.1.2   INTERSTITIAL LYMPHATICS

Ultrafiltrate from the peritoneal capillaries reaches the peritoneal cavity via pathways through the interstitium and so must bypass absorption by the interstitial lymphatics. Small increments in interstitial hydrostatic pressure increase the lymphatic flow rate until a maximum is reached when tissue hydrostatic pressure is 2 mmHg above normal (151). Although the hydrostatic pressure within the peritoneal interstitium is unknown, submesothelial oedema is often observed in specimens of peritoneum from patients on peritoneal dialysis (15,16) and may enhance fluid absorption by the interstitial lymphatics.

4.1.3   KINETICS OF HYPOTONIC INTRAPERITONEAL FLUID

Intraperitoneal fluid is absorbed continuously by convective flow via the peritoneal cavity lymphatics, regardless of the prevailing tonicity of the dialysis solution. In contrast net transcapillary absorption of fluid only occurs if the dialysis solution is hypotonic to plasma (110,152,153). After instillation of hypotonic or hypertonic solutions into the peritoneal cavity, net transcapillary absorption or ultrafiltration respectively continue until osmotic equilibrium is reached (Figures 5 and 6). Thereafter, the intraperitoneal fluid remains isosmotic, net transcapillary fluid transport is nearly zero and the intraperitoneal fluid volume is decreased at a linear rate by lymphatic absorption. Consequently only
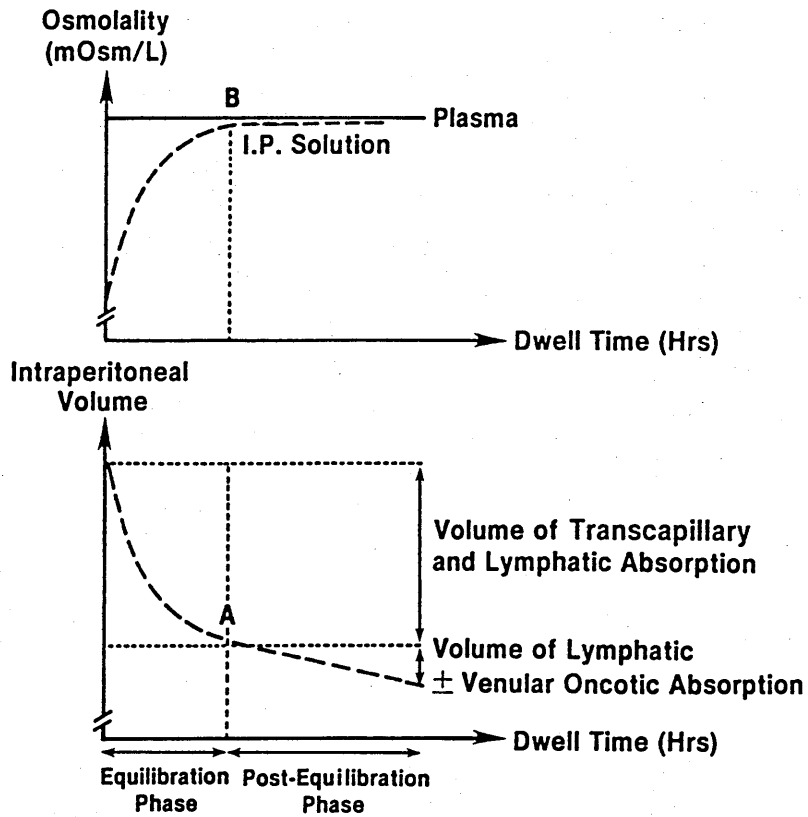
Figure 5: Changes in osmolality and intraperitoneal fluid volume following infusion of a hypotonic crystalloid solution. The equilibration volume (point A) coincides with crystalloid osmotic equilibrium (point B).
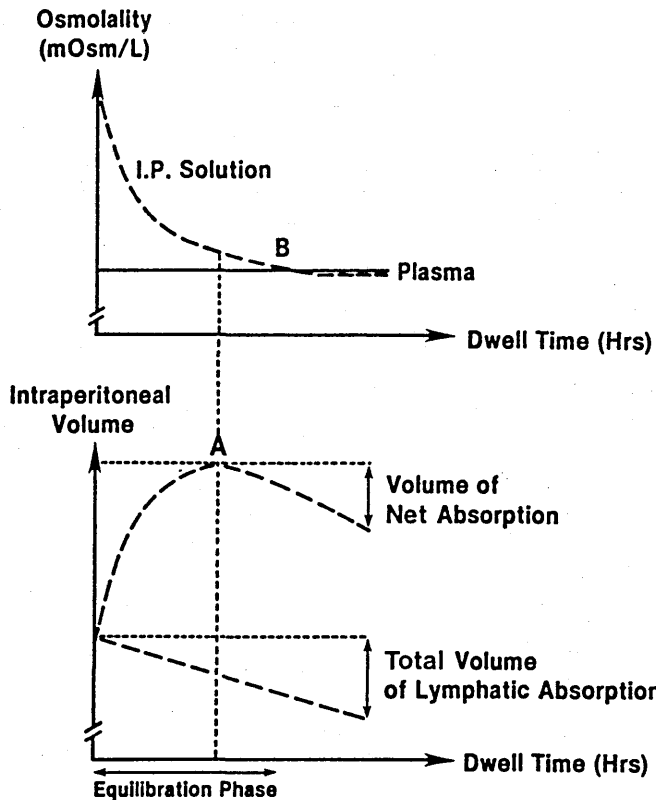
Figure 6: Changes in osmolality and intraperitoneal fluid volume following infusion of a hypertonic dextrose dialysis solution. The peak intraperitoneal volume (point A) precedes osmolar equilibrium (point B). Thereafter net fluid absorption represents lymphatic absorption in excess of net transcapillary ultrafiltration.

with initially hypotonic solutions is intraperitoneal fluid absorption both transcapillary and translymphatic (Figure 5).

An average of 546 ml of fluid was absorbed over seven hours in 9 uraemic patients after infusion of almost 2 litres of 0.9% saline (308 mOsm/L) into the peritoneal cavity (154). The average absorption rate of 78 ml per hour may, however, include net transcapillary absorption of fluid early in the dwell time due to an osmotic gradient between the infused 0.9% saline and uraemic plasma.

4.1.4 KINETICS OF ISOTONIC INTRAPERITONEAL FLUID

Absorption of intraperitoneal isosmotic fluid is primarily translymphatic (86-88,108-114). After 1 litre of 0.9% saline reached osmotic equilibrium with the plasma at two hours, the isosmotic fluid was then absorbed at a linear rate, which ranged from 30 to 37 ml per hour and averaged 33 ml per hour in the four patients (110). This study, however, overestimated the intraperitoneal volume later in the dwell time and thus underestimated actual fluid absorption. The intraperitoneal volumes during the eight hour dwell time were calculated from the dilution of a single dose of radio-iodinated serum albumin. The quantity of the tracer colloid remaining in the peritoneal cavity was corrected for losses in dialysate samples and urine and for mass transfer to the estimated plasma volume. However only 10-30% of the radio-iodinated serum albumin absorbed from the peritoneal cavity appears in the plasma volume during the study time (64,102,128,154-156). Consequently the

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calculations overestimated the quantity of the radio-labelled colloid remaining in the peritoneal cavity, and so overestimated the intraperitoneal volume later in the dwell time. Nevertheless, even at less than half of the usual intraperitoneal fluid volume of adults on CAPD, the rate of lymphatic absorption from the peritoneal cavity is considerable.

4.1.5 KINETICS OF HYPERTONIC INTRAPERITONEAL FLUID

In hypertonic peritoneal dialysis, the intraperitoneal volume begins to decrease before isosmolality of the dialysis solution and plasma is observed (26,29), indicating that net fluid absorption occurs before net transcapillary ultrafiltration is complete (Figure 6). Moreover, osmolar equilibrium is reached before osmotic and glucose equilibrium (26). The dialysis solution becomes isosmolar with the plasma before glucose equilibrium because of solute sieving with transcapillary ultrafiltration. The total transperitoneal osmotic pressure gradient is the sum of the products of the concentration gradient and the peritoneal reflection coefficient of each solute. Consequently the higher peritoneal reflection coefficient of glucose than other small molecular weight solutes (157) maintains the osmotic pressure gradient into the dialysis solution and allows net transcapillary ultrafiltration to continue at a slow rate until crystalloid osmotic equilibrium is approached later in the dwell time. The dialysis solution may become hyposmolar to plasma and this further suggests that net transcapillary

ultrafiltration continues after osmolar equilibrium is first reached. Thus the decrease in intraperitoneal volume (ΔV) after peak ultrafiltration really represents the lymphatic absorption rate (F_L) in excess of the net transcapillary ultrafiltration rate (J_W in equation 2). That is:

$$\Delta V = L_P \, A \, (\Delta \pi + \Delta P) \; - \; F_L \qquad \ldots \ldots \text{equation 6}$$

Direct measurements of drain volumes after sequential dwell times in 29 CAPD patients showed that the rate of decrease in the intraperitoneal volume ranged from 8 to 89 ml per hour and averaged 39 ml per hour (25,26). The net absorption rate was not significantly different, irrespective of whether 2 litre volumes of 1.5%, 2.5% or 4.25% dextrose dialysis solution were infused (25,26). Furthermore, net fluid absorption rates during dialysis with 2.5 litre infusion volumes also averaged 37 ml per hour in 16 CAPD patients (23). Thus, the daily average lymphatic absorption rate during CAPD may be indirectly estimated to exceed 1 litre per day.

4.2 DEFINITIONS

4.2.1   NET ULTRAFILTRATION

The measurable net ultrafiltration volume represents the net change in the intraperitoneal volume at the end of the dwell time and, assuming the residual volume is constant, equals the drain

volume minus the infusion volume. However the net ultrafiltration volume is, in effect, the difference between cumulative net transcapillary ultrafiltration into the peritoneal cavity and lymphatic absorption during the dwell time out of the peritoneal cavity (Figure 7). These two formulations of net ultrafiltration may be designated directly measured and calculated net ultrafiltration (UF), respectively. That is:

measured net UF = drain volume - infusion volume

calculated net UF = cumulative net transcapillary UF - lymphatic
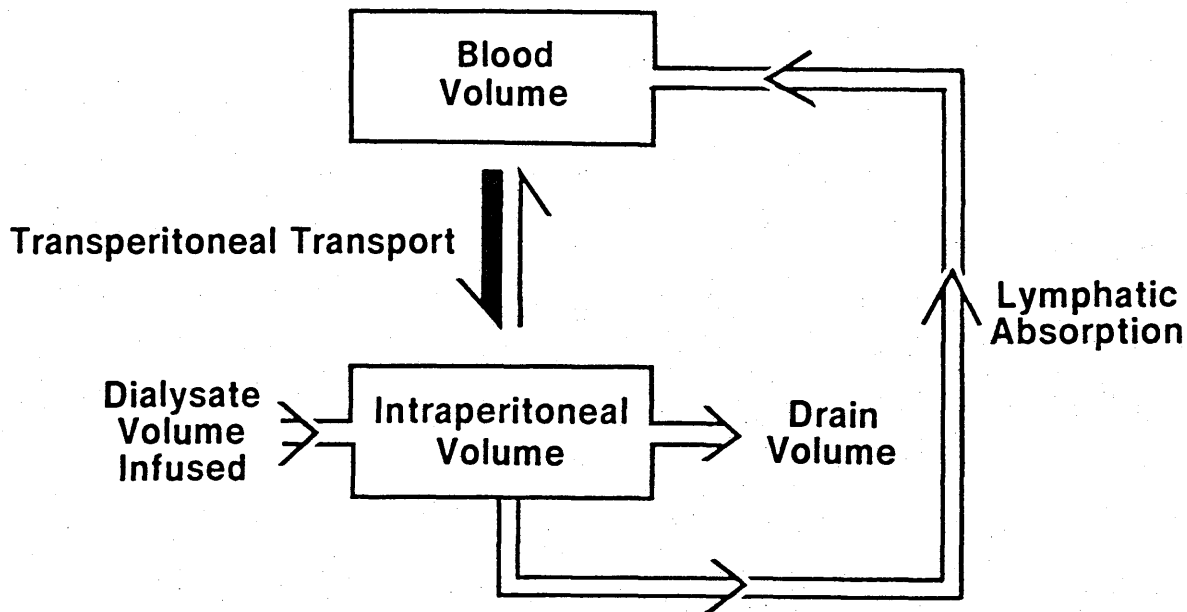
absorption

Figure 7: Schematic representation of the role of lymphatic absorption in the kinetics of peritoneal dialysis.

4.2.2   CUMULATIVE NET TRANSCAPILLARY ULTRAFILTRATION

Cumulative net transcapillary ultrafiltration defines the total net influx of fluid from the peritoneal microcirculation into the peritoneal cavity during the dwell time in response to the osmotic pressure of the dialysis solution. This definition allows for bidirectional transcapillary water movement during the dwell time and acknowledges that inflow into the peritoneal cavity dominates and that only the net fluid flux can be measured. Measured net ultrafiltration would equal the resultant net inflow of fluid if it was not for lymphatic absorption during the dwell time.

4.3   EFFECT OF LYMPHATIC ABSORPTION ON PERITONEAL DIALYSIS

The physiological roles of the peritoneal cavity lymphatics in the absorption of intraperitoneal isosmotic fluid, macromolecules, particles and bacteria are normally beneficial but have adverse consequences in peritoneal dialysis.

4.3.1   ABSORPTION OF INTRAPERITONEAL FLUID

Lymphatic drainage of intraperitoneal fluid throughout the dwell time will reduce the potential drain volume and consequently decrease both net ultrafiltration and solute mass transfer (18,22). During long-dwell exchanges lymphatic absorption is continuous, whereas net transcapillary ultrafiltration occurs mainly in the first hours of

the exchange. Thus fluid absorption via the lymphatics has a greater influence on ultrafiltration kinetics in CAPD than in intermittent peritoneal dialysis with rapid exchanges. In short-dwell exchanges cumulative net transcapillary ultrafiltration greatly exceeds lymphatic drainage and the reduction in the drain volume, due to lymphatic absorption, is relatively minor.

The contribution of lymphatic absorption to ultrafiltration kinetics in CAPD has several implications. Firstly, even if lymphatic drainage remains unchanged with time on CAPD, reduction of the intraperitoneal fluid volume by lymphatic absorption may become relatively more important if increases in peritoneal permeability x area reduce cumulative net transcapillary ultrafiltration, as in Type I membrane failure (41-43). The contribution of intraperitoneal fluid absorption to reduced net ultrafiltration in Type I membrane failure can be demonstrated by decreasing the dwell time, which captures maximum, albeit relatively low, ultrafiltration (158).

Secondly, calculations of transperitoneal ultrafiltration and solute transport which are based on the drain volume and drain dialysate solute concentration are erroneously low, since no allowance has been made for translymphatic absorption during the dwell time. Thus the efficiency of the peritoneal dialysing membrane is greater than previously recognised (24,57-59,64-66) and reduction of lymphatic absorption may provide an alternative means of increasing net ultrafiltration and solute mass transfer without increasing transperitoneal transport of water and solutes.

4.3.2 ABSORPTION OF INTRAPERITONEAL POLYMERS AND PARTICLES

Less absorbable osmotic agents than glucose have been sought to reduce the undesired metabolic sequelae of absorbed glucose and, most importantly, to induce sustained net transcapillary ultrafiltration (28,32,159-161). Despite initial promising results, the routine clinical use of glucose polymer has been limited by its systemic absorption and potential toxicity (159,160). Lymphatic drainage results in the systemic absorption of all polymer osmotic agents, regardless of their molecular weight, and will hinder, if not prevent, the development of effective and safe, alternative osmotic agents. Likewise any particulate matter which enters the peritoneal cavity in the dialysis solution will be potentially absorbed by the peritoneal cavity lymphatics. Thus contaminants in commercial dialysis solutions should be avoided to prevent their systemic accumulation and toxicity as well as their potentially adverse effects on the peritoneal membrane (39,162).

4.3.3 ABSORPTION OF INTRAPERITONEAL BACTERIA

Despite the uptake of bacteria by the peritoneal cavity lymphatics (105,106), blood cultures are infrequently positive during CAPD associated peritonitis (163). Moreover, secondary pulmonary infections or right sided endocarditis are very rare complications of peritonitis (104,163). The bacteria are presumably filtered and effectively trapped by the mediastinal lymph nodes. The

contractility of the lymphatic trunks is increased by leukotrienes and prostaglandins (164) and these mediators of inflammation are transiently elevated in the dialysis solution of patients with peritonitis (165). Consequently loss of ultrafiltration during peritonitis may be related to an increase in lymphatic absorption as well as to enhanced peritoneal permeability and increased absorption of glucose from the dialysate.

4.4 ESTIMATION OF LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS

Peritoneal lymphatic flow rates in hypertonic peritoneal dialysis cannot be quantitated directly since, later in the dwell time, net fluid absorption represents the lymphatic absorption rate in excess of the net transcapillary ultrafiltration rate (equation 6). Thus, lymphatic drainage in peritoneal dialysis, as in ascites, can only be estimated indirectly.

Since the peritoneal cavity lymphatics drain intraperitoneal fluid by convective flow without increase or decrease in the concentration of index colloids (111,112,126-128) and intraperitoneal macromolecules are returned to the systemic circulation almost exclusively by the lymphatics (89,102,124,125), the lymphatic absorption rate may be estimated either from the mass transfer rate of index colloids from the peritoneal cavity to the blood or from their rate of disappearance from the peritoneal cavity.

4.4.1 MASS TRANSFER RATES OF INTRAPERITONEAL COLLOIDS TO THE BLOOD

Peritoneal to blood clearances of radio-labelled colloids (equation 5) are a good index of the relative lymphatic absorption rates in different forms of ascites (128,130-134). However, the plasma appearance rate of an intraperitoneal index colloid underestimates the absolute lymphatic absorption rate for several reasons:

a) A significant proportion of the tracer colloid, absorbed by the subdiaphragmatic lymphatics, does not reach the systemic circulation during the study time due to delayed transit or permanent entrapment in the diaphragmatic and mediastinal lymph nodes. Indeed, this physiological function of the draining lymph nodes is utilised in mediastinal lymphoscintigraphy (131-134,166).

b) During the study time there is an initial lag phase before the plasma concentration of the radio-colloid begins to increase linearly as predicted (92,128,130,131).

c) The rise in concentration of the tracer colloid must be corrected for redistribution of the tracer out of the blood volume during the study interval (92,102,128).

Consequently, the lymphatic absorption rate calculated by this method is significantly lower than direct observations of absorption of plasma or whole blood in the same animal model (86,92). Likewise in 10 CAPD patients the average lymphatic flow rate, calculated from the peritoneal to blood clearance of radio-iodinated serum albumin, was only 11 ml per hour (156), compared with the aforementioned estimate from serial drain volumes of at least 39 ml per hour (25, 26). The lymphatic absorption rate in this study was underestimated since the plasma volume of the CAPD patients was extrapolated from their body weight, the elimination rate of the radio-colloid from the plasma was not included in the calculations and, most importantly, the plasma appearance rate of radio-iodinated serum albumin during the dwell time was only 20% of the peritoneal disappearance rate. Spencer and Farrell have shown that the mass transfer of intraperitoneal radio-iodinated albumin to the blood in CAPD patients is significantly greater after 24 hours than at the end of a four hour study exchange (64). Thus lymphatic transfer of radio-colloids continues after the washout exchange at the end of the study time and cannot be related accurately to the duration of the exchange.

4.4.2 MASS TRANSFER RATES OF COLLOIDS FROM THE PERITONEAL CAVITY

Alternatively the lymphatic absorption rate (F_L) may be estimated from the mass transfer rate of intraperitoneal macromolecules from the peritoneal cavity. That is:

$$F_L = \frac{(V_O \times C_O) - (V_t \times C_t)}{C_P} \qquad \dots\dots\text{equation 7.}$$

where V_O and V_t = intraperitoneal fluid volumes at times O and t

C_O and C_t = intraperitoneal concentration of marker colloid at times O and t

C_P = time averaged mean intraperitoneal concentration of marker colloid

This mass balance equation avoids the error in calculating lymphatic flow in the previous method due to delayed transfer of radio-labelled colloids from the diaphragm and interstitial lymphatics to the blood. This formulation, however, not only depends on the assumption that intraperitoneal macromolecules are absorbed exclusively from the peritoneal cavity by convective flow via the lymphatics (124-128), but further assumes that all of the intraperitoneal marker colloid lost from the peritoneal cavity is absorbed by the non-restrictive pathways of the lymphatics. In connective tissue spaces, back diffusion of colloids into capillaries is negligible (167), the osmolality and the concentration of protein in the tissue fluid and end lymphatic lymph are equal (151,168) and absorption of tissue protein is fully accounted for by lymphatic flow (167,169). Several observations indicate that these findings also pertain to intraperitoneal fluid and colloid kinetics and that intraperitoneal fluid absorption may be estimated from the rate of loss of an intraperitoneal marker colloid.

a) The concentration of marker colloids or albumin remains unchanged during absorption of intraperitoneal isosmotic fluid (86,92,102), suggesting that colloids are absorbed with fluid by convective transport through lymphatic pathways.

b) Fractional peritoneal absorption of albumin and IgG (128) and dextrans of different molecular weight (170) are similar, further suggesting that absorption of macromolecules is by convective flow.

c) The intraperitoneal content of radio-iodinated serum albumin during hypertonic peritoneal dialysis decreases at a linear rate averaging 3% per hour (156) and, late in the dwell time, correlates with net fluid absorption (29,156).

However, with microquantities of radio-colloid, a significant proportion of the administered dosage may be adsorbed to the peritoneal mesothelium, dialysis bag and administration set or be absorbed by the adjacent subperitoneal tissues (92,129,156). The addition of a large quantity of unlabelled colloid, such as albumin, instead of microamounts of radio-labelled colloid should only allow an insignificant fraction to be sequestered and so obviate this potential error. Thus, peritoneal cavity lymphatic absorption theoretically may be most accurately estimated from the net rate of removal of a high intraperitoneal concentration of unlabelled colloid from the peritoneal cavity (equation 7).

This method also requires accurate estimates of the intraperitoneal volume and serial samples of the intraperitoneal fluid. Both of these requirements can be achieved more easily in peritoneal dialysis patients than in patients with ascites, since CAPD patients have peritoneal catheters in-situ and both the infusion and drain volumes can be directly measured.

4.5 CONCLUSION

The role of lymphatic absorption in the kinetics of peritoneal dialysis has been disregarded (Chapter 1), even though the physiological absorptive capacity of the peritoneal cavity lymphatics is considerable (Chapter 2) and the importance of peritoneal lymphatic drainage in the pathophysiology of ascites is well established (Chapter 3). Indirect evidence indicates that lymphatic absorption in peritoneal dialysis may exceed 1 litre per day and thereby significantly alter ultrafiltration kinetics and reduce the efficiency of long-dwell peritoneal dialysis (Chapter 4).

The following studies were performed to resolve these issues.

PART II   STUDIES OF LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS

Chapter 5. LYMPHATIC ABSORPTION IN PERITONEAL DIALYSIS IN THE RAT

5.1 INTRODUCTION

The role of lymphatic absorption in the kinetics of ultrafiltration in peritoneal dialysis was evaluated during single six hour exchanges in rats using 15% dextrose dialysis solution. In this small animal model of peritoneal dialysis, serial direct measurements of net ultrafiltration and intraperitoneal volume can be compared with concurrent lymphatic absorption rates calculated from peritoneal clearances of rat albumin added to the dialysis solution. The albumin method for estimating the lymphatic absorption rate was assessed in parallel studies in rats using Ringer's lactate instead of hypertonic dextrose dialysis solution, where the net fluid absorption rate from the peritoneal cavity can be measured directly.

5.2 METHODS

5.2.1 THE RAT MODEL OF PERITONEAL DIALYSIS

After induction of anaesthesia with 50 mg per Kg subcutaneous pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, Illinois), male Sprague-Dawley rats were placed supine on a heating pad maintained at 37° C. Body temperature was monitored with a rectal temperature probe (Yellow Springs Instruments Inc., model 402), and anaesthesia maintained with supplemental subcutaneous

injections of 2.5 mg Nembutal as required. A Tenckhoff-type peritoneal catheter was passed through a small midline incision 1 cm below the xiphoid process and advanced into the peritoneal cavity with the tip directed towards the right lower quadrant of the abdomen. The external jugular vein was cannulated using Intramedic polyethylene tubing (Clay Adams, Parsippany, New Jersey), and the animals hydrated by infusion of warm Ringer's lactate solution (Baxter-Travenol Co., Deerfield, Illinois) at a rate of 8.8 to 13.3 ml per hour using a syringe pump (Sage instruments, model 341). Peritoneal exchanges were not begun until each rat was undergoing spontaneous diuresis, usually 60 to 90 mins after the intravenous infusion was commenced. The infusion rate was continued during the hypertonic exchanges to replace losses due to peritoneal ultrafiltration but was discontinued during the exchanges using Ringer's lactate solution.

5.2.2 EXCHANGES USING 15% DEXTROSE DIALYSIS SOLUTION

Six rats (mean weight 530g, range 489-627g) underwent a six hour exchange using 16 ml of 15% anhydrous dextrose dialysis solution. An immediate in and out exchange with 40 ml of Ringer's lactate solution was performed in each rat prior to the study exchange to determine the intraperitoneal residual volume. The hypertonic dialysis fluid was prepared by adding glucose to standard Dianeal PD-2 dialysis solution (Baxter-Travenol Co., Deerfield, Illinois). In three of the animals, rat albumin (Sigma Chemical Co.,

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St. Louis, Missouri) was added to the study dialysis solution to approach concentrations of 2.5% albumin.

The intraperitoneal volume at 30 mins and at the end of each hour was directly measured by rapid drainage into a graduated cylinder. Immediately after obtaining a sample (0.3 ml) from each drain volume, the dialysate was reinfused and the timer restarted. Complete drainage, sampling and reinfusion were performed within 5 minutes and this interval was considered as a total interruption of peritoneal transport. Baseline and serial dialysate samples were analysed for glucose and albumin concentrations and osmolality. Tail vein blood was obtained at each time interval for haematocrit and glucose concentration and at baseline and 360 min for estimation of osmolality and albumin concentration. Porcine insulin, 0.1 U per g % dialysate dextrose, was administered intraperitoneally and thereafter as needed to maintain serum glucose less than 200 mg/dl. The intravenous infusion rate of Ringer's lactate was altered as required to keep the haematocrit constant.

5.2.3 EXCHANGES WITH RINGER'S LACTATE SOLUTION

Six rats (mean weight 355g, range 319-404g) underwent a similar six hour exchange using 40 ml Ringer's lactate solution (273 mOsm/L). Rat albumin was added to the dialysis solution in three of the rats as before to give a 2.5% albumin solution. The protocol was the same as during the 15% dextrose exchanges with the exceptions that the

intravenous infusion was discontinued before beginning the study exchanges and no intraperitoneal insulin was administered.

5.2.4 LABORATORY METHODS

Albumin concentrations in serum and dialysate were measured in duplicate by the bromcresol green method (171). Glucose concentrations in the dialysate were measured by the ortho-toluidine method (172). Serum glucose concentrations were measured immediately with a Chemstrip BG (Boehringer Mannheim Diagnostic Inc., Indianapolis, Indiana). Serum and dialysate osmolalities were measured using a Wescor 5100 B vapour pressure osmometer (Wescor Inc., Logan, Utah).

5.2.5 CALCULATIONS

The intraperitoneal residual volume was calculated as the difference between the instillation and drainage volumes of the in and out exchange. Consequently, the intraperitoneal fluid volume at the end of each study interval equalled the drainage volume for that time interval plus the residual volume of the in and out exchange.

The net ultrafiltration volume after each study interval during the exchanges with hypertonic glucose was calculated as the drain volume for the time interval minus the initial infusion volume. The net absorption volume after each time interval during the exchanges

with Ringer's lactate was calculated as the instillation volume minus the drainage volume after the study interval.

Lymphatic absorption was estimated from the mass transfer rate of intraperitoneal albumin from the peritoneal cavity as described previously (equation 7). During each short time interval the average intraperitoneal albumin concentration (C_p) was calculated as the arithmetic mean of the corrected dialysate albumin concentrations at the beginning and end of the study interval. The corrected albumin concentration at each time interval in rats with albumin added to the dialysis solution was calculated as the measured albumin concentration minus the mean albumin concentration at the same time interval in rats without albumin added to the dialysis solution. Although these corrections were small, they allowed for the albumin entry from the peritoneal microcirculation during the dwell time, which would otherwise result in slight underestimates of net removal of intraperitoneal albumin by the lymphatics. Accordingly lymphatic absorption (ml) per time interval (t to t' min) equals

$$\frac{(C_t \times V_t) - (C_t' \times V_t')}{(C_t + C_t')/2}$$

where C_t and C_t' = corrected albumin concentrations at times t and t', respectively.

V_t and V_t' = intraperitoneal volumes at times t and t', respectively.

Cumulative lymphatic absorption was calculated as the progressive sum of interval measurements. Cumulative net transcapillary ultrafiltration was estimated by adding cumulative lymphatic absorption and cumulative measured net ultrafiltration. Mean interval rates were calculated by dividing changes per interval by the duration of the interval. Results were compared by Student's non-paired t test and correlation coefficients were calculated by linear regression.

5.3 RESULTS

5.3.1   EXCHANGES WITH 15% DEXTROSE DIALYSIS SOLUTION

Cumulative net ultrafiltration, cumulative lymphatic absorption and laboratory results during exchanges with 15% dextrose dialysis solution are summarised in Table 4. The mean intraperitoneal residual volume was 1.0 ± 0.3 (SEM) ml. The intraperitoneal fluid volume increased from 17 ± 0.3 ml at the beginning of the exchange to 41 ± 2.4 ml after six hours. Cumulative net ultrafiltration and cumulative lymphatic absorption related to dwell time are shown in Figure 8. Cumulative net ultrafiltration began to decrease after four hours while cumulative lymphatic absorption increased progressively throughout the dwell time. Cumulative net transcapillary ultrafiltration (actual transperitoneal ultra-filtration) was calculated as the sum of net ultrafiltration and lymphatic absorption after each time interval (Figure 9). At the end

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TABLE 4: ULTRAFILTRATION KINETICS AND LABORATORY RESULTS IN RATS DURING EXCHANGES WITH 15% DEXTROSE DIALYSIS SOLUTION (n=6)

DWELL TIME (min)	0	30	60	120	180	240	300	360
Cumulative Net UF (ml)	-	14.5 ± 1.1	23.3 ± 1.4	30.1 ± 2.4	31.4 ± 1.4	31.7 ± 2.2	28.8 ± 2.3	24.1 ± 2.2
Cumulative Lymph Flow (ml) *	-	3.4 ± 1.0	5.9 ± 1.4	11.0 ± 1.4	14.7 ± 3.2	17.3 ± 3.2	26.2 ± 4.7	28.4 ± 5.5
Dialysate Osmolality (mOsm/L)	1176 ± 7	489 ± 6	374 ± 2	326 ± 4	306 ± 3	292 ± 2	288 ± 2	280 ± 2
Serum Osmolality (mOsm/L)	302 ± 1	-	-	-	-	-	-	288 ± 2
Dialysate Glucose (mg/dl)	-	-	-	-	1150 ± 56	622 ± 64	322 ± 42	162 ± 4
Serum Glucose (mg/dl)	80 ± 1	160 ± 18	170 ± 9	110 ± 15	80 ± 9	60 ± 8	47 ± 6	40 ± 1
Haematocrit (%)	48 ± 0.6	51 ± 0.8	54 ± 0.8	52 ± 1.0	51 ± 1.2	49 ± 1.4	48 ± 1.2	48 ± 1.1

All results are expressed as mean ± SEM; * n=3.
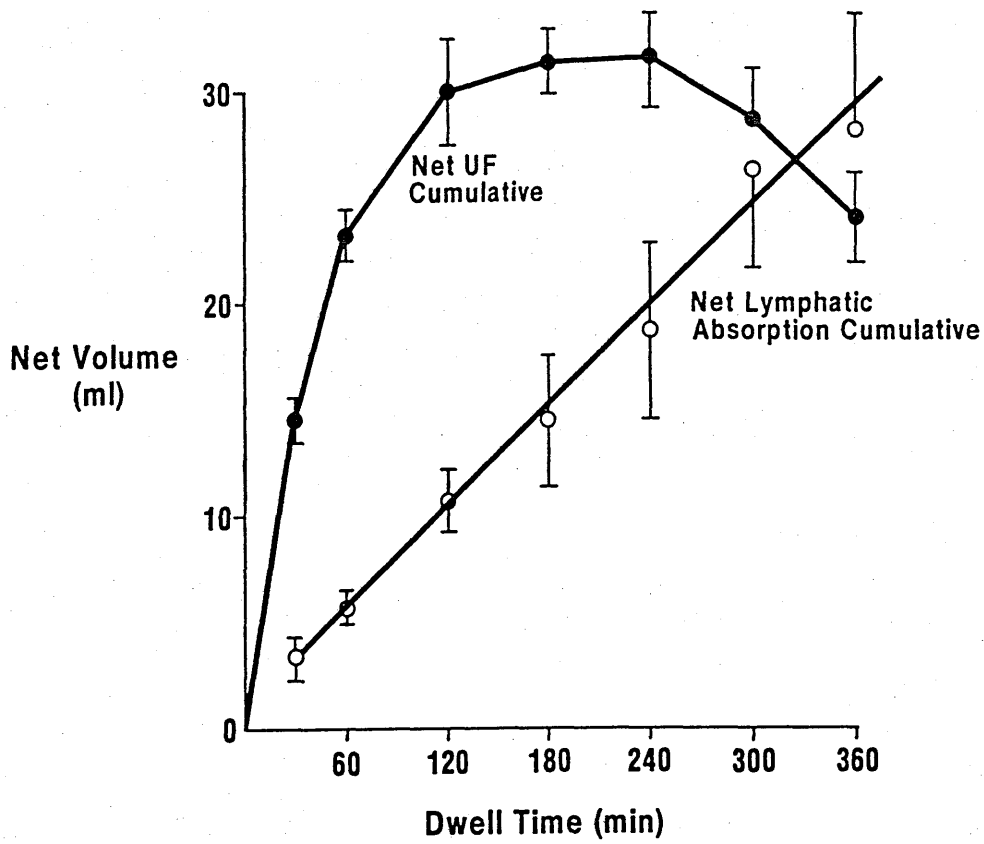
Figure 8: Cumulative lymphatic absorption and cumulative net ultrafiltration (mean ± SEM) during six hour exchanges with 15% dextrose dialysis solution in rats.

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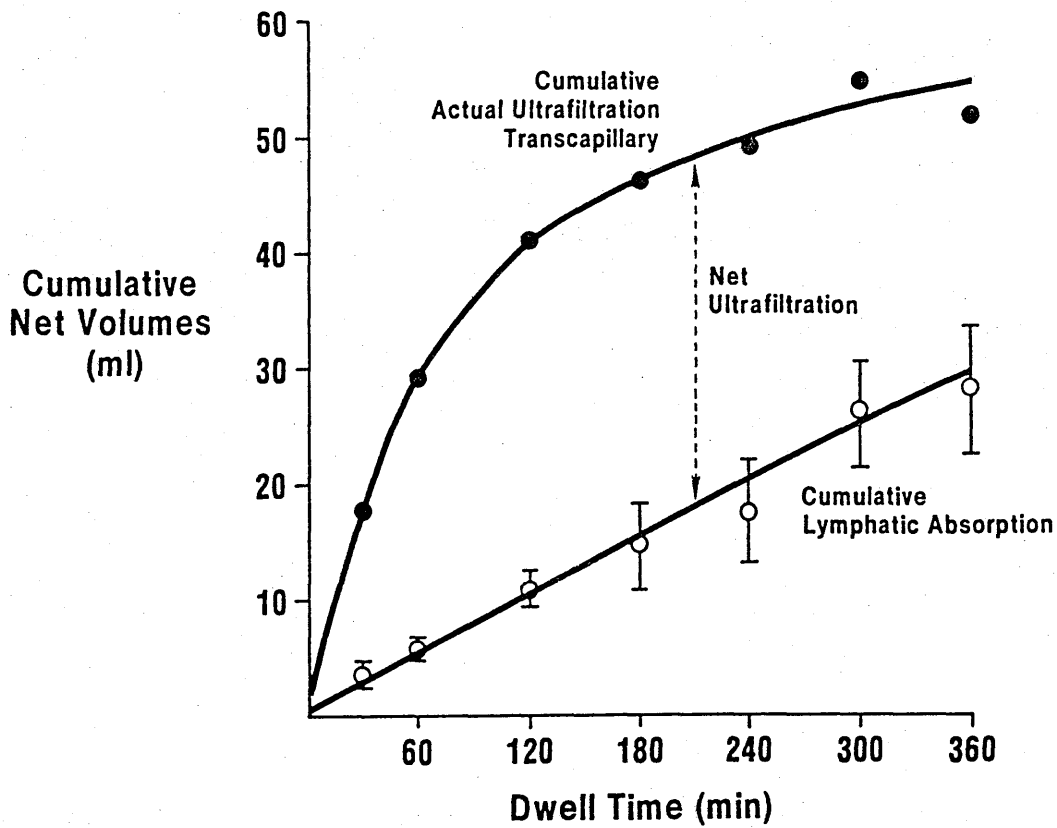
Figure 9: Cumulative lymphatic absorption (mean ± SEM) and cumulative net transcapillary ultrafiltration during six hour exchanges with 15% dextrose dialysis solution in rats.

of the six hour exchanges 54 ± 5% of the actual transperitoneal ultrafiltration had been reabsorbed by the peritoneal cavity lymphatics. That is, net ultrafiltration at the end of the dwell averaged 24 ± 2 ml whereas actual transperitoneal ultrafiltration averaged 52 ± 5 ml.

The net ultrafiltration volume and, thus the intraperitoneal volume, reached a maximum during the relative plateau phase between two and four hours dwell time (Table 4, Figure 8). The relationships of dialysate and serum osmolality and glucose concentrations to this intraperitoneal volume plateau phase are shown in Figure 10. Dialysate osmolality decreased almost exponentially and at six hours was significantly below serum osmolality (p < 0.05) even though dialysate glucose was still significantly higher than serum glucose (p < 0.01). As a result, the intraperitoneal volume plateau phase precedes osmolar equilibrium between serum and the dialysis solution and osmolar equilibrium precedes glucose equilibrium (Figure 10, Table 4).

Net transcapillary ultrafiltration and lymphatic absorption rates during each time interval are plotted in Figure 11. The net transcapillary ultrafiltration rate decreased at an exponential rate but did not reach zero until near the end of the dwell time. Lymphatic absorption is depicted as a mean rate over the entire dwell time since cumulative lymphatic absorption proceeded at a linear rate averaging 4.7 ± 0.9 ml per hour (Figure 9). The temporal relationships to peak intraperitoneal volume, osmolar equilibrium and glucose equilibrium are also shown in Figure 11. The maximum

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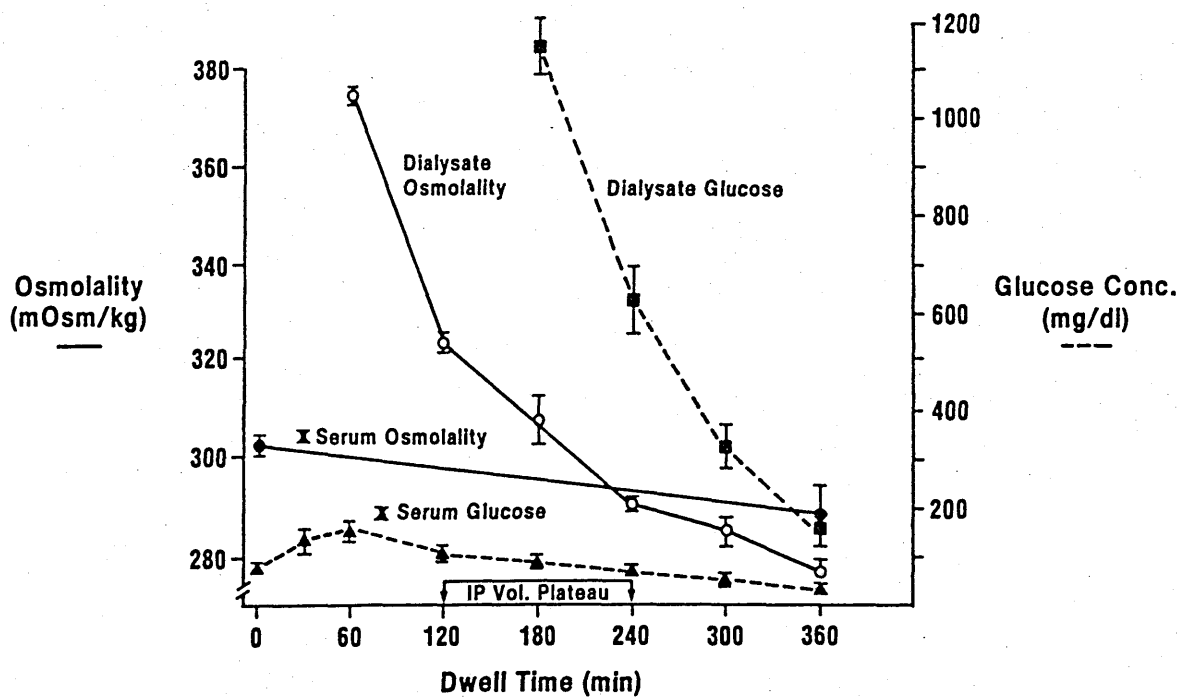
Figure 10: Dialysate and serum osmolalities and glucose concentrations (mean ± SEM) are related to dwell time and intraperitoneal volume in exchanges with 15% dextrose dialysis solution in rats. The period of maximum intraperitoneal volume is indicated.
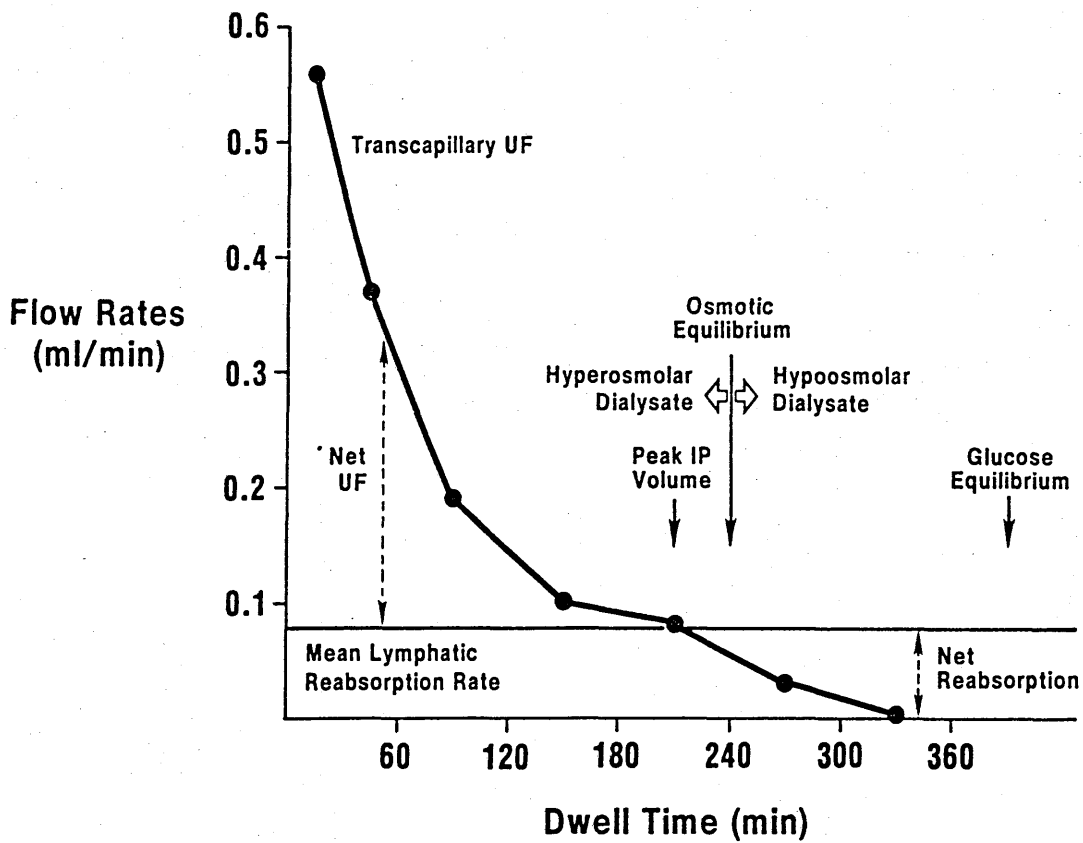
Figure 11: The net transcapillary ultrafiltration rate (mean ± SEM) and mean lymphatic absorption rate are related to dwell time and key events during exchanges with 15% dextrose dialysis solution in rats. The peak intraperitoneal volume precedes osmotic equilibrium which in turn precedes glucose equilibrium.

intraperitoneal volume occurred when the net transcapillary ultrafiltration rate equalled the lymphatic absorption rate. At this point the net ultrafiltration rate was zero. Thereafter the intraperitoneal volume decreased, even though net transcapillary ultrafiltration continued at a slow rate and osmolar equilibrium was not reached until slightly later in the dwell time. After osmolar equilibrium the dialysate became hyposmolar to serum, presumably as a result of continued net transcapillary ultrafiltration and solute sieving. Glucose dysequilibrium persisted throughout this hyposmolar phase until the end of the study dwell time.

Despite attempts at preventing hyperglycaemia using intraperitoneal insulin, a minor rise in serum glucose was observed early in the dwell time during peak glucose absorption from the dialysate (Table 4). However, this transient rise in serum glucose did not significantly reduce the transperitoneal osmolar gradient. The haematocrit also rose transiently during peak net transcapillary ultrafiltration early in the dwell time in spite of the intravenous infusion of Ringer's lactate to replace ultrafiltration losses as they occurred (Table 4).

5.3.2   EXCHANGES WITH RINGER'S LACTATE

Cumulative net fluid absorption, cumulative lymphatic absorption and laboratory results during exchanges with Ringer's lactate solution are summarised in Table 5. The initial mean intraperitoneal fluid volume decreased from 41 ± 0.4 ml to 25 ± 1.4 ml at the end of

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TABLE 5: INTRAPERITONEAL FLUID ABSORPTION AND LABORATORY RESULTS DURING EXCHANGES WITH RINGER'S LACTATE (n=6)

DWELL TIME (MIN)	0	30	60	120	180	240	300	360
Cumulative Net Absorption (ml)	-	2.0 ± 0.3	5.3 ± 1.0	6.5 ± 0.7	9.3 ± 0.8	11.9 ± 1.0	13.8 ± 1.3	15.5 ± 1.2
Cumulative Lymphatic Flow (ml)*	-	0.1 ± 0.2	4.1 ± 1.3	7.3 ± 0.4	6.7 ± 2.3	11.5 ± 2.8	14.8 ± 2.9	15.8 ± 3.0
Dialysate Albumin Concentration (g/dl)*	3.10 ± 0.06	3.27 ± 0.05	3.21 ± 0.07	3.06 ± 0.09	3.10 ± 0.12	2.98 ± 0.09	2.91 ± 0.09	2.92 ± 0.1
Dialysate Osmolality (mOsm/L)	268 ± 1	272 ± 1	282 ± 1	289 ± 3	290 ± 3	283 ± 8	292 ± 2	297 ± 3
Serum Osmolality (mOsm/L)	301 ± 3	-	-	-	295 ± 3	-	-	303 ± 3
Haematocrit (%)	52 ± 3	49 ± 2	48 ± 2	48 ± 1	48 ± 1	47 ± 1	47 ± 1	49 ± 2

All values are expressed as mean ± SEM; * n=3.

the dwell time. Cumulative directly measured net fluid absorption and cumulative lymphatic absorption are compared in Figure 12. Measured net fluid absorption slightly exceeded lymphatic absorption early in the dwell time due to the initial transperitoneal osmotic gradient favouring net transcapillary absorption. Thereafter net fluid absorption proceeded at an almost linear rate, the corrected dialysate albumin concentration remained relatively constant as the intraperitoneal volume decreased, and measured net fluid absorption was closely correlated with the calculated lymphatic absorption rate ($r = 0.98$; $p < 0.01$) (Figure 12, Table 4). The haematocrit and serum osmolality also remained almost constant during the absorption of Ringer's lactate solution from the peritoneal cavity, indicating that the animals were able to diurese most of the fluid and electrolytes as they were absorbed (Table 5).

Cumulative lymphatic absorption rates during exchanges with Ringer's lactate were lower than in the studies with 15% dextrose dialysis solution (Figure 13). However, when expressed in relation to the animal's body weight, mean lymphatic absorption rates with the two dialysis solutions were nearly identical (Figure 14) even though the intraperitoneal volume decreased from 41 to 25 ml with Ringer's lactate and increased from 17 to 41 ml with hypertonic dextrose dialysis solution.

LACTATED RINGER'S ABSORPTION

r = 0.98
p < 0.01

Cumulative Lymphatic Absorption (ml)

Cumulative Directly Measured Absorption (ml)
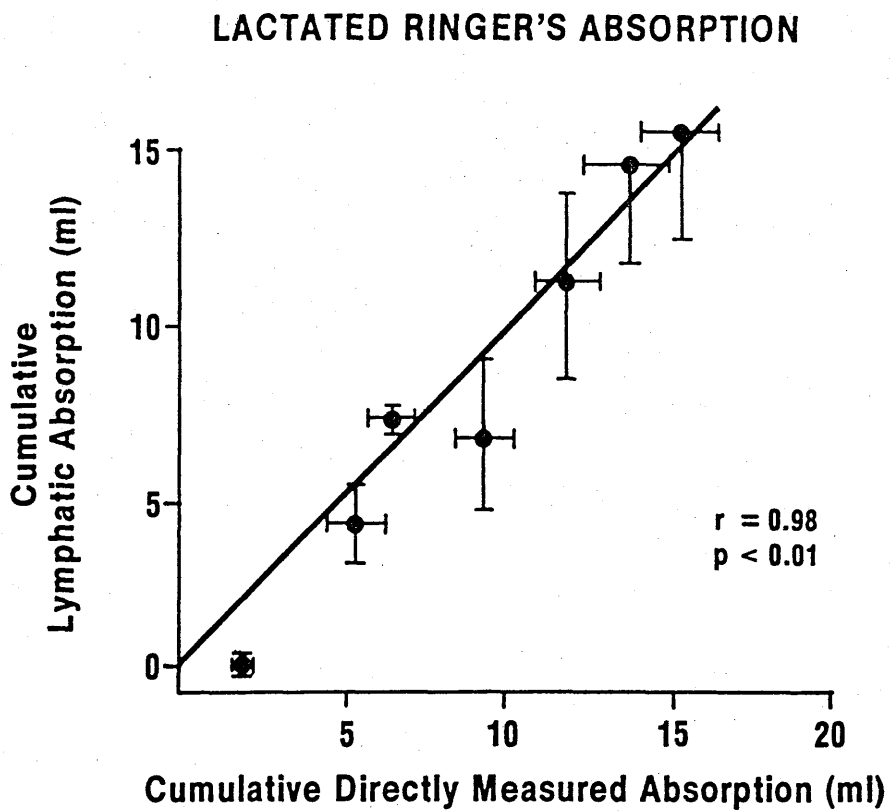
Figure 12:   Cumulative lymphatic absorption (mean ± SEM),
calculated by the albumin absorption method, is
related to directly measured fluid absorption (mean ±
SEM) during six hour exchanges with Ringer's lactate
solution in rats.   The identity line is shown.
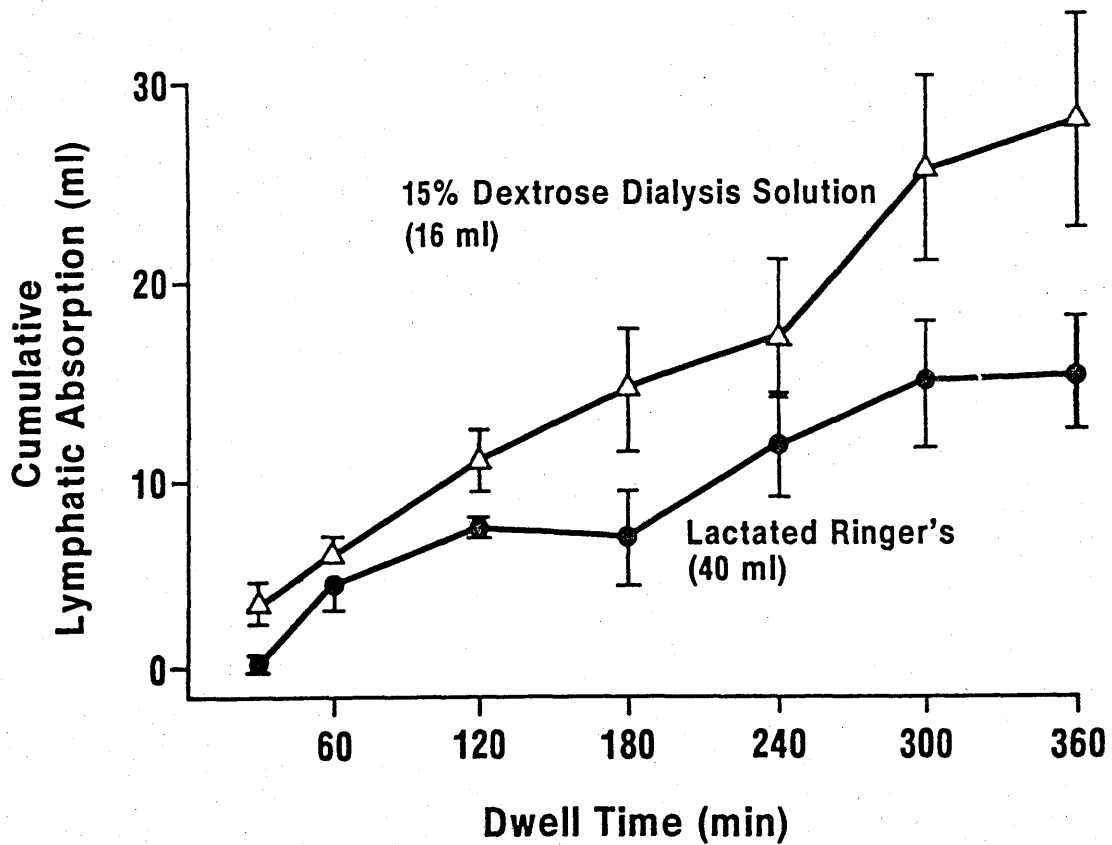
Figure 13: Comparison of cumulative lymphatic absorption (mean ± SEM) during six hour exchanges with Ringer's lactate and 15% dextrose dialysis solution in rats. The instillation volumes of each solution are shown in parentheses.
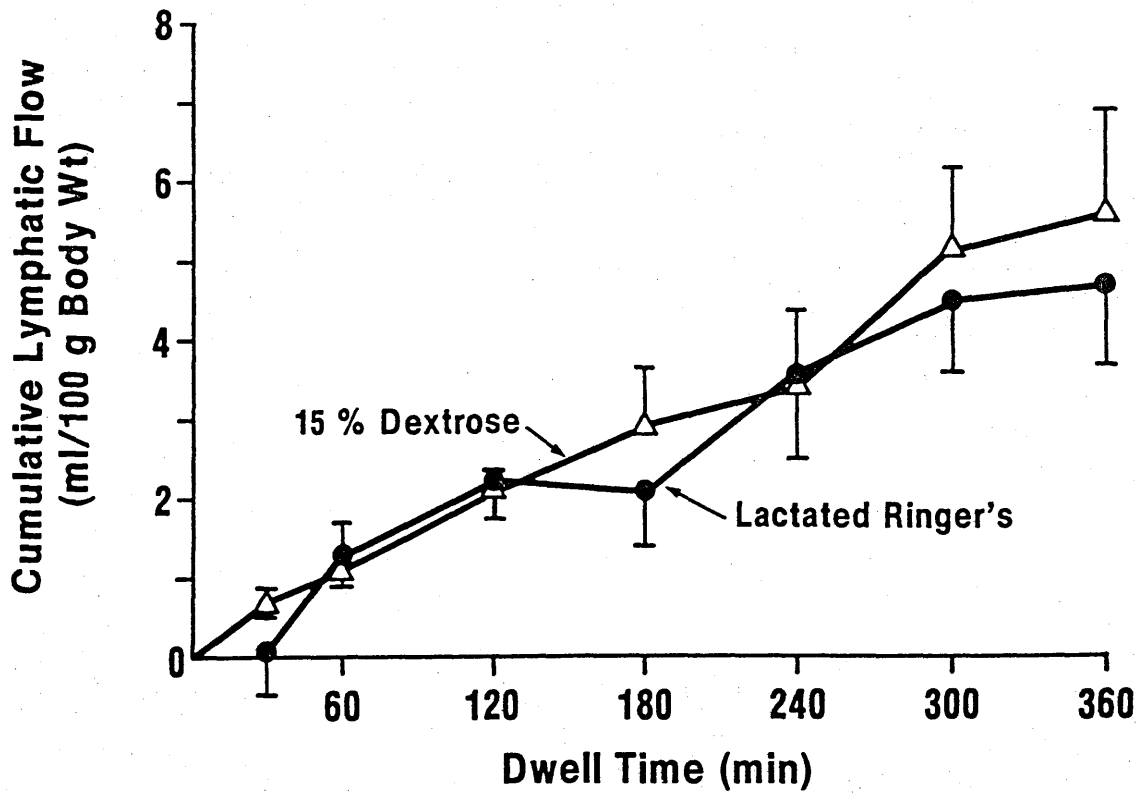
Figure 14: Comparison of cumulative lymphatic absorption (mean ± SEM) in exchanges with Ringer's lactate and 15% dextrose dialysis solution when corrected for the animals' body weight.

5.4 DISCUSSION

This study indicates that lymphatic absorption of intraperitoneal fluid during peritoneal dialysis exchanges with 15% dextrose dialysis solution is substantial, and significantly reduces the potential net ultrafiltration volume at the end of the exchanges. Thus, measured net ultrafiltration at the end of the six hour exchanges averaged only 46% of the calculated cumulative net transcapillary ultrafiltration over the dwell time. Since the net transcapillary ultrafiltration rate decreases exponentially during the dwell time while the lymphatic absorption rate remains almost constant throughout the exchange, prolonging the dwell time or reducing the dialysate glucose load (osmolality or volume of dextrose dialysis solution) in the above studies would be expected to increase the proportion of net transcapillary ultrafiltration reabsorbed by the peritoneal cavity lymphatics.

A very high dialysate dextrose concentration was used in these studies to permit more accurate measurements of net ultrafiltration in the small animal model of peritoneal dialysis, and thereby more clearly delineate the kinetics of ultrafiltration during the dwell time. In previous studies using 15% dextrose dialysis solution in the rat, no acute alterations in peritoneal morphology or transport characteristics were observed (173). Maximum net ultrafiltration rates in that study were between 0.4 and 0.5 ml/hour and are comparable with the results presented herein (Figure 11).

The peak ultrafiltration volume precedes transperitoneal osmolar equilibrium which in turn precedes glucose equilibrium (Figure 11). The maximum intraperitoneal volume is observed when the net transcapillary ultrafiltration rate falls to equal the lymphatic absorption rate. In the above studies maximum intraperitoneal volume occurred while the transperitoneal osmotic gradient was still high enough to induce net transcapillary ultrafiltration at approximately 4.7 ml per hour. Thus peak ultrafiltration occurs before net transcapillary ultrafiltration ceases and before osmolar equilibrium is reached. After peak ultrafiltration, the net fluid absorption rate represents lymphatic absorption in excess of concurrent net transcapillary ultrafiltration. The hyposmolar phase after osmolar equilibrium most likely reflects solute sieving with continued net transcapillary ultrafiltration. Since osmotic pressure is the sum of the products of the osmolar gradient and peritoneal reflection coefficient of each solute, the minor net transcapillary ultrafiltration rate after osmolar equilibrium is presumably related to glucose dysequilibrium and the higher peritoneal reflection coefficient of glucose than other small molecular weight solutes (157,174). This mechanism also has been invoked to explain the net ultrafiltration observed following intraperitoneal instillation of electrolyte-free 5% dextrose solution (252 mOsm/L) (157).

During the exchanges with Ringer's lactate solution net fluid absorption proceeded at a linear rate after osmolar equilibrium was approached during the first two hours of the dwell time. The lymphatic absorption rate, calculated by the albumin method, was also

relatively constant during Ringer's lactate exchanges and closely correlated with the directly measured net fluid absorption rate (r = 0.98) (Figure 12). Moreover, in the present and in previous studies (92,102), the concentrations of intraperitoneal marker colloids remained almost constant during absorption of near isosmotic crystalloid solutions, and further suggest that the intraperitoneal fluid was absorbed by convective flow via the peritoneal cavity lymphatics. Thus, under the conditions of these long-dwell studies, the absorption of intraperitoneal Ringer's lactate is mainly translymphatic. These findings also validate the method of estimating lymphatic flow from the peritoneal clearance of albumin added to the dialysis solution.

The net fluid absorption rates after infusion of 40 ml of Ringer's lactate averaged 2.6 ml per hour (7.3 ml/Kg/hour) throughout the exchange and 2.2 ml per hour (6.2 ml/Kg/hour) during the final four hours of the dwell time and are in accord with previous studies in rats (92,108). Following infusion of 33 to 40 ml of Krebs-Ringer solution with 5% bovine serum albumin, net fluid absorption averaged 2.1 ml per hour (10 ml/Kg/hour) (92) and following infusion of 20 ml/Kg of homologous plasma, net absorption averaged 6 ml/Kg/hour (108).

The lymphatic absorption rates during the exchanges with hypertonic dextrose and Ringer's lactate solution were comparable when corrected for body weight. The physiological factors which are known to influence the lymphatic absorption rate of intraperitoneal fluid were also similar in both groups of rats (Table 2). All of the

study rats were maintained supine under general anaesthesia and the average intraperitoneal volumes were comparable in each group of rats, even though the intraperitoneal volume was decreasing with Ringer's lactate and increasing during 15% dextrose exchanges.

5.5 CONCLUSIONS

1. Cumulative lymphatic absorption significantly alters ultrafiltration kinetics in peritoneal dialysis with hypertonic dextrose dialysis solutions in rats:

    a) As a result of the substantial lymphatic absorption rate, net ultrafiltration at the end of the dwell time is much lower than cumulative net transcapillary ultrafiltration during the exchange.

    b) The net transcapillary ultrafiltration rate decreases exponentially during the dwell time whereas lymphatic absorption proceeds at an almost constant rate.

    c) The peak ultrafiltration volume is observed when the net transcapillary ultrafiltration rate equals the lymphatic absorption rate. Thereafter, net fluid absorption represents lymphatic absorption in excess of concurrent net transcapillary ultrafiltration.

2. The absorption of intraperitoneal Ringer's lactate solution
    during long-dwell exchanges in rats is primarily translymphatic:

    a) Lymphatic absorption of Ringer's lactate solution proceeds
        at a linear rate and closely correlates with directly
        measured net fluid absorption.

    b) The corrected dialysate albumin concentration remains
        relatively constant during absorption of the intra-
        peritoneal fluid.

Chapter 6. LYMPHATIC ABSORPTION IN ADULT PATIENTS ON CAPD

6.1 INTRODUCTION

Cumulative lymphatic absorption from the peritoneal cavity may significantly reduce net ultrafiltration and peritoneal solute clearances at the end of long-dwell CAPD exchanges. Moreover, since net ultrafiltration represents cumulative net transcapillary ultrafiltration minus cumulative lymphatic drainage over the dwell time, lymphatic absorption may be relatively more important in CAPD patients with high peritoneal permeability x area, rapid absorption of glucose from the dialysis solution and lower net transcapillary ultrafiltration. This study was performed to evaluate the contribution of lymphatic absorption to the loss of ultrafiltration and solute mass transfer during standardised exchanges in adult CAPD patients, and to compare the influence of lymphatic drainage on the kinetics of ultrafiltration in CAPD patients with normal and high peritoneal permeability x area.

6.2 METHODS

6.2.1   PATIENTS

Standardised, four hour study exchanges using 2 litres of 2.5% hydrated dextrose dialysis solution were performed in 18 stable CAPD patients (11 men, 7 women). The study was approved by the

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Institutional Review Board for Human Experimentation, University of Missouri-Columbia, and each patient gave informed, written consent. At the time of study, the mean age of patients was 59 ± 3 (SEM) years (range 28-81) and the mean duration of CAPD was 26 ± 7 months (range 1-105). Although seven patients had had 20 prior episodes of peritonitis, none of the patients had peritonitis within three months of study. All of the patients had used only lactate containing dialysis solutions. None of the patients had clinical features of chronic liver disease and liver function tests (serum bilirubin, serum alanine aminotransferase and serum aspartate aminotransferase) were within normal limits in all of the patients. None of the patients had evidence of an extraperitoneal dialysate leakage and only one of the patients (patient 12) was slightly fluid overloaded (minimal ankle oedema, recent weight gain of 3 Kg) at the time of study.

The patients were divided into two groups using peritoneal transport rates (dialysate/serum urea and creatinine and effluent/initial dialysate glucose ratios) during the study exchanges as indices of peritoneal permeability x area (Figure 2) (60,61). Ten patients (group 1) had urea, creatinine and glucose ratios within the normal range (± 1 SD) and eight patients (group 2) had dialysate/serum urea and creatinine ratios greater than 1 SD above the mean and effluent/initial dialysate glucose ratios more than 1 SD below the mean of the CAPD population at the University of Missouri-Columbia (Figures 15,16 and 17). The demographic features of groups 1 and 2 are summarised in Tables 6 and 7, respectively. The mean age,
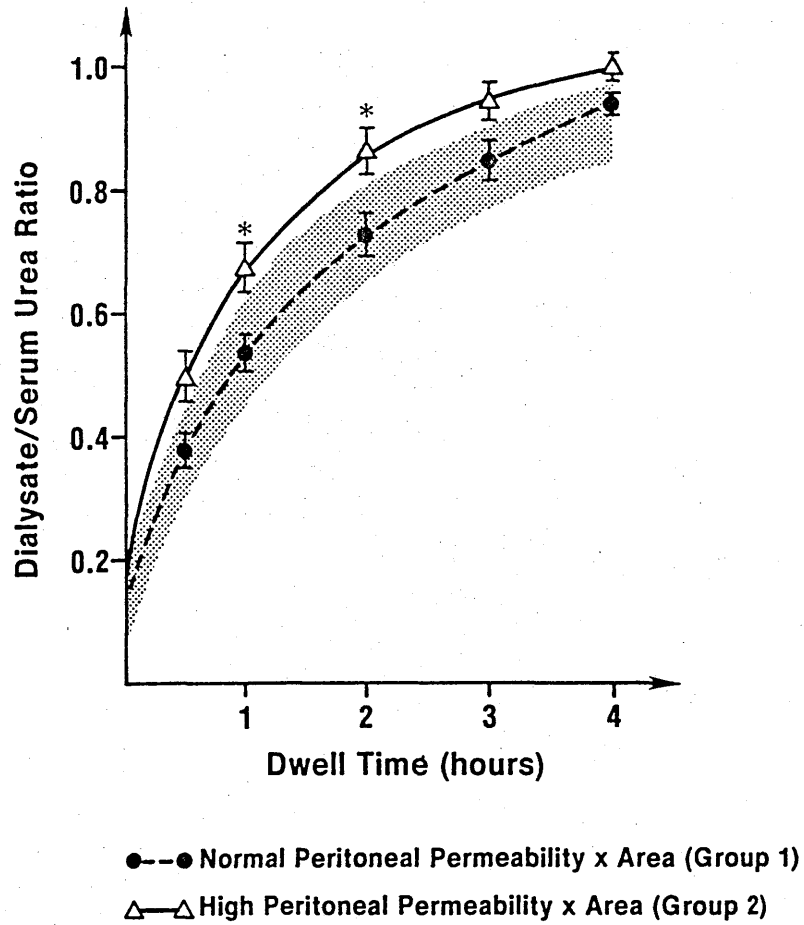
Figure 15: Dialysate/serum urea ratios (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in groups 1 and 2. The reference range (mean ± 1 SD) is shaded.

* $p < 0.05$.

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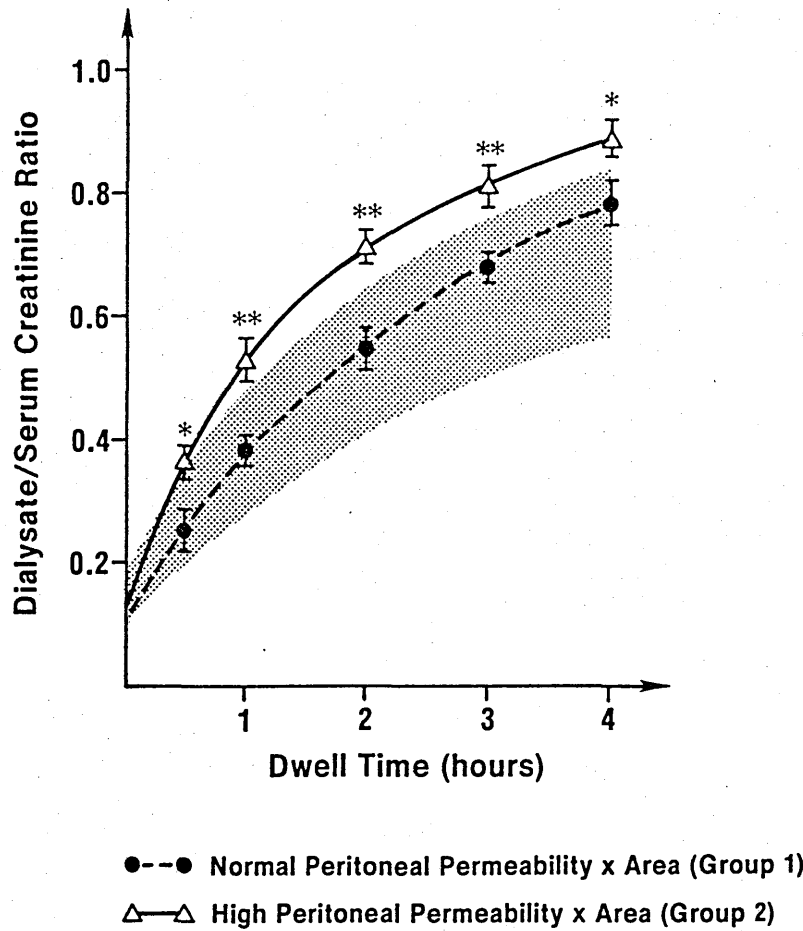
Figure 16: Dialysate/serum creatinine ratios (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in groups 1 and 2.

** $p < 0.01$; * $p < 0.05$.
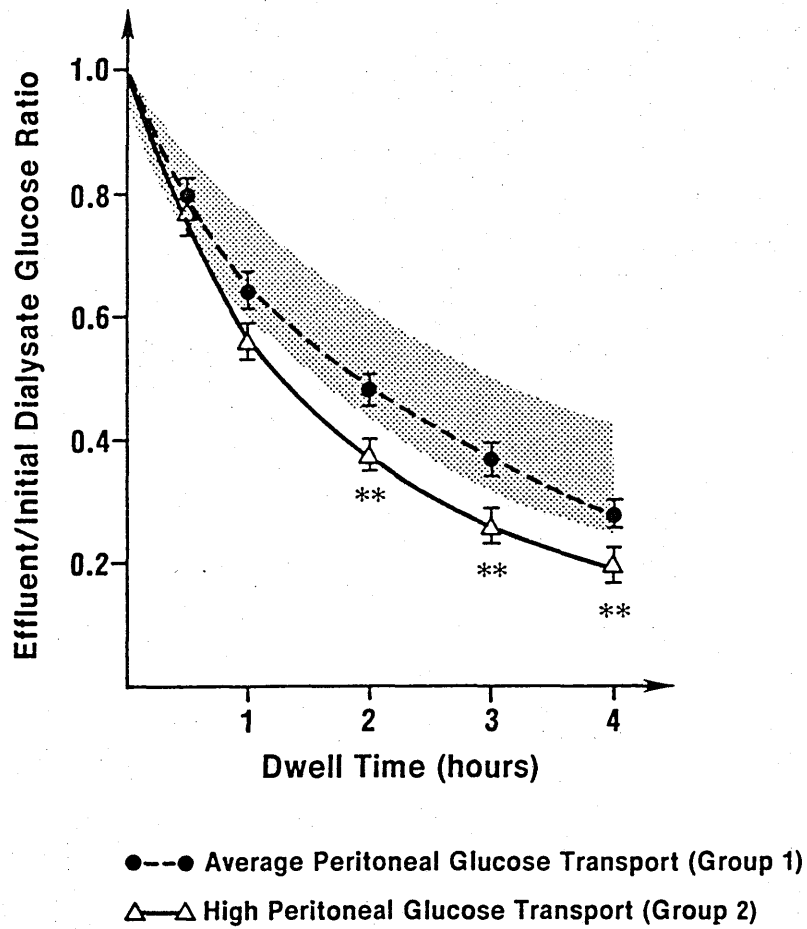
Figure 17:   Effluent/initial dialysate glucose ratios (mean ± SEM)
during four hour exchanges with 2 litres of 2.5%
dextrose dialysis solution in groups 1 and 2.

** p < 0.01.

TABLE 6: CLINICAL FEATURES OF CAPD PATIENTS WITH AVERAGE PERITONEAL PERMEABILITY X AREA (GROUP 1)

PATIENT NO.	AGE (YEARS)	SEX	CAUSE OF END-STAGE RENAL FAILURE	MONTHS ON CAPD	PRIOR EPISODES OF PERITONITIS
1	63	F	Chronic Glomerulonephritis	10	1
2	72	M	Chronic Glomerulonephritis	13	2
3	48	M	Diabetic Nephropathy	52	2
4	31	F	Chronic Pyelonephritis	63	1
5	73	M	Unknown	36	0
6	64	M	Interstitial Nephropathy	61	0
7	28	F	Hypoplastic Kidneys	4	0
8	81	M	Hypertensive Nephrosclerosis	12	0
9	62	M	Obstructive Uropathy	1	0
10	66	F	Diabetic Nephropathy	14	0

The mean age of patients was 59 ± 6 (SEM) years and the mean duration of CAPD at the time of study was 27 ± 8 months.

TABLE 7: CLINICAL FEATURES OF CAPD PATIENTS WITH HIGH PERITONEAL PERMEABILITY X AREA (GROUP 2)

PATIENT NO.	AGE (YEARS)	SEX	CAUSE OF END-STAGE RENAL FAILURE	MONTHS ON CAPD	PRIOR EPISODES OF PERITONITIS
11	52	F	Chronic Glomerulonephritis	105	9
12	59	M	Obstructive Uropathy	1	0
13	65	M	Chronic Glomerulonephritis	5	0
14	49	F	Renal Cortical Necrosis	5	1
15	46	M	Obstructive Uropathy	46	0
16	72	F	Unknown	26	4
17	68	M	Hypertensive Nephrosclerosis	12	0
18	66	M	Renovascular Disease	6	0

The mean age of patients was 60 ± 3 (SEM) years and the mean duration of CAPD at the time of study was 26 ± 12 months.

duration of CAPD and number of previous episodes of peritonitis did not differ between the two patient groups.

All of the patients in group 2 exhibited poor peritoneal ultrafiltration and only patients 13 and 14, with residual urine volumes in excess of 1 litre per day, did not require 2.5% or 4.25% dextrose dialysis solution for all CAPD exchanges. Patients 16 and 17 needed short-dwell exchanges overnight using a cycler machine (nightly peritoneal dialysis) to maintain fluid balance (175).

6.2.2   STUDY EXCHANGES

Each study was performed after an overnight exchange (10-12 hours) with 2 litres of 2.5% dextrose dialysis solution (Dianeal, Baxter-Travenol, Deerfield, Illinois). After complete drainage of the overnight dwell over 20 minutes, 2 litres of 2.5% dextrose dialysis solution with 30 g added albumin was infused over 10 minutes. The study dialysis solution was prepared by preheating 2 litres of 2.5% Dianeal PD-2 solution to 37°C, withdrawing 120 ml of the dialysate under aseptic technique and adding 120 ml of 25% human serum albumin (Baxter-Travenol, Deerfield, Illinois). During infusion of the dialysis solution the patient rolled from side to side every two minutes to promote intraperitoneal mixing of the dialysate. After complete infusion (time 0), the patient remained supine for the four hour study exchange. After four hours the dialysate was drained over 20 minutes, the drain volume measured in a

graduated cylinder and 2 litres of fresh dialysis solution without albumin instilled.

Dialysate samples (10 ml) were obtained serially at times 0, 30, 60, 120, and 180 minutes during the study dwell time, from each infusion and drain volume, and immediately following complete infusion of the wash-out exchange after the study dwell. Serial samples during the exchange were taken after draining 200 ml into the drain bag and inverting the bag three times. Immediately after each serial sample was obtained, the dialysate remaining in the drain bag was reinfused. Blood samples (10 ml) were drawn at the beginning and end of the study exchange.

Repeat study exchanges were performed in four patients within the next four days. Three of the patients were from group 1 and one from group 2 (patients 1, 3, 5 and 13). The above protocol was followed using 2 litres of Ringer's Lactate solution with 25 g albumin instead of 2 litres of 2.5% dextrose, 1.5% albumin solution. 23.4% Na Cl was added to the Ringer's Lactate solution (273 mOsm/L) to correct the dialysate osmolality to each patient's measured serum osmolality. These studies enabled comparison of lymphatic absorption rates during four hour exchanges with equal volumes of isotonic and hypertonic intraperitoneal fluid. A third two hour study using 2 litres of 2.5% dextrose dialysis solution without albumin was performed in the same four patients to compare peritoneal permeability x area during exchanges with and without added albumin as well as calculated and measured two hour intraperitoneal volumes.

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Albumin, glucose, urea, creatinine, and potassium concentrations, osmolality and colloid osmotic pressure were measured in all samples.   Serum glucose and dialysate and serum urea and creatinine concentrations were determined by standard automated methods (Autoanalyser II, Technicon Instruments Corp., Tarrytown, New York). Dialysate creatinine values were corrected for high dialysate glucose concentrations (176).   Serum and dialysate potassium concentrations were measured using a flame photometer (model 343, Instrumentation Laboratories Inc., Lexington, Maryland). Serum and dialysate albumin concentrations and osmolality and dialysate glucose concentrations were measured as described previously (5.2.4). The mean difference between duplicate determinations of albumin concentrations was 2.5 ± 0.3%. Colloid osmotic pressure was measured using a Weil osmometer (Instrumentation Laboratories Inc.).

6.2.4   CALCULATIONS

Since intraperitoneal marker colloids are removed from the peritoneal cavity almost exclusively by convective flow via the peritoneal cavity lymphatics (124-128), cumulative lymphatic absorption over the four hour dwell time was calculated from the net mass transfer of albumin from the peritoneal cavity during the exchange as discussed previously (equation 7, 4.4.2). Moreover, since lymphatic drainage during peritoneal dialysis in the rat

proceeds at almost a linear rate throughout the exchange, the lymphatic absorption rate during CAPD exchanges was also assumed to be constant and was calculated by dividing cumulative lymphatic absorption by the dwell time.

Cumulative net transcapillary ultrafiltration was estimated from the dilution of the initial dialysate albumin concentration since the intraperitoneal albumin concentration is unchanged by lymphatic absorption of intraperitoneal fluid (111,112,126-128) and any decrease in the dialysate albumin concentration during the dwell time results from net influx of fluid from the peritoneal microcirculation.

The calculated net ultrafiltration volume at time intervals during the exchange was calculated as the difference between cumulative net transcapillary ultrafiltration and lymphatic absorption after each time interval. The calculated intraperitoneal volume at each time interval was calculated as the intraperitoneal volume at 0 hour dwell time plus the calculated net ultrafiltration after the time interval. The measured net ultrafiltration volume at the end of the four hour dwell time equalled the difference between the intraperitoneal volumes at the beginning and end of the exchange. That is:

1. Lymphatic absorption during the 4 hour dwell (ml) =

$$\frac{(A_O \times IPV_O) - (A_4 \times IPV_4)}{A_G}$$

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2. Cumulative net transcapillary UF (ml) after time t (hours) =

$$A_0/A_t \; (IPV_0) \; - \; (IPV_0)$$

3. Calculated net UF (ml) at time t (hours) =

Cumulative net transcapillary UF - lymphatic absorption after time t

4. Calculated intraperitoneal volume (ml) after time t (hours) =

$$IPV_0 + \text{calculated net UF at time } t$$

5. Measured net UF at 4 hours (ml) = $IPV_4 - IPV_0$

where A_0, A_4, A_t = dialysate albumin concentrations at times 0, 4 and t hours dwell time, respectively

A_G = geometric mean dialysate albumin concentration

= $\sqrt{A_0 \times A_4}$

IPV_0 = intraperitoneal volume at 0 hour dwell time

= infusion volume (2070 ml) + pre-exchange residual volume

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IPV_4 = intraperitoneal volume at 4 hour dwell
time

= drain volume + sample volume + post-
exchange residual volume

The geometric mean (A_G) was utilised to represent the time averaged mean intraperitoneal albumin concentration since the dialysate albumin concentration decreases almost exponentially during the exchange due to ultrafiltration. The pre- and post-exchange residual volumes were derived from the mean of each residual volume calculated from the change induced in the drain dialysate urea, potassium, glucose and albumin concentrations by the known volume and solute concentrations of the newly infused dialysis solution. The average volume of dialysis solution in 2 litre bags of Dianeal PD-2 was 2070 ml and was therefore used in all calculations based on the infusion volume.

The percentage glucose absorption from the dialysis solution during the exchange was calculated as:

$$\left(1 - \frac{IPV_4 \times G_4}{IPV_0 \times G_0}\right) \times 100\%$$

where G_0 and G_4 = dialysate glucose concentrations at 0
and 4 hours, respectively

Daily net transcapillary ultrafiltration and drain volumes using four exchanges of 2 litres, 2.5% dextrose dialysis solution per day were extrapolated by assuming that net transcapillary ultrafiltration was complete after four hours dwell time and lymphatic absorption was continuous at the observed rate. Daily solute clearances were calculated as the product of daily drain volume and drain dialysate solute concentration divided by the mean serum solute concentration, where the drain dialysate concentration was assumed to equal the four hour dwell time solute concentration. Reverse solute clearances (via lymphatics) were similarly calculated based on the mean dialysate solute concentration per exchange. Thus, reverse solute clearances equalled the product of daily lymphatic drainage and mean dialysate solute concentration divided by the mean serum solute concentration.

The results in groups 1 and 2 were compared by Student's non-paired t test and correlation coefficients were derived by linear regression.

6.3 RESULTS

6.3.1 EXCHANGES WITH 2.5% DEXTROSE DIALYSIS SOLUTION WITH ADDED ALBUMIN

ALL PATIENTS

The mean cumulative lymphatic absorption from the peritoneal cavity during the four hour exchanges with 2 litres of 2.5% dextrose dialysis solution was 343 ± 39 (SEM) ml (Table 8). Cumulative net

TABLE 8: CUMULATIVE LYMPHATIC ABSORPTION AND NET TRANSCAPILLARY ULTRAFILTRATION DURING FOUR HOUR EXCHANGES WITH 2 LITRES OF 2.5% DEXTROSE DIALYSIS SOLUTION

FLUID TRANSPORT (ml)	GROUPS 1 & 2 (n=18)	GROUP 1 (n=10)	GROUP 2 (n=8)	*
Net TCUF at 30 min.	302 ± 34	334 ± 41	269 ± 49	
Net TCUF at 60 min.	455 ± 42	468 ± 59	441 ± 64	
Net TCUF at 120 min.	589 ± 56	639 ± 67	532 ± 88	
Net TCUF at 180 min.	629 ± 60	692 ± 77	548 ± 90	
Net TCUF at 240 min.	686 ± 66	806 ± 77	536 ± 92	$p < 0.05$
Lymphatic Absorption over 240 min.	343 ± 39	332 ± 65	356 ± 36	
Lymphatic Absorption/ Net TCUF at 240 min.(%)	54.4 ± 5.9	40.1 ± 6.0	72.1 ± 7.3	$p < 0.005$

* Mean ± SEM values in groups 1 and 2 were compared by Student's non-paired t test. Net TCUF denotes cumulative net transcapillary ultrafiltration after the stated dwell time.

transcapillary ultrafiltration and calculated net ultrafiltration at each time interval during the exchanges are summarised in Tables 8 and 9, respectively. The influence of cumulative lymphatic absorption on the kinetics of ultrafiltration during the four exchanges is derived from these data and is shown in Figure 18. As a result of cumulative lymphatic absorption, peak net ultrafiltration occurred near the two hour dwell time and calculated net ultrafiltration at the end of the exchanges averaged only 44 ± 6% of the total net transcapillary ultrafiltration volume during the dwell time (Figure 18 and Table 8). The calculated and directly measured net ultrafiltration volumes at the end of the exchanges correlated closely ($r = 0.97$; $p < 0.0001$), supporting the validity of the methods (Table 9).

The mean rates of net transcapillary ultrafiltration and lymphatic absorption during the exchanges are depicted in Figure 19. The net transcapillary ultrafiltration rate decreased exponentially during the dwell time as expected, whereas lymphatic absorption was assumed to be constant throughout the exchange (5.3.1). Consequently the peak ultrafiltration volume occurred when the net transcapillary ultrafiltration rate had decreased to equal the lymphatic absorption rate. At this point the net ultrafiltration rate was zero. Subsequently, the net absorption rate represented the lymphatic absorption rate in excess of the concurrent net transcapillary ultrafiltration rate (Figure 19).

Dialysate and serum osmolalities and glucose concentrations at each time interval during the exchanges are summarised in Table 10

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TABLE 9: CALCULATED AND MEASURED NET ULTRAFILTRATION DURING FOUR HOUR EXCHANGES WITH 2 LITRES OF 2.5% DEXTROSE DIALYSIS SOLUTION

CALCULATED NET UF (ml)	GROUPS 1 & 2 (n=18)	GROUP 1 (n=10)	GROUP 2 (n=8)	*
30 min.	261 ± 33	297 ± 36	220 ± 42	
60 min.	373 ± 40	393 ± 53	351 ± 63	
120 min.	426 ± 50	489 ± 55	354 ± 76	
180 min.	389 ± 49	467 ± 60	289 ± 72	
240 min.	343 ± 57	474 ± 64	180 ± 71	$p < 0.01$

MEASURED NET UF (ml)	GROUPS 1 & 2	GROUP 1	GROUP 2	
240 min.	310 ± 58	452 ± 54	133 ± 64	$p < 0.005$

* Mean ± SEM values in groups 1 and 2 were compared by Student's t test. Measured and calculated net UF at the end of the exchanges correlated closely (r=0.96).
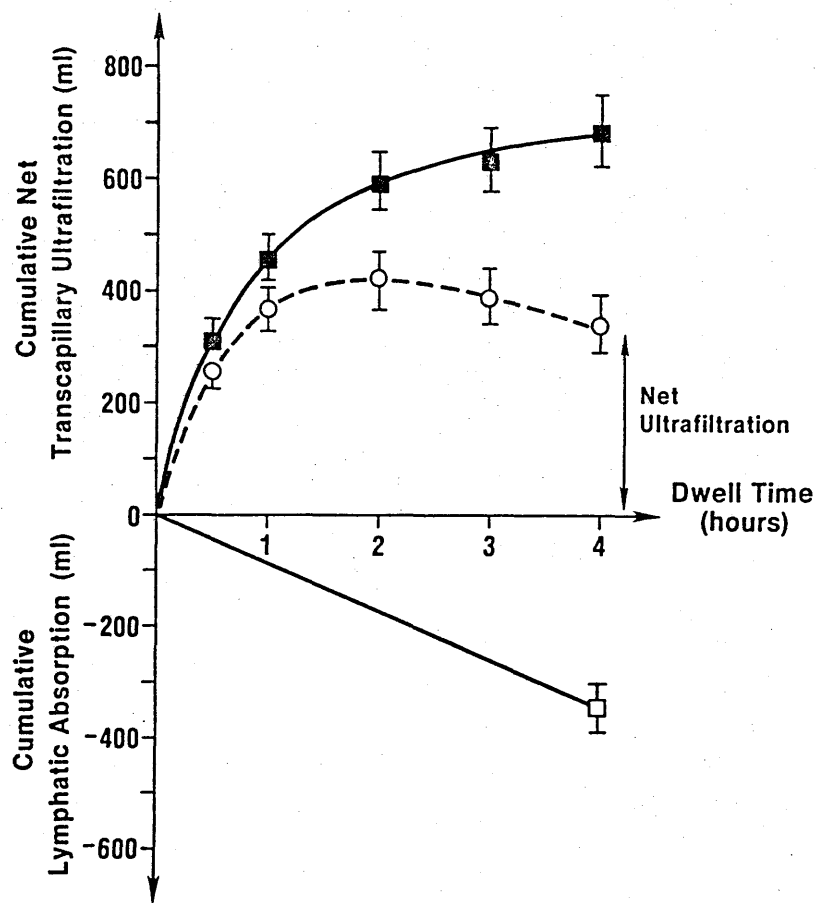
Figure 18: Cumulative lymphatic absorption, net ultrafiltration and cumulative net transcapillary ultrafiltration (mean ± SEM) during four hour exchanges in 18 CAPD patients using 2 litres of 2.5% dextrose dialysis solution.
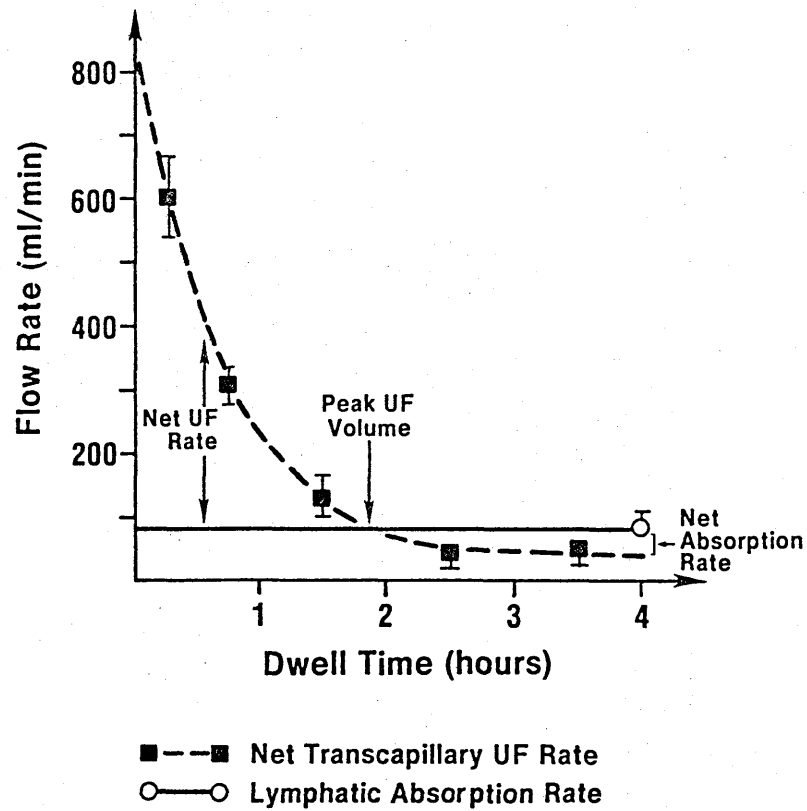
Figure 19:   Net   transcapillary   ultrafiltration   and   lymphatic
              absorption rates (mean ± SEM) during   four   hour
              exchanges using 2 litres of 2.5% dextrose dialysis
              solution (n = 18).   Peak ultrafiltration volume
              (arrowed)   occurs   when   the   net   transcapillary
              ultrafiltration rate equals the lymphatic absorption
              rate.

TABLE 10: DIALYSATE AND SERUM OSMOLALITIES AND GLUCOSE CONCENTRATIONS DURING FOUR HOUR EXCHANGES WITH 2 LITRES OF 2.5% DEXTROSE DIALYSIS SOLUTION

OSMOLALITY (mOsm/L)		GROUPS 1 & 2	GROUP 1	GROUP 2
D I A L Y S A T E	0 min.	358 ± 3	362 ± 4	354 ± 3
	30 min.	335 ± 3	337 ± 4	332 ± 4
	60 min.	324 ± 3	327 ± 6	321 ± 3
	120 min.	310 ± 4	314 ± 7	304 ± 3
	180 min.	302 ± 4	306 ± 8	297 ± 4
	240 min.	294 ± 4	295 ± 6	294 ± 4
S E R U M	0 min.	295 ± 4	294 ± 7	297 ± 6
	240 min.	294 ± 4	290 ± 6	300 ± 6

GLUCOSE CONCENTRATION (mg/dl)		GROUPS 1 & 2	GROUP 1	GROUP 2	*
D I A L Y S A T E	0 min.	2140 ± 75	2147 ± 102	2132 ± 110	
	30 min.	1683 ± 59	1709 ± 76	1651 ± 95	
	60 min.	1300 ± 53	1367 ± 71	1216 ± 81	
	120 min.	936 ± 35	1038 ± 42	808 ± 60	$p < 0.01$
	180 min.	690 ± 33	803 ± 40	549 ± 59	$p < 0.005$
	240 min.	535 ± 35	609 ± 45	443 ± 61	$p < 0.05$
S E R U M	0 min.	146 ± 16	172 ± 29	114 ± 14	
	240 min.	147 ± 19	164 ± 26	125 ± 29	

* The mean ± SEM values of groups 1 and 2 were compared by Student's t test.

and are related to the intraperitoneal volume in Figure 20. The peak intraperitoneal volume was observed before the two hour dwell time even though osmolar equilibrium did not occur until near the end of the exchanges. Glucose dysequilibrium persisted until the end of the study dwell time.

GROUPS 1 AND 2

Ultrafiltration kinetics in the patients with average (group 1) and high (group 2) peritoneal permeability x area are compared in Figure 21 and Tables 8 and 9. Net transcapillary ultrafiltration continued throughout the four hour dwell time of exchanges in group 1 but was virtually complete within the first two hours of the exchanges in group 2 (Figure 21). Cumulative net transcapillary ultrafiltration at the end of the exchanges was 806 ± 77 ml in group 1 and 536 ± 92 ml in group 2 ($p < 0.05$), whereas cumulative lymphatic absorption over the four hour dwell time did not differ between the two groups (332 ± 65 ml in group 1 and 356 ± 36 ml in group 2) (Table 8). Thus, although lymphatic absorption was similar in both groups, lymphatic drainage caused a proportionately greater reduction in net ultrafiltration at the end of the exchanges in group 2 ($p < 0.005$) (Figure 22).

Peak net ultrafiltration was observed earlier in the exchanges in group 2 (Figure 21). The net absorption rate after peak ultrafiltration was greater in group 2 since lymphatic absorption later in the dwell time in these patients was unopposed by

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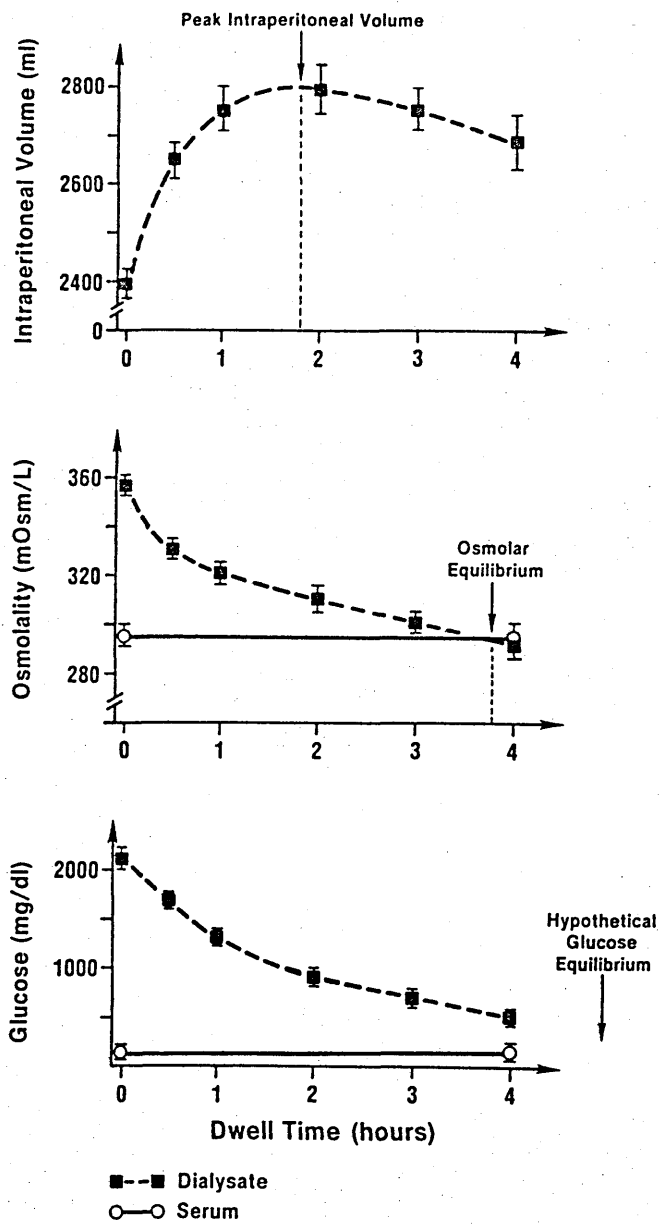
Figure 20: Intraperitoneal volume, serum and dialysate osmolality and glucose concentrations (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution (n = 18). Arrows indicate peak intraperitoneal volume, osmolar equilibrium and hypothetical glucose equilibrium.
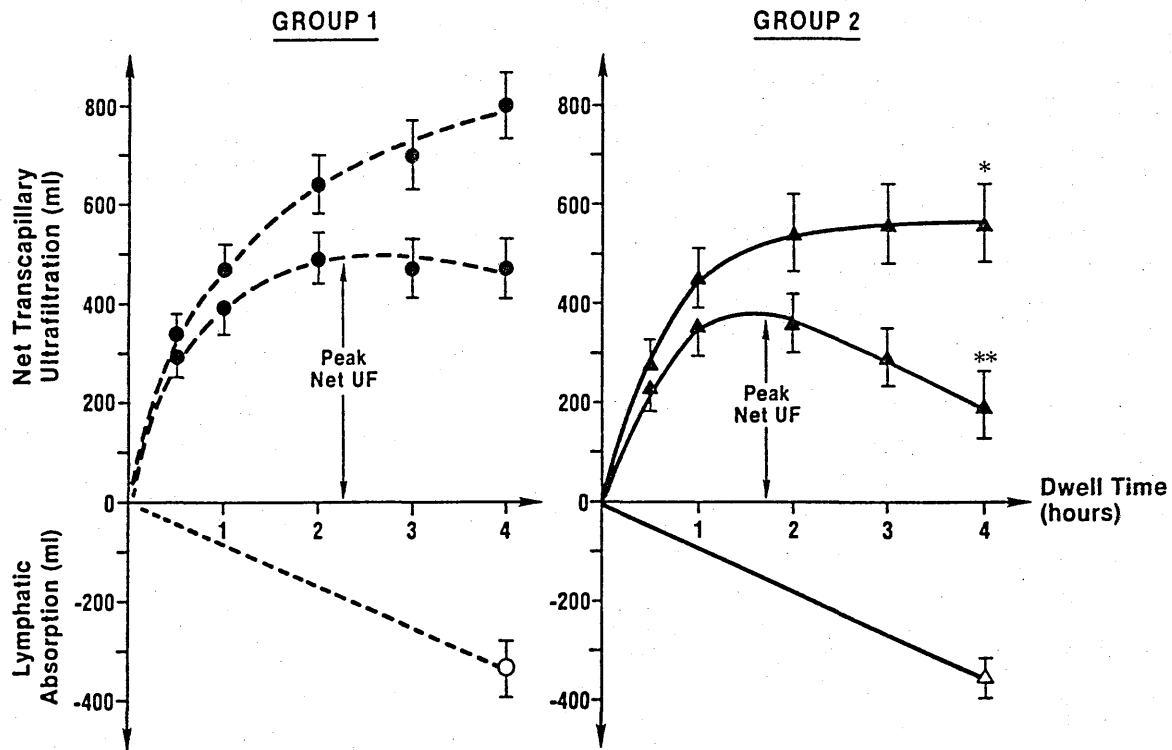
Figure 21: Comparison of cumulative lymphatic absorption, net ultrafiltration and cumulative net transcapillary ultrafiltration (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in groups 1 and 2.

** p < 0.01 ; * p < 0.05
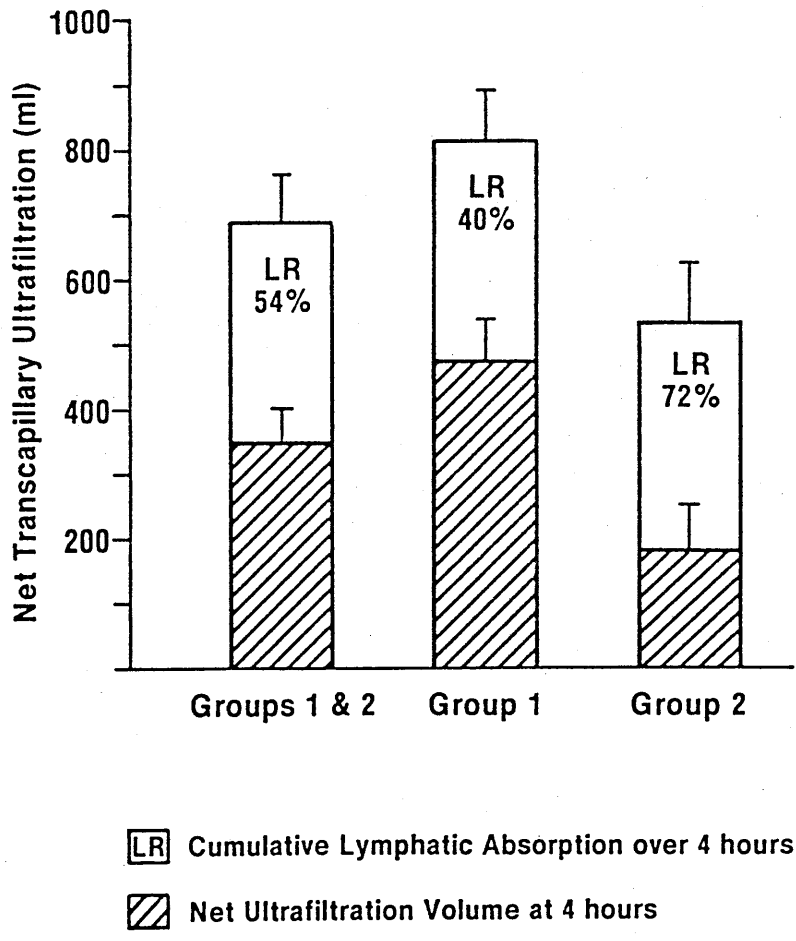
Figure 22: Cumulative net transcapillary ultrafiltration, lymphatic reabsorption and net ultrafiltration (mean ± SEM) at the end of four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in groups 1 and 2.

concurrent net transcapillary ultrafiltration (Figure 23). Consequently calculated and measured net ultrafiltration volumes after the four hour dwell time were significantly lower in group 2 (Table 9).

Dialysate and serum osmolalities and glucose concentrations during the exchanges in group 1 and 2 are compared in Figure 24 and Table 10. In group 1 the maximum intraperitoneal volume was observed after the two hour dwell time even though osmolar and glucose equilibrium did not occur during the four hour exchange (Figure 24). In group 2 the peak ultrafiltration volume was observed before the two hour dwell time, osmolar equilibrium occurred before the three hour dwell time and glucose dysequilibrium continued until the end of the exchanges. Thus peak ultrafiltration and osmolar equilibrium both occurred earlier in the dwell time in group 2. These data also indicate that the maximum intraperitoneal volume was observed before net transcapillary ultrafiltration ceased due to dissipation of the transperitoneal osmotic gradient and that isosmolality was approached before glucose equilibrium.

Rapid absorption of glucose from the dialysate was confirmed in the group 2 patients with high peritoneal permeability x area and reduced cumulative net transcapillary ultrafiltration (Figure 24). Despite the infusion of 2 litres of 2.5% dextrose dialysis solution in each exchange, 79 ± 2% of the initial intraperitoneal glucose load was absorbed over the four hour dwell time in group 2 compared with 67 ± 2% in group 1 (p < 0.001). Glucose absorption via the peritoneal cavity lymphatics did not differ between the two groups

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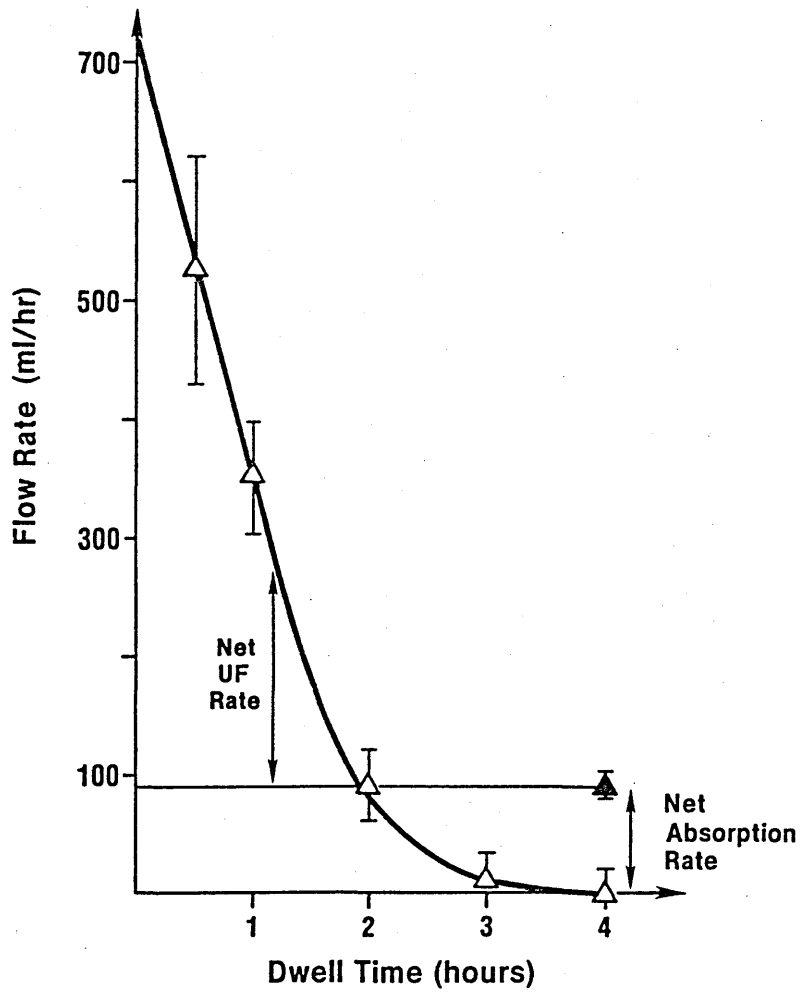
Figure 23: Net transcapillary ultrafiltration and lymphatic absorption rates (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in patients with high peritoneal permeability x area (group 2). The net absorption rate represents lymphatic absorption in excess of concurrent net transcapillary ultrafiltration.

GROUP 1 / **GROUP 2**

Osmolality (mOsm/L) · Glucose (mg/dl) · Dwell Time (hours)

Peak Intraperitoneal Volume · Osmolar Equilibrium
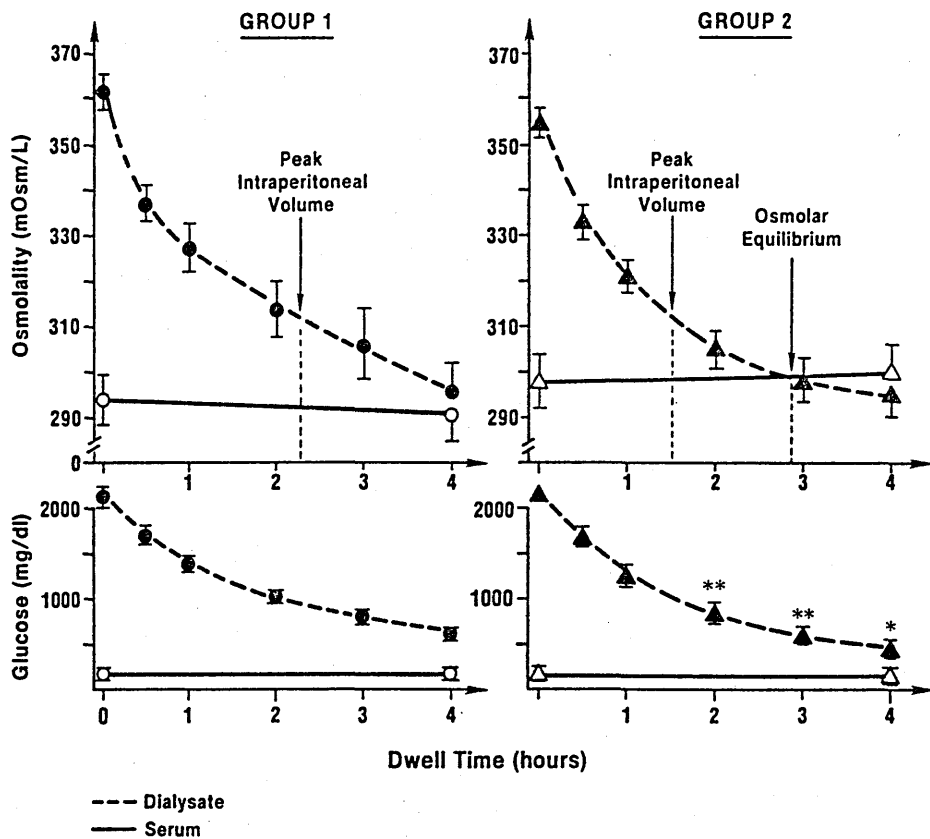
- - - Dialysate
—— Serum

Figure 24: Comparison of serum and dialysate osmolality and glucose concentrations (mean ± SEM) during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution in groups 1 and 2. Arrows indicate peak intraperitoneal volume and osmolar equilibrium.

** p < 0.01 ; * p < 0.05

(3.2 ± 0.4 g in group 2 and 3.6 ± 0.7 g in group 1), even though 41 ±

2 g of glucose was absorbed from the dialysate during the exchanges

in group 2 and 34 ± 2 g in group 1.   These results confirm that the

more rapid absorption of glucose from the dialysis solution in group

2 can be attributed solely to increased transperitoneal absorption

secondary to high peritoneal permeability x area, and is unrelated to

the absorption rate via the lymphatics.   The importance of the

intraperitoneal   glucose   concentration   in maintaining the

transperitoneal osmotic gradient for ultrafiltration is emphasised by

the inverse correlation between the percentage of glucose absorbed

from the dialysis solution over the exchange and both cumulative net

transcapillary ultrafiltration (r = - 0.54) and measured net

ultrafiltration (r = - 0.69).   The serum albumin and glucose

concentrations, serum colloid osmotic pressure, serum osmolality,

body surface area, duration of CAPD and number of previous episodes

of peritonitis were similar in both groups (Table 11).   Thus the only

factor which was identified to explain the reduced cumulative net

transcapillary ultrafiltration in the group 2 patients was their

higher peritoneal permeability x area (Figures 15,16 & 17), more

rapid transcapillary absorption of dialysate glucose (Figure 24,

Table 11) and earlier dissipation of the transperitoneal osmotic

gradient (Figure 24).

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TABLE 11: FACTORS WHICH MAY INFLUENCE CUMULATIVE NET TRANSCAPILLARY
ULTRAFILTRATION IN CAPD PATIENTS

	GROUP 1	GROUP 2	*
% Glucose Absorption	67 ± 2	79 ± 2	p < 0.001
Dialysate/Serum Creatinine (2 Hours)	0.54 ± 0.03	0.71 ± 0.02	p < 0.001
Dialysate/Serum Urea (2 Hours)	0.73 ± 0.03	0.86 ± 0.04	p < 0.02
Effluent/Initial Dialysate Glucose (2 Hours)	0.49 ± 0.02	0.38 ± 0.02	p < 0.001
Body Surface Area (m^2)	1.89 ± 0.07	1.81 ± 0.06	
Serum Albumin (g/dl)	2.9 ± 0.3	3.2 ± 0.4	
Serum Colloid Osmotic Pressure (mmHg)	19.8 ± 1.2	19.9 ± 1.1	
Serum Osmolality (mOsm/L)	292 ± 6	299 ± 6	
Serum Glucose (mg/dl)	168 ± 27	120 ± 22	
Duration of CAPD (Months)	27 ± 8	26 ± 12	
Prior Episodes of Peritonitis	0.6 ± 0.3	1.75 ± 1.1	

* Mean ± SEM values were compared by Student's t test. Body surface
area was calculated from Dubois nomograms of the patient's height and weight.
Serum values are the mean of measurements at the beginning and end of the
exchanges.

6.3.2 DAILY LYMPHATIC ABSORPTION AND REVERSE SOLUTE CLEARANCES

Extrapolated to four exchanges with 2 litres of 2.5% dextrose dialysis solution per day, daily lymphatic absorption in the 18 patients was 2.1 ± 0.2 litres and daily drain volumes were 8.8 ± 0.3 litres. Thus, lymphatic drainage reduced the potential daily net ultrafiltration volume by 82 ± 9% (Figure 25) and the potential daily drain volume by 18 ± 2% (Figure 26). Daily lymphatic (reverse) urea and creatinine clearances were 1.5 ± 0.2 litres and 1.1 ± 0.1 litres, respectively and reduced the potential daily urea and creatinine clearances by 14.0 ± 1.4% and 13.3 ± 1.5%, respectively (Figure 26).

Daily net ultrafiltration and drain volumes and daily peritoneal clearances of urea and creatinine in groups 1 and 2 are compared in Table 12. Patients in group 2 had significantly lower daily net ultrafiltration (p < 0.005), daily drain volumes (p < 0.005) and daily urea clearances (p < 0.05). The peritoneal clearances of creatinine were not significantly different between the two groups since the reduced drain volumes in group 2 were compensated for by higher dialysate/serum creatinine ratios after the four hour dwell time (Figure 16). In contrast dialysate/serum urea ratios approached unity at the end of the exchanges in both groups (Figure 15) and the peritoneal clearances of urea were related primarily to the daily drain volume. The average daily negative ultrafiltration in group 2 (Figure 25) is consistent with previous clinical observations of these patients using four exchanges of 2 litres of 2.5% dextrose dialysis solution per day (6.2.1).

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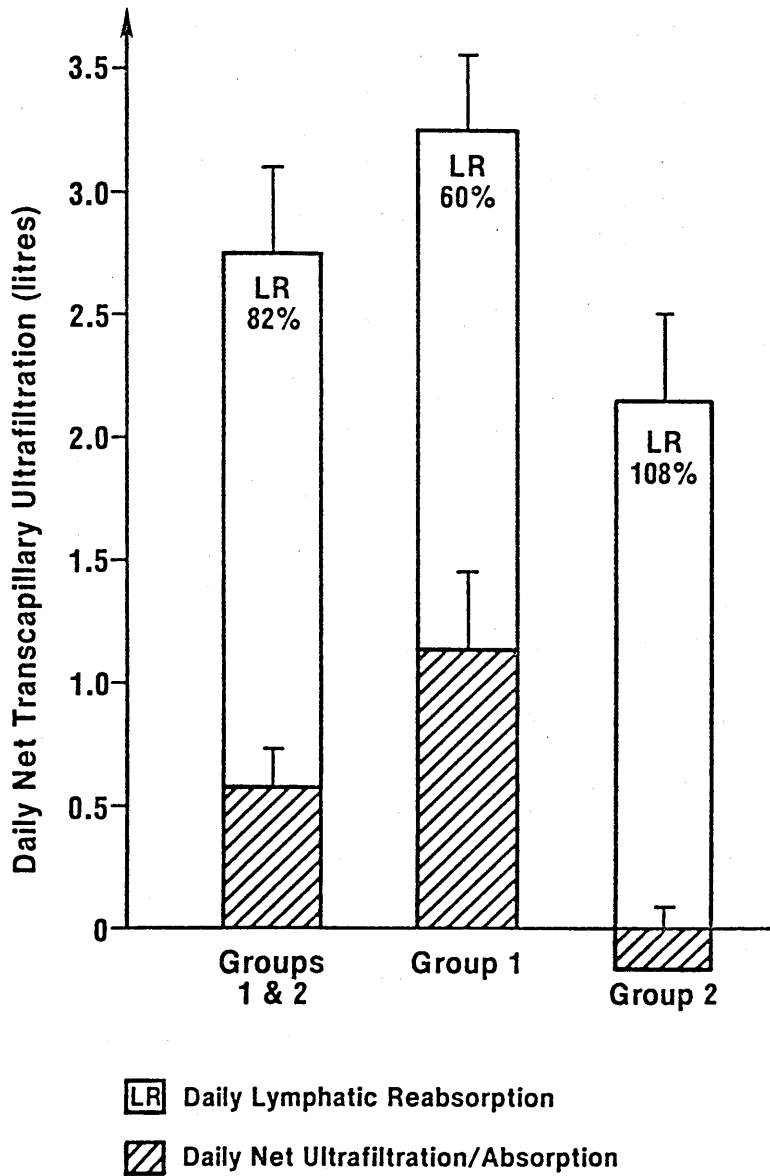
Figure 25: Daily net transcapillary ultrafiltration, lymphatic absorption and net ultrafiltration (mean ± SEM) in groups 1 and 2 using four exchanges of 2 litres, 2.5% dextrose dialysis solution per day.
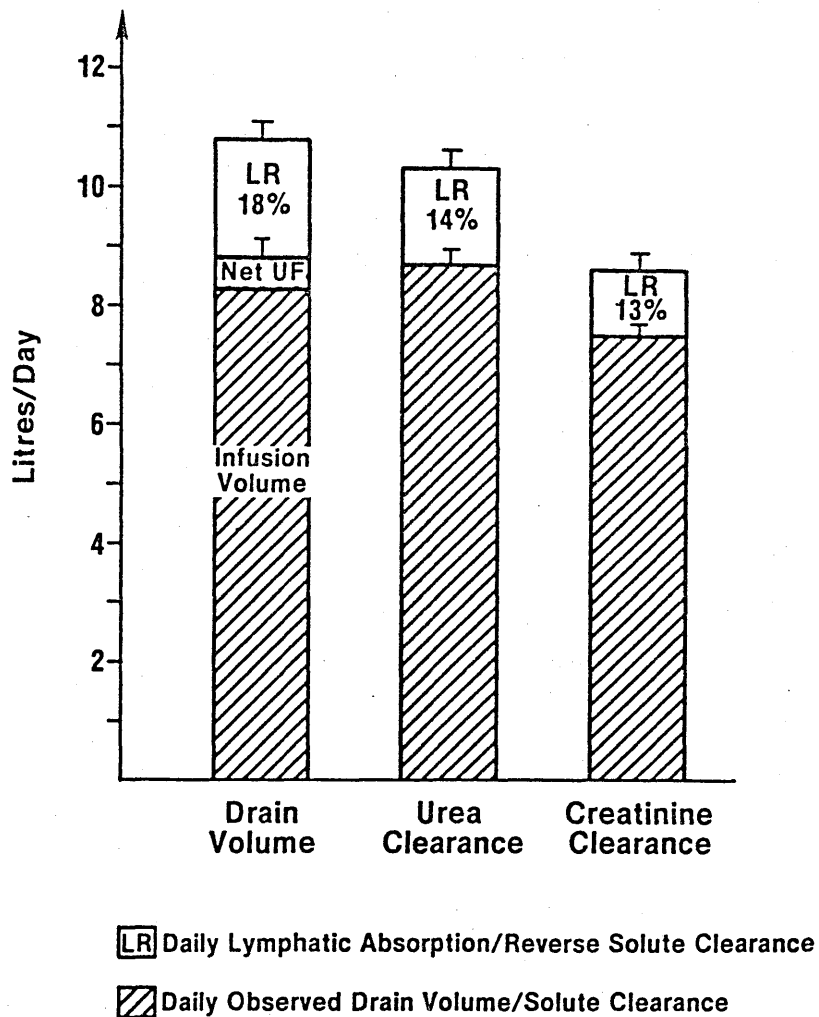
Figure 26: Contribution of lymphatic absorption to loss of potential daily drain volume, urea clearance and creatinine clearance (mean ± SEM) in CAPD patients (n = 18) using four exchanges of 2 litres of 2.5% dextrose dialysis solution per day.

TABLE 12: DAILY NET ULTRAFILTRATION, DRAIN VOLUMES AND OBSERVED AND REVERSE SOLUTE CLEARANCES IN GROUPS 1 AND 2

FLUID AND SOLUTE KINETICS (L/DAY)	GROUPS 1 & 2	GROUP 1	GROUP 2	*
Net Ultrafiltration	0.6 ± 0.2	1.2 ± 0.3	-0.2 ± 0.1	p < 0.005
Drain Volume	8.8 ± 0.3	9.4 ± 0.3	8.1 ± 0.3	p < 0.005
Observed Urea Clearance	8.7 ± 0.2	9.1 ± 0.3	8.1 ± 0.3	p < 0.05
Observed Creatinine Clearance	7.5 ± 0.2	7.7 ± 0.4	7.2 ± 0.2	
Reverse Urea Clearance	1.5 ± 0.2	1.3 ± 0.2	1.7 ± 0.2	
Reverse Creatinine Clearance	1.1 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	

* Mean ± SEM values in groups 1 and 2 were compared by Student's t test. Results were extrapolated from four hour exchanges with 2 litres of 2.5% dextrose dialysis solution.

6.3.3 EXCHANGES WITH RINGER'S LACTATE SOLUTION WITH ADDED ALBUMIN

Measured net fluid absorption at the end of four hour exchanges with 2 litres of near isosmotic Ringer's lactate solution in four of the patients was 255 ± 38 ml (Figure 27). The reduction in the intraperitoneal volume was observed without a significant change in the concentration of albumin added to the dialysis solution (Figure 27). In comparison cumulative lymphatic absorption during the previous study exchanges with 2.5% dextrose dialysis solution was 333 ± 87 ml in the four patients.

6.3.4 EXCHANGES WITH 2.5% DEXTROSE DIALYSIS SOLUTION WITHOUT ADDED ALBUMIN

In the same four patients, measured intraperitoneal volumes after two hour exchanges with 2 litres of 2.5% dextrose dialysis solution without albumin were 2604 ± 51 ml and calculated intraperitoneal volumes at the two hour dwell time during exchanges with 2 litres of 2.5% dextrose dialysis solution with added albumin were 2663 ± 154 ml ($r = 0.98$). Drain dialysate solute ratios after the two hour exchanges with 2.5% dextrose dialysis solution without albumin correlated with dialysate solute ratios at the same dwell time during the study exchanges with 2.5% dextrose dialysis solution and Ringer's lactate with added albumin in these patients ($r = 0.75$; $p < 0.05$) (Figure 28).
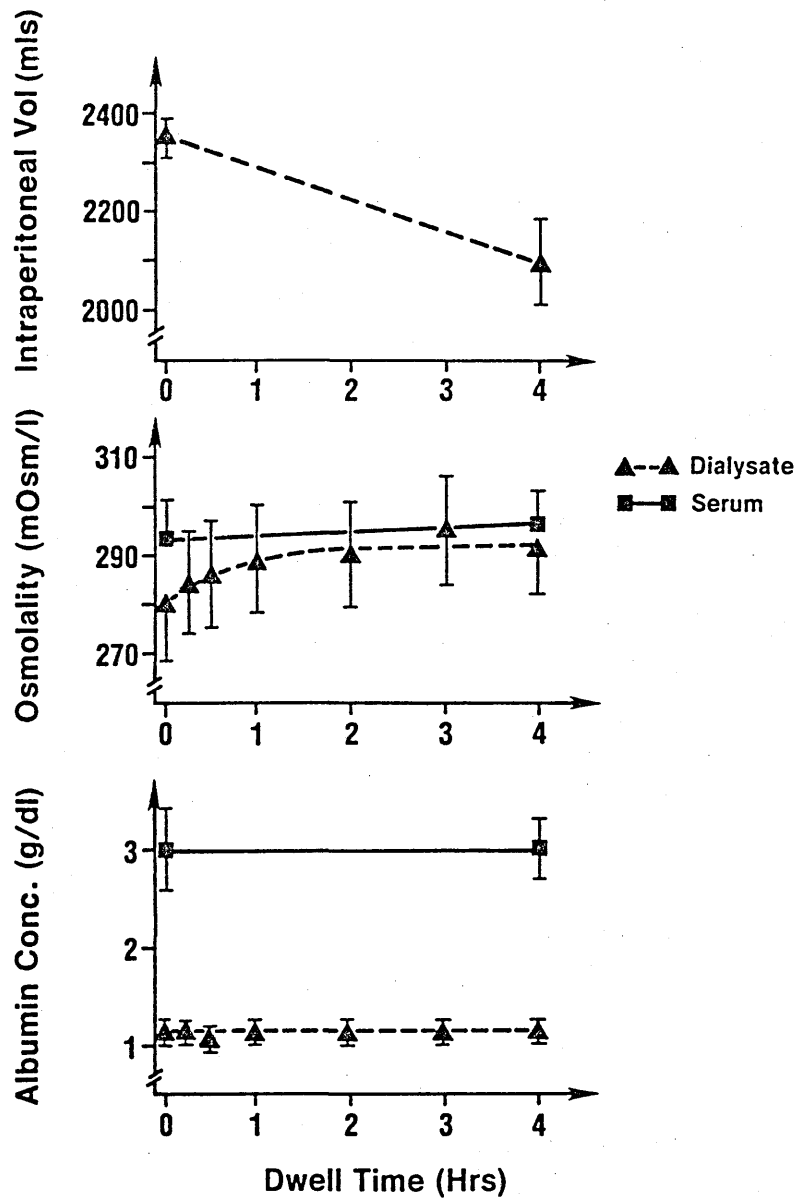
Figure 27: Net fluid absorption, serum and dialysate osmolality and albumin concentrations (mean ± SEM) during four hour exchanges with near isosmotic Ringer's lactate solution with added albumin (n = 4).
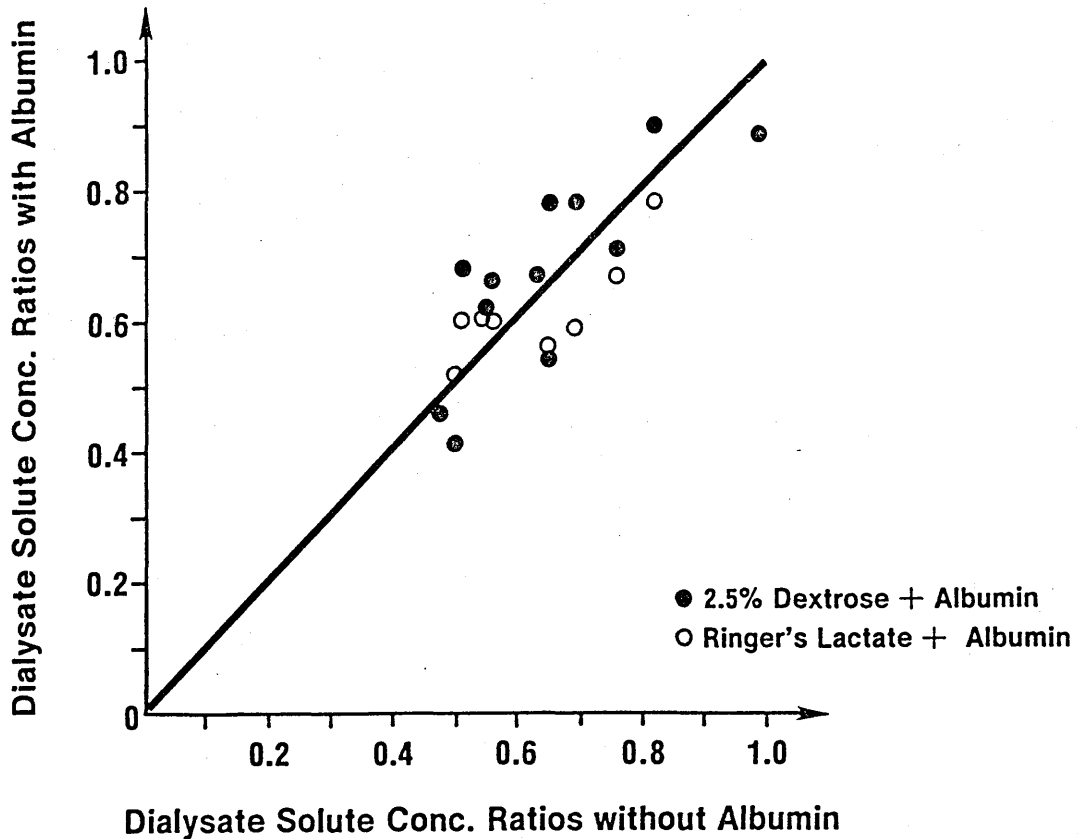
Figure 28: Dialysate/serum urea and creatinine ratios and effluent/initial dialysate glucose ratios at two hour dwell time during exchanges with 2 litres of 2.5% dextrose dialysis solution without albumin (n=4) correlated with dialysate solute ratios during exchanges using 2 litres of 2.5% dextrose dialysis solution (n=4) and Ringer's lactate (n=4) with added albumin performed within the preceding four days (r = 0.75).

Moreover, dialysate/serum urea and creatinine ratios and effluent/initial dialysate glucose ratios during exchanges with 2 litres of 2.5% dextrose dialysis solution without albumin, which were performed within the preceding year in 11 of the patients, also correlated with dialysate solute ratios during the 2.5% dextrose exchanges with added albumin (Figures 29,30 and 31). Net ultrafiltration (drain + sample volumes - infusion volume) during these exchanges without albumin averaged 284 ± 107 ml compared with 230 ± 74 ml during the study exchanges with albumin in the same 11 patients (r = 0.66; p < 0.05). These results all suggest that the addition of 30 g albumin to 2.5% dextrose dialysis solution did not significantly change peritoneal permeability x area or net ultrafiltration.

6.4 DISCUSSION

These data indicate that cumulative lymphatic absorption from the peritoneal cavity significantly reduces net ultrafiltration and solute clearances in CAPD patients. The peritoneal lymphatic absorption rates in the 18 CAPD patients in this study averaged 86 ± 10 ml per hour, which is consistent with prior observations in patients with ascites who have no fibrosis or tumour invasion of the diaphragmatic and mediastinal lymphatics (128,131-134,148).

This considerable rate of lymphatic absorption has a major influence on ultrafiltration kinetics in long-dwell peritoneal dialysis (CAPD) exchanges. The maximum intraperitoneal volume occurs

DIALYSATE/SERUM UREA RATIOS

r = 0.90

(y-axis) Exchanges with Albumin

(x-axis) Exchanges without Albumin
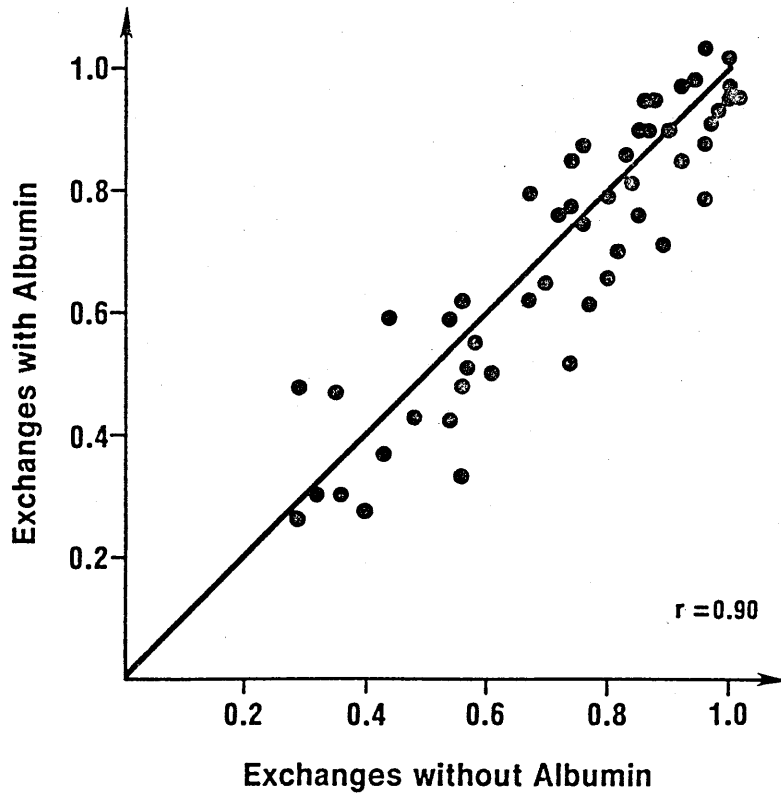
Figure 29: Dialysate/serum urea ratios during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution with added albumin(n=11) correlated with dialysate solute ratios during exchanges with 2 litres of 2.5% dextrose dialysis solution without albumin (n=11) performed within the preceding year (r = 0.90).

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DIALYSATE/SERUM CREATININE RATIOS

$r = 0.75$

Exchanges with Albumin (y-axis)

Exchanges without Albumin (x-axis)
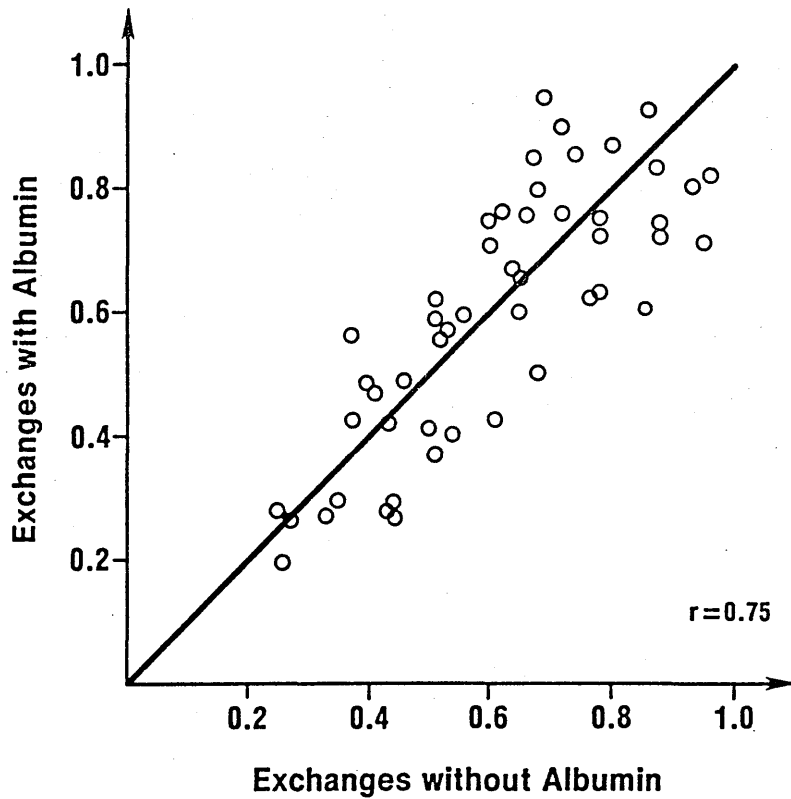
Figure 30: Dialysate/serum creatinine ratios during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution with added albumin are correlated with exchanges using 2 litres of 2.5% dextrose dialysis solution (n=11), which were performed within the preceding year (r=0.75).

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EFFLUENT/INITIAL DIALYSATE GLUCOSE RATIOS
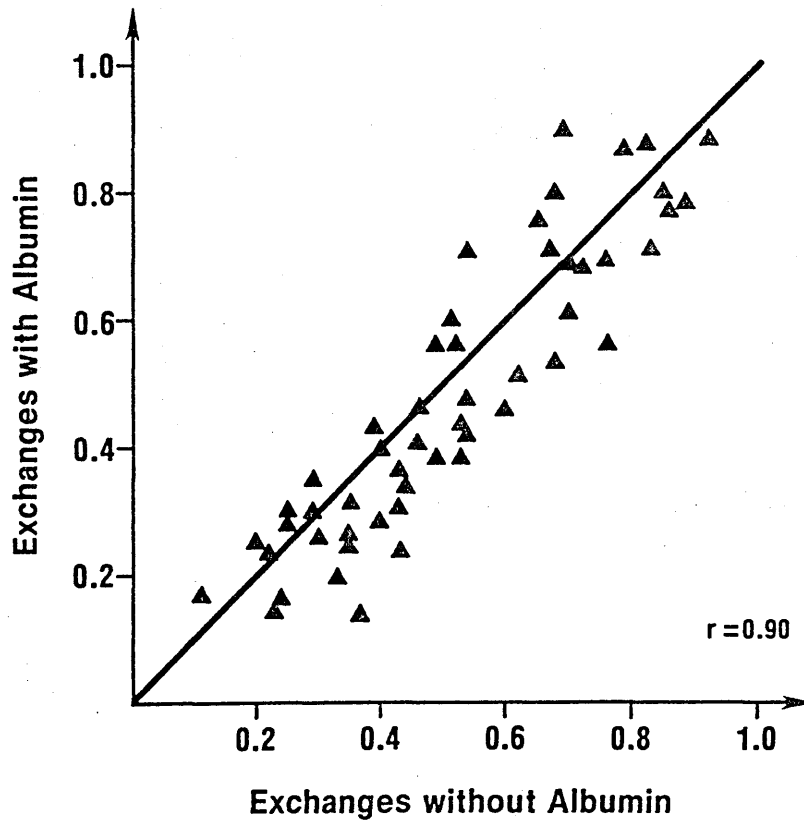
Figure 31:   Effluent/initial dialyate glucose ratios during four hour exchanges with 2 litres of 2.5% dextrose dialysis solution with added albumin are correlated with exchanges performed in the same patients (n=11) within the preceding year using 2 litres of 2.5% dextrose dialysis solution without albumin (r=0.90).

when the net transcapillary ultrafiltration rate decreases to equal the lymphatic absorption rate and the net ultrafiltration rate is zero (Figure 19). Consequently peak net ultrafiltration is observed during CAPD exchanges while net transcapillary ultrafiltration continues and before osmolar equilibrium between serum and dialysate is reached (Figures 19 & 20). Thereafter, the lymphatic absorption rate exceeds the net transcapillary ultrafiltration rate and the intraperitoneal volume decreases. Thus the net absorption rate after maximum intraperitoneal volume represents lymphatic absorption minus concurrent net transcapillary ultrafiltration (Figures 19 & 23). Osmolar equilibrium precedes glucose equilibrium during CAPD exchanges (Figures 20 & 24) because of solute sieving with ultrafiltration. These findings are similar to the kinetics of ultrafiltration during exchanges with hypertonic dextrose dialysis solution in rats (5.3.1). The calculated net fluid absorption rates in this study (Figure 19) are in agreement with previous sequential, direct measurements of drain volumes in CAPD patients (25,26) and infer that fluid absorption in CAPD is mainly via the peritoneal cavity lymphatics.

The peritoneal lymphatic absorption rates reported herein are higher than in a previous study of CAPD patients (156). However, peritoneal lymphatic flow rates in the prior study were underestimated since lymphatic absorption was calculated from the mass transfer rate of radio-iodinated serum albumin from the peritoneal cavity to the blood (4.4.1). Lymphatic absorption in the present study was estimated from the rate of removal of

intraperitoneal albumin from the peritoneal cavity (4.4.2) and thus it was important to exclude the presence of extraperitoneal dialysate leaks in the study patients. None of the patients had clinical evidence of an external dialysate leak, and three of the patients with poor peritoneal ultrafiltration and clinical suspicion of an internal dialysate leak had negative studies after intraperitoneal infusion of radio-contrast (177).

Two findings in this study suggest that there was no significant adsorption of the administered albumin to the mesothelium of the peritoneal membrane. Firstly, only 1.8 ± 0.4 g of albumin was removed in the post-study in and out exchange, which can be accounted for by the albumin content of the study residual volume. Secondly, the albumin concentration remained constant during absorption of isotonic intraperitoneal fluid in four of the patients (Figure 27). The exchanges with 2.5% dextrose dialysis solution without added albumin show that the addition of 30 g albumin had no significant effect on peritoneal permeability x area or net ultrafiltration (Figures 28-31). The validity of the calculations is also supported by the close correlation between measured and calculated intraperitoneal volumes at the two hour dwell time in four of the patients ($r = 0.96$) and measured and calculated net ultrafiltration at the end of the four hour exchanges in all of the patients ($r = 0.97$).

The lymphatic absorption rates in the patients with average and high peritoneal permeability x area did not differ. However, lymphatic absorption causes a proportionately greater reduction in

net ultrafiltration in patients with high peritoneal permeability x
area (Table 8) since these patients have more rapid absorption of
glucose from the dialysis solution (Figure 24), earlier dissipation
of the transperitoneal osmolar gradient and lower cumulative net
transcapillary ultrafiltration (Figure 21). Extrapolated to four
exchanges with 2 litres of 2.5% dextrose dialysis solution per day,
the group 2 patients with high peritoneal permeability x area had
negative daily ultrafiltration even though daily net transcapillary
ultrafiltration averaged 2.1 ± 0.4 litres (Figure 25). Thus, in the
absence of a dialysate leak, loss of peritoneal ultrafiltration in
CAPD patients occurs when daily lymphatic absorption equals or
exceeds daily net transcapillary ultrafiltration.

Lymphatic absorption rates in this study may be higher than in
active CAPD patients since the exchanges were all performed in the
supine position and fluid contact with the diaphragm may have been
more extensive than in the upright posture (108). Alternatively, the
increase in intraperitoneal pressure with upright posture (36,178)
may tend to increase lymphatic absorption in active CAPD patients
(112). Further studies are needed to compare lymphatic absorption
rates in the supine and upright positions. The lymphatic absorption
rates of the CAPD patients in this study were unrelated to the number
of prior episodes of peritonitis. This suggests that CAPD associated
peritonitis has no long-term effect on the patency or function of the
peritoneal cavity lymphatics. None of the patients were studied
within three months of peritonitis since acute peritonitis may alter
the lymphatic absorption rate (118). However, the influence of CAPD

associated peritonitis on lymphatic absorption has still to be evaluated.

Although the physiological role of the peritoneal lymphatics in maintaining a small volume of isosmotic fluid in the peritoneal cavity and improving host defences is advantageous, lymphatic absorption has adverse sequelae in all CAPD patients. Net ultrafiltration with four exchanges of 2 litres of 2.5% dextrose dialysis solution per day averaged only 18% of daily cumulative net transcapillary ultrafiltration (Figure 25) and daily reverse (lymphatic) solute clearances reduced daily peritoneal clearances of urea and creatinine by greater than 13% (Figure 26). Thus, previous estimates of peritoneal mass transfer, which were based on dialysate drain volumes and solute concentrations and neglected translymphatic transport during the dwell time, are erroneously low (57-59,64-66). Consequently net ultrafiltration and solute clearances in CAPD may be increased if daily lymphatic drainage is reduced. Pharmacological reduction of lymphatic absorption would alleviate the problem of loss of ultrafiltration observed in some CAPD patients and may provide a means for future improvement in the efficiency of CAPD.

6.5 CONCLUSIONS

1. Lymphatic absorption in CAPD is considerable and averages 86 ± 10 ml per hour during exchanges with 2 litres of 2.5% dextrose dialysis solution.

2. Peak net ultrafiltration is observed during CAPD exchanges before net transcapillary ultrafiltration ceases due to dissipation of the transperitoneal osmotic gradient.

3. The net absorption rate after peak intraperitoneal volume represents the lymphatic absorption rate minus the concurrent net transcapillary ultrafiltration rate.

4. Cumulative lymphatic absorption significantly reduces net ultrafiltration and solute clearances after four hour exchanges in all CAPD patients.

5. Cumulative lymphatic absorption causes a proportionately greater reduction in net ultrafiltration in patients with high peritoneal permeability x area since these patients have more rapid absorption of glucose from the dialysis solution, earlier dissipation of the transperitoneal osmotic gradient during the dwell time and less cumulative net transcapillary ultrafiltration.

6. Failure of peritoneal ultrafiltration occurs in CAPD patients when daily lymphatic absorption equals or exceeds daily net transcapillary ultrafiltration.

Chapter 7 LYMPHATIC ABSORPTION IN CHILDREN ON PERITONEAL DIALYSIS

7.1 INTRODUCTION

Peritoneal dialysis has several advantages over haemodialysis in the treatment of children with azotaemia and thus has become the preferred dialytic modality for most children developing end-stage renal disease (179,180). Previous studies of ultrafiltration kinetics in CAPD suggest that net ultrafiltration, scaled for body surface area, is lower in children than in adults (181-183). The relative reduction in net ultrafiltration volumes in children on CAPD has been attributed to more rapid absorption of glucose from the dialysis solution and earlier dissipation of the transperitoneal osmotic gradient (181,182). However, in a paediatric CAPD population with poor ultrafiltration capacity, net ultrafiltration did not correlate with the duration of CAPD, the patient's age at the onset of dialysis, the occurrence of prior episodes of peritonitis or the use of 4.25% dextrose or acetate containing dialysis solutions (184). Thus, as in adults, the pathophysiology of the loss of peritoneal ultrafiltration capacity in children on long-dwell peritoneal dialysis remains incompletely understood.

Intraperitoneal blood transfusions have been performed in children for over fifty years (185-187). Following intraperitoneal infusion red blood cells are absorbed intact by the peritoneal cavity lymphatics at a rate only slightly lower than intraperitoneal plasma (78,86). Lymphatic absorption from the peritoneal cavity may also

significantly reduce net ultrafiltration during long-dwell peritoneal dialysis exchanges in children. This study was performed to evaluate the contribution of cumulative lymphatic absorption during the dwell time to the loss of ultrafiltration and solute clearances in children on peritoneal dialysis.

7.2 PATIENTS AND METHODS

7.2.1   PATIENTS

Standardised four hour exchanges using 2.5% dextrose dialysis solution were performed in 6 children on CAPD or CCPD (continuous cyclic peritoneal dialysis) (188). The study was approved by the Institutional Review Board for Human Experimentation, University of Missouri-Columbia and the parents of each child gave written, informed consent. At the time of study, the mean age of the children was 9 ± 2 (SEM) years (range 2-13) and the mean duration of CAPD was 19 ± 6 months (range 10-47).   None of the children had clinical evidence of dialysate leaks or fluid overload. Although all of the children had had at least one prior episode of peritonitis (mean 2.3 ± 2.0 episodes), none had peritonitis within three months of the study. All of the patients had always used lactate containing dialysis solutions and none of the children had clinical or biochemical features of acute or chronic liver disease. The clinical data of each patient at the time of study are summarised in Table 13.

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TABLE 13: **CLINICAL DATA OF CHILDREN AT TIME OF STUDY EXCHANGES**

PATIENT NO.	AGE (YEARS)	SEX	CAUSE OF END-STAGE RENAL FAILURE	MONTHS ON CAPD	PRIOR EPISODES OF PERITONITIS	WEIGHT (Kg)	HEIGHT (cm)	BODY SURFACE AREA (m^2)	PERITONEAL DIALYSIS SCHEDULE*
1	8	M	Haemolytic-Uraemic Syndrome	12	1	21	115	0.84	CAPD
2	12	F	Chronic Glomerulonephritis	10	1	33	143	1.19	CCPD
3	11	M	Medullary Cystic Disease	11	1	30	135	1.07	CCPD
4	2	M	Dysplastic Kidneys	24	3	10	76	0.44	CAPD
5	10	M	Haemolytic-Uraemic Syndrome	47	6	29	129	1.03	CAPD
6	13	M	Chronic Glomerulonephritis	12	2	30	130	1.04	CCPD

* CAPD; all children used four 1 litre exchanges per day except patient 4 who used volumes of 0.5 litre.
CCPD; all children used four 1 litre exchanges overnight and a 0.5 litre exchange during the day.

Each study was performed after a four hour exchange using the child's usual infusion volume (Table 13) of 2.5% dextrose dialysis solution (Dianeal PD-2). To standardise the dialysis mechanics of the study exchanges and thereby permit interpatient comparisons, the study infusion volume was 40 ml/Kg body weight for each child. The study dialysis solution was prepared by preheating 1.0 or 1.5 litres of 2.5% Dianeal PD-2 to 37° C, removing the required volume of dialysate with aseptic technique and adding 25% human serum albumin (Baxter-Travenol, Deerfield, Illinois) to give a volume of 1.5% albumin dialysis solution equivalent to 40 ml/Kg body weight. The standardised study volumes were selected to correspond to infusion volumes routinely used in children on CAPD (179,180). The protocol for the study exchanges was thereafter the same as described previously for the adult CAPD patients (6.2.2). It is emphasised that all the dialysate samples were taken with strict aseptic technique using a povidone-iodine solution impregnated sponge (Connection Shield III, Baxter-Travenol, Deerfield, Illinois) over the sample port of the dialysis bag. Fortunately these precautions proved to be adequate since none of the patients (adults as well as children) developed peritonitis within one week of undergoing the study exchange. The laboratory methods, calculations and statistical analysis were the same as in Chapter 6.

7.3 RESULTS

7.3.1 EXCHANGES WITH 2.5% DEXTROSE DIALYSIS SOLUTION IN CHILDREN

Mean cumulative lymphatic absorption from the peritoneal cavity during four hour exchanges with 40 ml/kg of 2.5% dextrose dialysis solution averaged 257 ± 67 (SEM) ml in the six children. To enable the analysis of group data from exchanges with variable infusion volumes, cumulative net transcapillary ultrafiltration, lymphatic absorption and calculated net ultrafiltration during the exchanges in children are expressed in Figure 32 as a percentage of the intraperitoneal volume at the beginning of each exchange.   Net transcapillary ultrafiltration occurred mainly during the first two hours of the dwell time, whereas lymphatic absorption continued at a linear rate throughout the exchanges. Thus, peak ultrafiltration was observed at around the 90 minutes dwell time (Figure 32) and at the end of the exchanges calculated net ultrafiltration averaged only 27 ± 10% of total net transcapillary ultrafiltration during the exchange (Table 14).   Calculated net ultrafiltration correlated closely with measured ultrafiltration at the end of the four hour dwell time (r = 0.94; p < 0.02) (Table 14).   Maximum intraperitoneal volume, expressed as a percentage of the initial intraperitoneal volume, preceded osmolar equilibrium which in turn preceded the approach of glucose equilibrium (Figure 33).
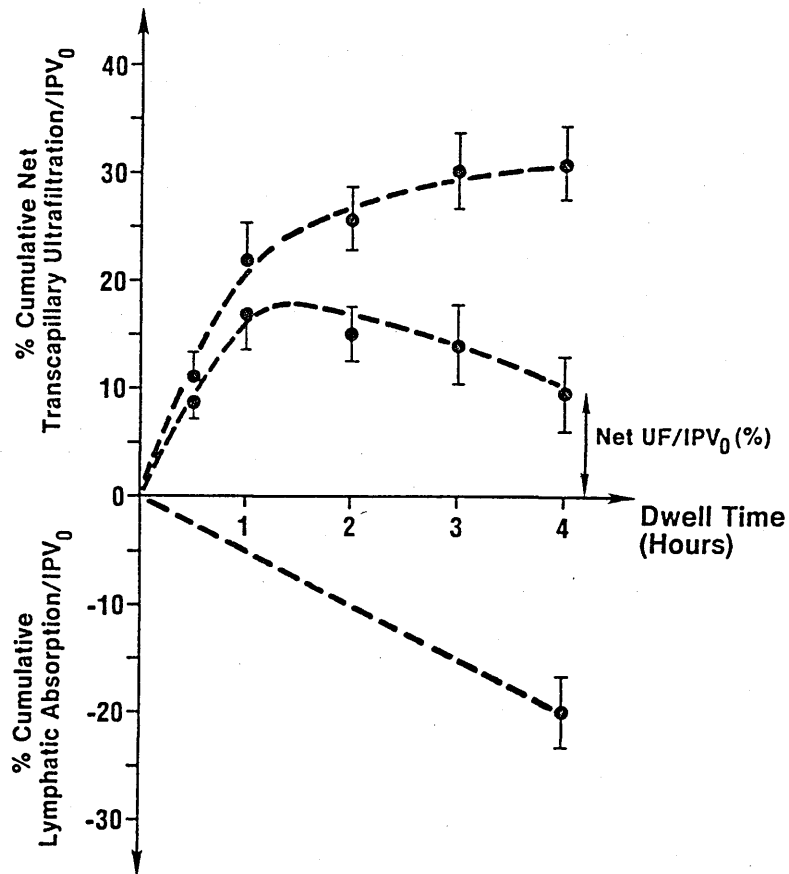
Figure 32: Cumulative net transcapillary ultrafiltration, lymphatic absorption and net ultrafiltration (mean ± SEM) during four hour exchanges in children using 40 ml/Kg of 2.5% dextrose dialysis solution. All values are expressed as a percentage of the intraperitoneal volume at the beginning of the exchange (IPV_o).

TABLE 14: ULTRAFILTRATION KINETICS IN CHILDREN AFTER FOUR HOUR EXCHANGES WITH 2.5% DEXTROSE DIALYSIS SOLUTION*

FLUID TRANSPORT (after 4 hrs)	ABSOLUTE VOLUMES (ml)	ABSOLUTE VOLUMES/INITIAL INTRAPERITONEAL VOLUME (%)
Net Transcapillary UF	383 ± 88	31 ± 4
Lymphatic Absorption	257 ± 67	20 ± 3
Calculated Net UF	125 ± 54	10 ± 4
Measured Net UF	104 ± 55	8 ± 4

* Infusion volumes of dialysis solution were 40 ml/Kg for each exchange and accordingly results (mean ± SEM) are also expressed as a percentage of the intraperitoneal volume at the beginning of the exchanges.
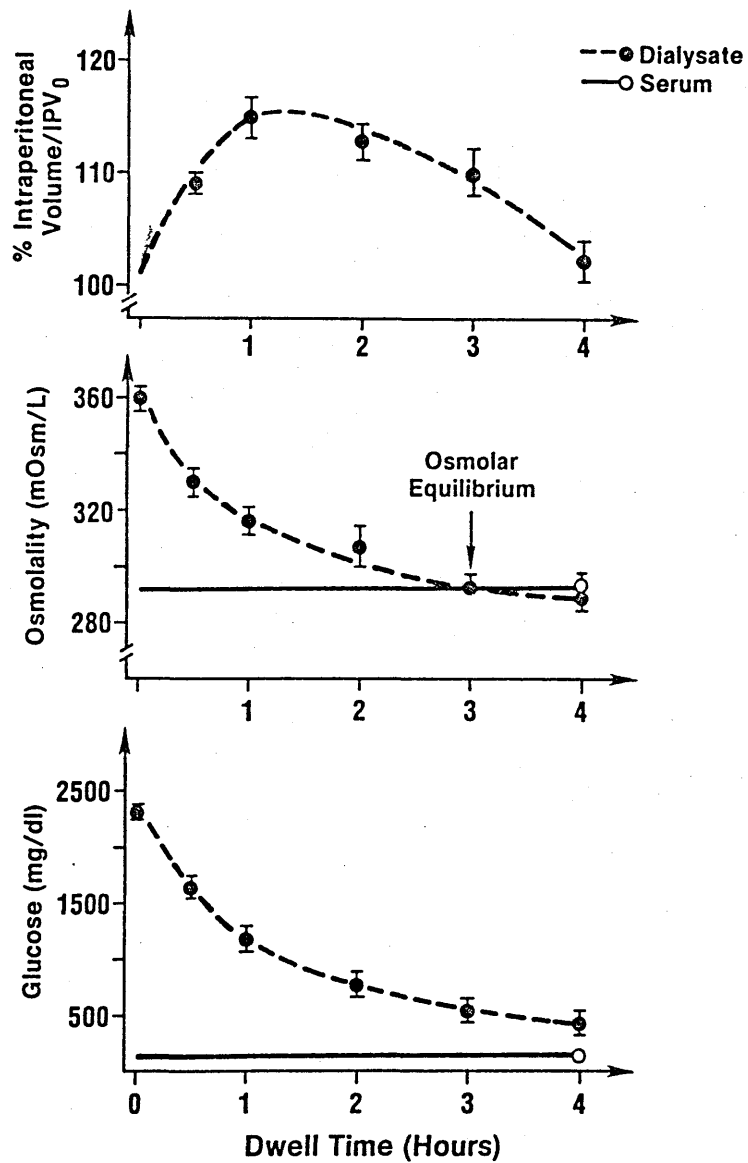
Figure 33: Intraperitoneal volume, dialysate and serum osmolalities and glucose concentrations (mean ± SEM) during four hour exchanges with 2.5% dextrose dialysis solution in children. Peak intraperitoneal volume, expressed as a percentage of the initial intraperitoneal volume (IPV_0), occurs before the 90 minutes dwell time.

7.3.2 COMPARISON OF ULTRAFILTRATION KINETICS IN CHILDREN AND ADULTS

Cumulative lymphatic absorption after four hour exchanges with 2.5% dextrose dialysis solution in children and in adults with average peritoneal permeability x area (group 1 from Chapter 6) are compared in Table 15. For valid comparisons the absolute lymphatic absorption rates in each group are expressed in relation to body weight, body surface area, intraperitoneal volume and dialysate glucose load. Lymphatic absorption was higher in children than in adults but the difference only reached significance when corrected for body weight ($p < 0.01$; Table 15).

Cumulative net transcapillary and measured net ultrafiltration volumes after the four hour exchanges in children and adults are compared in Table 16. Cumulative net transcapillary ultrafiltration tended to be lower in children when adjusted for body surface area, intraperitoneal volume or dialysate glucose load but was higher when expressed relative to body weight (Table 16). Consequently the proportion of total net transcapillary ultrafiltration reabsorbed by the peritoneal cavity lymphatics was greater during the exchanges in children (73 ± 10%) than in adults (40 ± 6%; $p < 0.01$). Measured net ultrafiltration, scaled for body surface area, intraperitoneal volume and dialysate glucose load, was significantly lower in children (Table 16).

The kinetics of ultrafiltration during the exchanges in the children in this study were similar to adult CAPD patients with high peritoneal permeability x area (group 2 from Chapter 6). Firstly,

TABLE 15: LYMPHATIC ABSORPTION AFTER FOUR HOUR EXCHANGES IN CHILDREN AND ADULTS RELATED TO BODY WEIGHT, BODY SURFACE AREA, INTRAPERITONEAL VOLUME AND DIALYSATE GLUCOSE LOAD.

LYMPHATIC ABSORPTION	CHILDREN	ADULTS	
ml/Kg body weight	10.3 ± 1.8	4.4 ± 0.9	* p < 0.01
ml/m^2 body surface area	271 ± 48	180 ± 36	
% initial intraperitoneal volume[+]	20.4 ± 3.0	13.9 ± 2.7	
ml/g dialysate glucose load	8.9 ± 1.7	6.9 ± 1.5	

[*] Mean ± SEM values were compared by Student's t test.

[+] The infusion volumes averaged 26 ml/Kg (1254 ± 63 ml/m^2 body surface area) in adults and 40 ml/Kg (1333 ± 156 ml/m^2) in children.

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TABLE 16: ULTRAFILTRATION VOLUMES IN CHILDREN AND ADULTS RELATED TO BODY WEIGHT, BODY SURFACE AREA, INTRAPERITONEAL VOLUME AND DIALYSATE GLUCOSE LOAD.

NET TRANSCAPILLARY UF	CHILDREN	ADULTS	*
ml/Kg body weight	15.4 ± 2.1	10.6 ± 1.2	$p < 0.05$
ml/m^2 body surface area	406 ± 61	430 ± 42	
% initial intraperitoneal volume	31 ± 4	34 ± 2.9	
ml/g dialysate glucose load	13.4 ± 2.0	16.1 ± 1.8	

MEASURED NET UF	CHILDREN	ADULTS	
ml/Kg body weight	4.1 ± 1.9	5.9 ± 0.7	
ml/m^2 body surface area	111 ± 52	237 ± 26	$p < 0.05$
% initial intraperitoneal volume	9.2 ± 3.8	19.1 ± 2.2	$p < 0.05$
ml/g dialysate glucose load	3.5 ± 1.9	8.8 ± 1.6	$p < 0.01$

* Mean ± SEM values compared by Student's t test.

the rate of absorption of dialysate glucose and the rate of equilibration of dialysate and serum urea were significantly above the reference range in adults (Figures 34 and 35, respectively). Secondly, peak ultrafiltration and osmolar equilibrium during the exchanges in this group of children were observed as early in the dwell time (Figure 33) as during the exchanges in adults with high peritoneal permeability x area (Figure 24). Thirdly, the percentage of the dialysate glucose load absorbed during the exchanges in children (79 ± 6%) was higher than in exchanges in adults with average peritoneal permeability x area (67 ± 2%; p < 0.025). Despite the differences in peritoneal transport rates, the duration of peritoneal dialysis and the number of prior episodes of peritonitis did not differ significantly between the children and adults with average peritoneal permeability x area.

7.3.3 DAILY LYMPHATIC ABSORPTION AND REVERSE SOLUTE CLEARANCES

Extrapolated to four exchanges with 40 ml/Kg of 2.5% dextrose dialysis solution per day, the daily drain volume in the children averaged 168 ± 15 ml/Kg while daily lymphatic absorption was 62 ± 11 ml/Kg (Figure 36). Thus lymphatic absorption produced a proportionately greater reduction in the potential daily drain volume in peritoneal dialysis in children (27 ± 5%) than in adults with average peritoneal permeability x area (16 ± 3% ; p < 0.05).

Daily peritoneal and reverse (lymphatic) clearances of urea and creatinine, scaled for body weight, are shown in Figure 36. Lymphatic
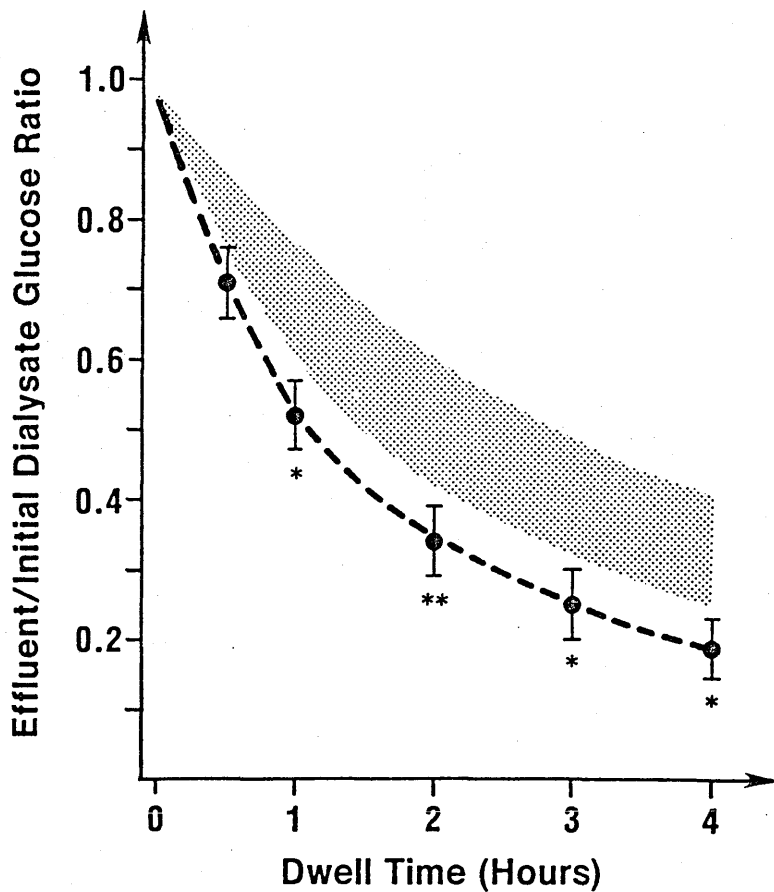
Figure 34: Effluent/initial dialysate glucose ratios (mean ± SEM) during exchanges with 2.5% dextrose dialysis solution in children. The reference range (mean ± 1SD) in adults is shaded. Glucose ratios in children and adults with average peritoneal permeability x area (Group 1, Figure 17) were compared by Student's t test.

** p < 0.01 ; * p < 0.05
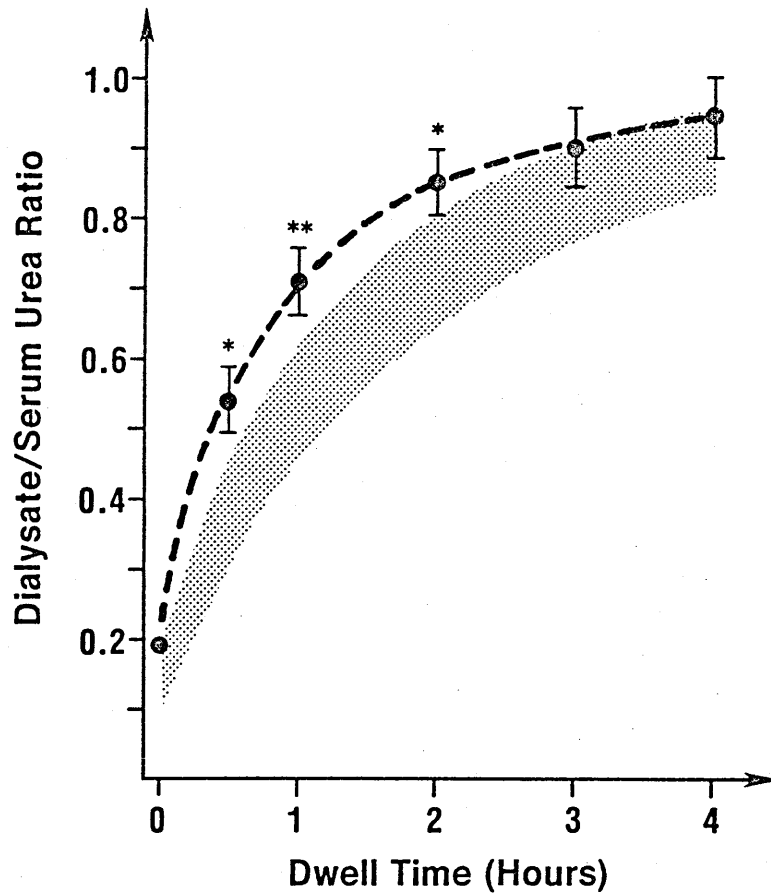
Figure 35: Dialysate/serum urea ratios (mean ± SEM) during exchanges with 2.5% dextrose dialysis solution in children. The reference range in adults is shaded. Solute ratios in children and adults with average peritoneal permeability x area (Group 1) were compared by Student's t test.

** $p < 0.01$; * $p < 0.05$

240 —

LA
27%

200 —

LA
24%

ml/Kg/day

LA
22%

160 —

120 —

80 —

40 —

Drain   Urea   Creatinine
Volume   Clearance   Clearance

|LA| Lymphatic Absorption/Reverse Solute Clearances
      (ml/Kg/day)

▨ Drain Volume/Solute Clearances (ml/Kg/day)
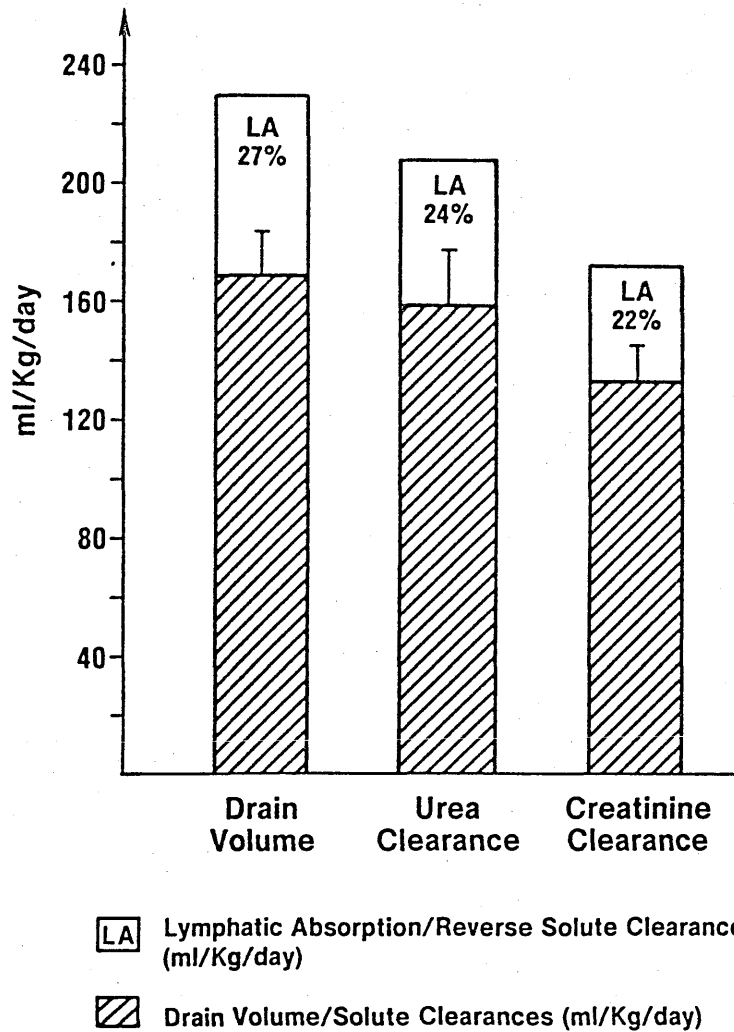
Figure 36:   Contribution of lymphatic absorption to loss of daily
drain volumes and solute clearances in children using
four exchanges with 40 ml/Kg of 2.5% dextrose dialysis
solution per day.   Observed drain volumes and solute
clearances (mean ± SEM) are expressed as ml/Kg/day.

absorption reduced the daily potential clearances of urea and creatinine in the children by 24 ± 4% and 22 ± 5%, respectively. Daily urea and creatinine clearances in children and adults with average peritoneal permeability x area are compared in Table 17. Peritoneal and reverse solute clearances, corrected for body weight, were significantly higher in children than in adults. However, when related to body surface area, urea and creatinine clearances in children (4.2 ± 0.4 and 3.5 ± 0.2 L/m^2/day, respectively) were not significantly different from peritoneal clearances of urea and creatinine in adults (4.9 ± 0.3 and 4.1 ± 0.3 L/m^2/day, respectively). Nevertheless, the reduction in daily potential solute clearances due to lymphatic absorption was significantly higher in children than in adults (Table 17).

7.4 <u>DISCUSSION</u>

This study indicates that cumulative lymphatic absorption from the peritoneal cavity significantly reduces net ultrafiltration and solute clearances in children on peritoneal dialysis. The kinetics of peritoneal dialysis in children of diverse body size can only be interpreted meaningfully if the dwell time, osmolality and volume of exchanges are standardised (179,189). The dialysis mechanics of the study exchanges in this group of children were kept constant and infusion volumes of 40 ml/Kg were selected to simulate exchange volumes used routinely in CAPD or CCPD in children (179,180,189). Consequently group data in the children were analysed in relation to

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TABLE 17: PERITONEAL AND REVERSE SOLUTE CLEARANCES IN CHILDREN AND ADULTS USING FOUR EXCHANGES WITH 2.5% DEXTROSE DIALYSIS SOLUTION PER DAY.

SOLUTE CLEARANCES	CHILDREN	ADULTS	*
Peritoneal urea clearances (ml/Kg/day)	158 ± 20	121 ± 9	$p < 0.05$
Reverse urea clearance (ml/Kg/day)	50 ± 11	17 ± 3	$p < 0.005$
Reverse/potential urea clearance (%)	24 ± 4	12 ± 2	$p < 0.01$
Peritoneal creatinine clearance (ml/Kg/day)	133 ± 13	104 ± 12	
Reverse creatinine clearance (ml/Kg/day)	39 ± 10	13 ± 2	$p < 0.005$
Reverse/potential creatinine clearance (%)	22 ± 5	11 ± 2	$p < 0.05$

* Mean ± SEM solute clearances or clearance ratios were compared by Student's t test.

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intraperitoneal volume or body weight (Figures 32 & 33, Tables 14-17).

Lymphatic absorption during the four hour exchanges in the children averaged 2.6 ± 0.4 ml/Kg per hour. This considerable rate of lymphatic drainage resulted in the peak intraperitoneal volume being observed well in advance of osmolar equilibrium (Figure 33). Although osmolar equilibrium occurred at the three hours dwell time, glucose dysequilibrium continued until the end of the exchanges (Figure 33). Similar findings were observed during exchanges in adults on CAPD (Figures 18 and 20).

The kinetics of peritoneal dialysis in children and adults were compared during four hour exchanges with 2.5% dextrose dialysis solution. The dialysis mechanics of the exchanges were the same except for the infusion volumes of dialysis solution, which in adults averaged 26 ml/Kg and in children were standardised at 40 ml/Kg. These infusion volumes, however, are similar to exchange volumes used clinically in both adults and children. Thus scaled comparisons will reflect differences in ultrafiltration and solute kinetics during standard peritoneal dialysis exchanges in children and adults.

Measured net ultrafiltration at the end of four hour exchanges is lower in children than adults with average peritoneal permeability x area, when scaled for body surface area (183), initial intraperitoneal volume or dialysate glucose load (Table 16). When adjusted for the same parameters, lymphatic absorption during the exchanges in children tended to be higher and net transcapillary ultrafiltration lower than in adults (Tables 15 & 16). Thus, since

net ultrafiltration equals cumulative net transcapillary ultrafiltration minus lymphatic absorption, the above combination of factors most likely explains the relatively low measured net ultrafiltration in children. However, this relatively low net ultrafiltration capacity in children is compensated to some extent by the routine use of higher infusion volumes (ml/Kg). In effect net ultrafiltration volumes in children and adults are equal when corrected for body weight, but are achieved at the expense of a relatively higher intraperitoneal volume, higher dialysate glucose load and higher transperitoneal glucose absorption. Impaired calorie intake and poor growth rates are major problems in uraemic children and the high obligatory dialysate glucose (and calorie) load per Kg in children may not produce metabolic complications as frequently as in adults (31).

Net transcapillary ultrafiltration and solute kinetics in the studied children were similar to adults with high peritoneal permeability x area. Glucose was absorbed rapidly from the dialysis solution (Figure 34) (181-183) and peak ultrafiltration and osmolar equilibrium occurred early in the dwell time (Figure 33). Likewise the rate of equilibration of dialysate and serum urea was also more rapid in children than adults with average peritoneal permeability x area (Figure 35) (183). These findings occurred despite higher infusion volumes per Kg body weight in children, which would tend to delay peritoneal solute transport by reducing peritoneal surface area to volume relationships (33,56). Thus the higher transperitoneal solute transport rates in the children cannot be attributed to, and

indeed should be lessened by, differences in dialysis mechanics between the children and adults. The apparently higher peritoneal permeability x area in children may be related to their higher peritoneal surface area/body weight ratio and presumably higher functional membrane transfer area/body weight ratio (14).

Peritoneal solute clearances, scaled for body weight, are higher in children than adults (Table 17). The augmented peritoneal clearances (ml/Kg/day) in children, however, are due to the routine use of higher dialysate volumes (ml/Kg) as well as the more rapid equilibration of small solutes. These factors may explain the success of CCPD, with three or four overnight exchanges and one daytime exchange, in maintaining adequate daily peritoneal solute clearances in most children (188). Moreover, with long-dwell CAPD exchanges in children, the rapid absorption of glucose from the dialysate, early dissipation of the transperitoneal osmolar gradient and early onset of net fluid absorption may result in inadequate daily net ultrafiltration. The kinetics of peritoneal dialysis in children may be more suited to short-dwell intermittent dialysis therapy such as nightly peritoneal dialysis (175). This form of peritoneal dialysis also allows the child freedom from daytime exchanges.

Lymphatic absorption during peritoneal dialysis in children causes a proportionately greater reduction in daily net ultrafiltration and solute clearances than in adults. Thus, the efficiency of peritoneal dialysis may be increased to a greater

extent in children than in adults if lymphatic absorption is reduced.

7.5 CONCLUSIONS

1. Lymphatic absorption significantly reduces net ultrafiltration and solute clearances in children on long-dwell peritoneal dialysis exchanges.

2. Net ultrafiltration volumes, scaled for body surface area, dialysate glucose load or intraperitoneal volume, are lower in children than adults due to a combination of relatively higher lymphatic absorption and lower net transcapillary ultrafiltration.

3. Lymphatic absorption produces a relatively greater reduction in solute clearances in children than in adults.

Chapter 8. EFFECT OF PERITONITIS ON ABSORPTION OF INSULIN AND GLUCOSE DURING PERITONEAL DIALYSIS IN DIABETIC RATS

8.1 INTRODUCTION

Diabetic nephropathy is a major and ever increasing cause of renal failure in patients beginning renal replacement therapy (9). CAPD rather than haemodialysis is often advocated for diabetic patients beginning dialysis since CAPD avoids the need for vascular access or systemic heparinisation, maintains steady state metabolite concentrations, can provide continuous control of fluid status and hypertension without inducing haemodynamic instability, and may retard the progression of neuropathy (190,191). Furthermore, despite the additional glucose load absorbed from the dialysis solution, glycaemic control in diabetic dialysis patients can be improved by the intraperitoneal administration of insulin with each CAPD exchange (191-194). In the USA more than one quarter of all patients now beginning CAPD have end-stage renal disease secondary to diabetic nephropathy (10).

Bacterial peritonitis continues to be the most frequent complication of CAPD therapy (163). The dosage of intraperitoneal insulin required for glycaemic control in diabetic CAPD patients may change during episodes of peritonitis. Increased absorption of glucose from the dialysis solution (44,195,196), insulin resistance secondary to infection and physical inactivity during acute illness will tend to increase insulin requirements during peritonitis.

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Alternatively, insulin demands may decrease if insulin absorption across the inflamed peritoneum and via the peritoneal cavity lymphatics increases or if oral carbohydrate intake is reduced. Although intraperitoneal insulin requirements are widely believed to increase during CAPD associated peritonitis (163,197), symptomatic hypoglycaemia has been observed in diabetic CAPD patients when the usual dosage of intraperitoneal insulin was continued during peritonitis (198).

The relative absorption rates of glucose and insulin during peritonitis in diabetic CAPD patients have not been studied. Insulin administered into the peritoneal cavity enters the portal venous circulation by diffusion across the visceral peritoneum and the capsule of the liver (199,200), and also reaches the systemic circulation directly by diffusion across the parietal peritoneum and by convective transport via the peritoneal cavity lymphatics (201). This study was performed to evaluate lymphatic and transperitoneal absorption of insulin and glucose during peritoneal dialysis in diabetic rats with and without peritonitis.

8.2 MATERIALS AND METHODS

8.2.1 ANIMAL MODEL

Diabetes mellitus was induced in 250-300g, male Sprague-Dawley rats by injecting streptozocin (Sigma Chemical Co., St. Louis, Missouri) 65 mg/Kg into the tail vein under ether anaesthesia (202).

Streptozocin is a listed carcinogen in the Federal Hazardous Waste Guide and therefore all materials used in the injection of streptozocin were double bagged and labelled with a biohazard warning prior to incineration. After recovering from anaesthesia, the animals were placed in metabolic cages and fed standard rat chow (Purina Ralston Co., St. Louis, Missouri) and water ad libitum for one week. Weight, food and water intake and urine output were recorded daily.

8.2.2   STUDY PROTOCOL

Seven days after streptozocin injection, six of the rats were anaesthetised with ether and inoculated with 1 ml Heart Brain suspension containing 10^6 colony forming units/ml Staphylococcus epidermidis. The other six diabetic rats acted as controls. The study exchanges were performed the next morning. Overnight the rats were allowed free access to water only. Following subcutaneous injection of 50 mg/Kg pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, Illinois), an external jugular venous catheter was inserted for infusion of intravenous fluids. A femoral arterial catheter (Intramedic polyethylene tubing, Clay Adams, Parsippany, New Jersey) was placed to monitor blood pressure and to permit serial blood sampling. Blood pressure was monitored using a P32/D pressure transducer (Gould Stratham, Hato Rey, Puerto Rico) connected to a Model 7 polygraph (Grass Instruments Co., Quincy, Massachusetts). The peritoneal dialysis catheter was inserted

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through a mid-line incision as described previously (5.2.1). All rats were hydrated with Ringer's lactate solution until voiding urine. Immediately before the study exchange, an in and out exchange was performed with 15 ml of 2.5% dextrose dialysis solution to estimate the intraperitoneal residual volume. White blood cell count (WBC/mm^3) and culture were performed on the washout dialysate to document the presence of infection in the peritonitis group (group 1) and exclude infection in the control rats (group 2).

Albumin (25% human serum albumin, Baxter-Travenol, Deerfield, Illinois) was added to 2.5% dextrose dialysis solution (Dianeal PD-2) to give a 2.5% albumin study dialysis solution. Two hour peritoneal dialysis exchanges were performed in all rats using 25 U regular insulin (Humulin, Eli Lilly Co., Indianapolis, Indiana) added to 15 ml of the study dialysis solution. Serial dialysate samples (0.5 ml) were obtained at baseline and time 0, 20, 60, 90 and 120 minutes after infusion. To obtain each dialysate sample, 1 ml of dialysis solution was aspirated into a sterile syringe to clear the catheter dead space, the sample taken, and then the aspirated dialysate reinfused. Serial blood samples (0.5 ml) were also taken at the same time intervals. Ringer's lactate solution was infused throughout the exchange using a syringe pump (Sage Instruments, model 341) to replace ultrafiltration and blood sample losses. Insulin, glucose and albumin concentrations were measured in all dialysate and serum samples. The haematocrit was also measured in each blood sample. Extra blood (1.2 ml) was taken at the beginning and end of the two hour exchanges to measure plasma C-peptide concentrations. At the

end of two hours, the dialysate solution was drained completely and measured in a graduated cylinder. Similar two hour exchanges using 15 ml of 2.5% dextrose dialysis solution without added albumin were also performed in five rats with peritonitis and five rats without peritonitis.

8.2.3 LABORATORY METHODS

Serum and dialysate glucose concentrations were measured by the glucose oxidase method using a YSI model 27 glucose analyser (Yellow Springs Instrument Co. Inc., Ohio). Serum and dialysate immunoreactive insulin (IRI) were measured by the coated charcoal radioimmunoassay (203). Plasma C-peptide concentrations were measured by a double antibody radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California). Bacterial cultures of the dialysate were performed on blood agar. Dialysate albumin concentrations were determined by the bromcresol method (5.2.4).

8.2.4 CALCULATIONS

The intraperitoneal volume at the beginning and end of the study exchanges was calculated as the residual volume, measured from the prior in and out exchange, plus the infusion and drain volume, respectively. Net ultrafiltration was measured as drain volume minus infusion volume. Cumulative lymphatic absorption and net transcapillary ultrafiltration during the exchanges were calculated

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as described previously (5.2.5 and 6.2.4). The drain dialysate albumin concentration was corrected for the influx of albumin during the dwell time by subtracting the mean drain dialysate albumin concentration of the corresponding group of rats without addition of albumin to the infused dialysis solution.

The percentages of glucose, insulin and albumin absorbed from the dialysis solution were calculated as:

$$\left(1 - \frac{(V_2 \times C_2) + (V_s \times \Sigma\, C_s)}{(V_0 \times C_0)} \right) \times 100\%$$

where V_2 and V_0 = intraperitoneal volumes at times 0 and 2 hours, respectively

C_2 and C_0 = solute concentrations at times 0 and 2 hours

V_s = sample volume (0.5 ml)

$\Sigma\, C_s$ = sum of solute concentrations in samples at times 0, 20, 60 and 90 mins

Results in groups 1 and 2 were compared by Student's non-paired and paired t test where appropriate.

8.3 RESULTS

Polyuria, polydipsia and weight loss were observed in all of the rats during the week following streptozocin injection (Table 18). Diabetes mellitus was confirmed in each rat by performing fasting

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TABLE 18: <u>FLUID BALANCE AND BODY WEIGHT OF RATS FOLLOWING STREPTOZOCIN INJECTION</u>

RAT GROUP	GROUP 1	GROUP 2	CONTROL
Water Intake (ml/day)			
Day 1	69 ± 8	59 ± 6	43 ± 2
Day 7	150 ± 11	150 ± 20	34 ± 1
Urine Output (ml/day)			
Day 1	47 ± 6	32 ± 6	4 ± 3
Day 7	97 ± 9	95 ± 19	4 ± 3
Weight Change (g)			
Days 1-7	-24 ± 5	-14 ± 4	+11 ± 6

Daily water intake and urine output (mean ± SEM) in diabetic rats during days 2-6 following streptozocin injection were intermediate to volumes observed on days 1 and 7.

blood glucose concentrations on the morning of the study (Table 19). The immediate in and out exchange in the group 1 rats yielded cloudy drain dialysate, which contained greater than 500 WBC/mm^3 and gave positive culture results. There was no evidence of peritonitis in the group 2 rats.

The drain and net ultrafiltration volumes after the two hour study exchanges were lower in the rats with peritonitis. Net ultrafiltration in group 1 averaged 1.4 ± 1.3 (SEM) ml compared with 3.3 ± 0.5 ml in group 2. Serial dialysate glucose and insulin concentrations in both groups of rats are depicted in Figure 37. Despite lower net ultrafiltration, dialysate glucose and insulin concentrations both decreased more rapidly in the rats with peritonitis. However, only the differences in dialysate glucose concentrations were statistically significant ($p < 0.05$) (Figure 37).

Glycaemic control was similar during the exchanges in both groups of rats even though serum insulin levels tended to be higher in group 1 (Table 19). This may be explained, at least in part, by the higher absorption of glucose from the dialysate in group 1 (Figure 37). Plasma C-peptide levels were low at the beginning and end of the exchanges in both groups, indicating that endogenous insulin secretion was minimal in spite of the presence of hyperglycaemia (Table 19).

Cumulative lymphatic absorption was higher during the exchanges in rats with peritonitis (7.8 ± 1.1 ml in group 1 and 5.9 ± 0.7 ml in group 2 ; $0.05 < p < 0.1$). Total net transcapillary ultrafiltration

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TABLE 19: SERUM INSULIN AND GLUCOSE AND PLASMA C-PEPTIDE
CONCENTRATIONS DURING 2 HOUR EXCHANGES IN DIABETIC
RATS WITH AND WITHOUT PERITONITIS

SERUM GLUCOSE (mg/dl)	GROUP 1	GROUP 2
0	323 ± 25	327 ± 27
20	322 ± 29	316 ± 33
60	212 ± 32	250 ± 44
90	174 ± 37	223 ± 51
120	163 ± 26	206 ± 56

SERUM INSULIN (µU/ML)	GROUP 1	GROUP 2
0	41 ± 12	26 ± 6
20	1808 ± 498	1048 ± 258
60	2936 ± 990	1944 ± 750
90	6384 ± 2717	4867 ± 1943
120	7720 ± 2630	7167 ± 868

PLASMA C-PEPTIDE (ng/ml)	GROUP 1	GROUP 2
0	0.21 ± 0.02	0.23 ± 0.02
120	0.22 ± 0.01	0.26 ± 0.01

All values are expressed as mean ± SEM.

Normal range of plasma C-peptide levels in rats is
0.8 - 4.0 ng/ml. Values in diabetic rats are < 0.5 ng/ml.
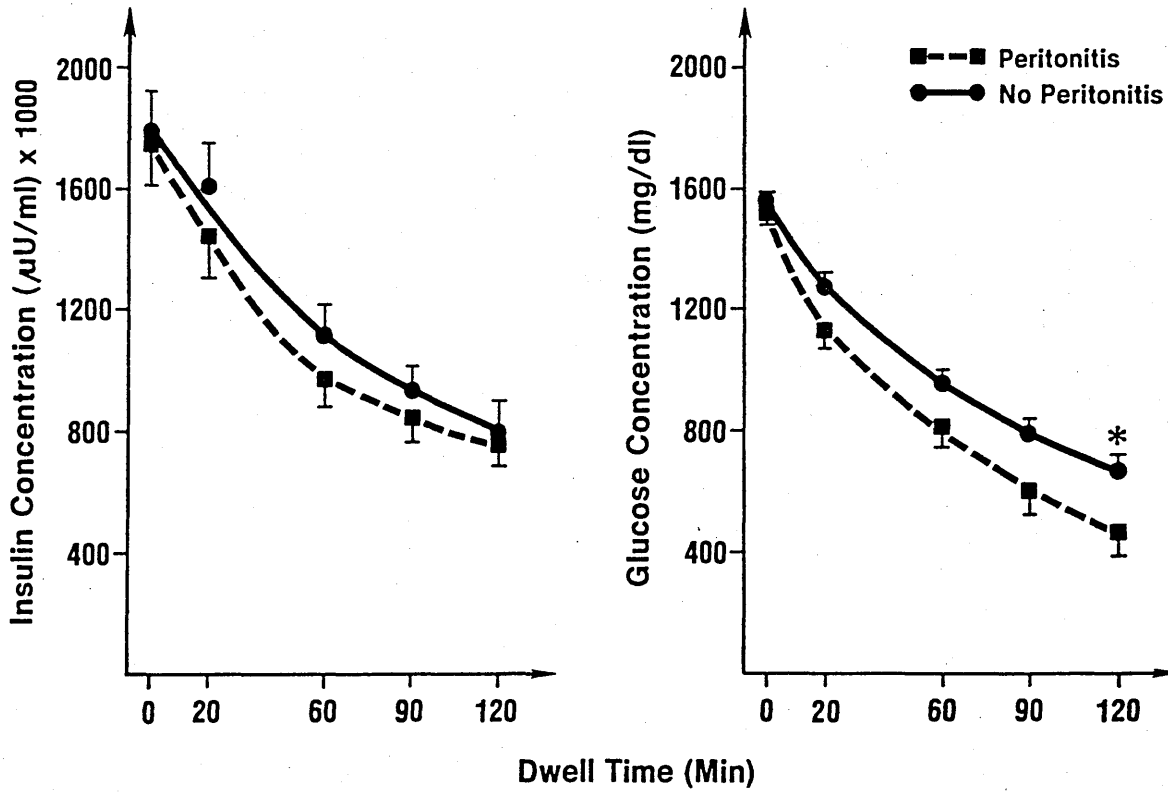
Figure 37: Dialysate insulin and glucose concentrations (mean ± SEM) during 2 hour exchanges using 15 ml, 2.5% dextrose dialysis solution in diabetic rats with and without peritonitis were compared by Student's t test.

* p < 0.05

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during the short exchanges was similar in both groups (9.5 ± 1.7 ml in group 1 and 9.4 ± 1.0 ml in group 2).

Fractional absorption rates (%) of glucose (M.W.=180), insulin (M.W.= 6,000) and albumin (M.W.= 68,000) from the dialysis solution during the exchanges are summarised in Table 20. Transperitoneal uptake of each solute was higher in the rats with peritonitis but only reached statistical significance for glucose (p < 0.05). Since solutes of molecular weight greater than 20,000 are only absorbed from the peritoneal cavity by convective transport, the percentage absorption of albumin in each group will also indicate the percentage of glucose and insulin absorbed by bulk flow. Thus, in the rats with peritonitis, 34% of the dialysate glucose, insulin and albumin were absorbed by convection via the lymphatics, whereas 51% of the dialysate glucose, 15% of insulin and none of the albumin were absorbed by diffusion (Table 20). In the rats without peritonitis, 26% of the dialysate glucose, insulin and albumin were absorbed by convective transport (Table 20).

8.4 DISCUSSION

In this study glucose and insulin were absorbed from the dialysis solution more rapidly during episodes of peritonitis in diabetic rats. However, the relative absorption rates of glucose and insulin and the hypoglycaemic response to absorbed insulin were not altered significantly by peritonitis. Thus, the same dosage of intraperitoneal insulin produced a comparable biological effect in

TABLE 20: PERCENTAGE ABSORPTION OF DIALYSATE GLUCOSE, INSULIN AND ALBUMIN DURING TWO HOUR EXCHANGES WITH 15 ML, 2.5% DEXTROSE DIALYSIS SOLUTION IN DIABETIC RATS

ABSORPTION (%)	GROUP 1	GROUP 2	*
Glucose	85 ± 8	47 ± 3	$p < 0.05$
Insulin	49 ± 5	44 ± 6	
Albumin	34 ± 4	26 ± 3	$p < 0.1$

* Mean ± SEM values were compared by Student's non-paired t test.

both groups. These data suggest that intraperitoneal insulin therapy should not be increased routinely during peritonitis in diabetic CAPD patients unless extra hypertonic dextrose exchanges are required to achieve adequate daily net ultrafiltration.

Diabetes mellitus and peritonitis were induced successfully in the animal model described in this study. Furthermore, endogenous release of pro-insulin from residual pancreatic β-cells was excluded by demonstrating very low plasma C-peptide concentrations at the beginning and end of the two hour exchanges (204). Thus, the rise in serum insulin (IRI) levels during the exchanges reflected transperitoneal absorption of insulin from the dialysis solution. Absorption kinetics of insulin from the peritoneal cavity cannot be evaluated quantitatively from peripheral blood insulin levels since 50% of portal venous insulin is removed during first pass through the liver (205). The increments in serum IRI were greater during the second half of the exchanges in both groups of rats, even though the transperitoneal insulin concentration gradient for diffusive uptake into the peritoneal vasculature had decreased (p < 0.001) (Table 19, Figure 37). This apparent delay in insulin absorption may be explained by a lag phase before insulin transported via the lymphatics reaches the systemic circulation (92,128,130,131).

Lymphatic absorption was higher during the exchanges in rats with peritonitis but the difference did not reach statistical significance in the small number of animals studied. These results indicate that the functional patency of the peritoneal cavity

lymphatics is well maintained during episodes of peritonitis in this animal model.

Previous studies in rats have shown that macromolecules (M.W.> 20,000) are absorbed from the peritoneal cavity almost exclusively by convective transport via the lymphatics (102,170). The fractional absorption rates of albumin and insulin in this study (Table 20) indicate that a significant proportion of peritoneal cavity to plasma transport of insulin is via convective transport. In rats with and without peritonitis, convection (via lymphatics) accounted for 69% and 59%, respectively of total insulin absorption from the dialysate. In a similar study in rats, absorption of inulin (M.W.= 5,200) from the dialysis solution was 81% translymphatic and only 19% by diffusion (170). These studies emphasise that lymphatic absorption is relatively more important in peritoneal cavity to plasma transport of molecules of increasing size and infer that diffusive uptake from the peritoneal cavity is through size discriminatory pores in the peritoneal capillaries and/or venules. (206)

The absorption of intraperitoneal insulin along with the obligatory dialysate glucose load is more physiological than subcutaneous insulin administration (207,208) and improves glycaemic control in diabetic CAPD patients (191,193,194). The addition of insulin to the infused dialysis solution avoids the need for subcutaneous injections and permits good patient compliance with administration of insulin four times per day. The intraperitoneal administration of insulin has no effect on solute clearances, net

ultrafiltration or dialysate glucose absorption in CAPD patients (209) and has not been found to increase the risk of CAPD associated peritonitis (10,191,193). For these reasons, intraperitoneal insulin therapy is the route of choice for insulin administration in diabetic CAPD patients.

This study suggests that insulin requirements are unaltered during peritonitis in diabetic CAPD patients provided the dialysate glucose load and carbohydrate intake are kept constant.

8.5 CONCLUSIONS

1. Glucose and insulin are absorbed from the dialysate more rapidly during episodes of peritonitis in diabetic rats.

2. Greater than 50% of the absorption of intraperitoneal insulin during peritoneal dialysis is via the peritoneal cavity lymphatics.

3. Peritonitis per se does not change intraperitoneal insulin requirements during peritoneal dialysis in diabetic rats.

Chapter 9: <u>NEOSTIGMINE REDUCES LYMPHATIC ABSORPTION IN PERITONEAL</u>

<u>DIALYSIS IN THE RAT</u>

9.1 <u>INTRODUCTION</u>

Pharmacological reduction of lymphatic absorption during the exchange time should augment net ultrafiltration and solute clearances in peritoneal dialysis. Most of the lymphatic drainage from the peritoneal cavity occurs by convective flow via the subdiaphragmatic stomata (Figure 3). Intraperitoneal administration of cholinergic drugs has been observed to induce fixed contraction of these stomata (100) and consequently may decrease lymphatic absorption of intraperitoneal fluid. Neostigmine, an anticholinesterase agent which is used therapeutically in myasthenia gravis, also ought to reduce the patency of the subdiaphragmatic stomata. This study was performed to evaluate if intraperitoneal neostigmine reduces lymphatic absorption and thus increases net ultrafiltration and solute clearances in peritoneal dialysis in rats.

9.2 <u>MATERIALS AND METHODS</u>

9.2.1 STUDY PROTOCOL

Two hour exchanges were performed in 19 ambulant, male Sprague-Dawley rats using 2.5% dextrose solution with and without added neostigmine. Albumin (25% human serum albumin) was added to 2.5%

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dextrose solution (Dianeal PD-2, Baxter-Travenol, Deerfield, Illinois) to give a 1.5% albumin solution. Under ether anaesthesia, 20 ml of the 2.5% dextrose solution containing 1.5% albumin was infused through a 22 gauge needle into the peritoneal cavity of each rat. In ten of the rats 0.0063 mg/ml of neostigmine methylsulphate (Prostigmin, Roche Laboratories, Nutley, New Jersey) was also mixed with the instilled dialysis solution. This administered dosage corresponds with therapeutic dosages in adult man (210). The animals were then permitted to recover from anaesthesia and were ambulant and allowed water and rat chow ad libitum during the two hour dwell time. Ten minutes before the end of the exchange a peritoneal catheter was inserted in the midline under ether anaesthesia (5.2.1) and the dialysis solution drained completely.

Pre-dialysis blood was drawn from the tail vein and post-dialysis blood was collected during sacrifice of the rats. The peritoneal cavity was opened to ensure complete drainage of the dialysate and the drain volume was measured in a graduated cylinder. Albumin, urea and creatinine concentrations and osmolality in the pre- and post-dialysis serum and dialysate samples were measured as described previously (6.2.3). Dialysate and serum phosphate were measured using the Autoanalyser II (Technicon Instruments Corporation, Tarrytown, New York).

CALCULATIONS

Cumulative lymphatic absorption and net transcapillary ultrafiltration were calculated as before (6.2.4). Measured net ultrafiltration equalled the drain volume minus the infusion volume. Solute clearances of urea, creatinine and phosphate during the single peritoneal dialysis exchanges were calculated as the solute mass transfer rate (equation 1) divided by the mean serum solute concentration. Dialysate/serum solute ratios were determined at the end of the two hour exchanges to compare transperitoneal solute transport with and without neostigmine added to the dialysis solution. Results in each group of rats were compared by Student's non-paired t test.

9.3 RESULTS

The dialysate drain volumes after the two hour dwell time were significantly higher in the neostigmine treated rats (23.7 ± 0.5 (SEM) ml) than in the control rats (21.5 ± 0.4 ml; $p < 0.01$). Thus, measured net ultrafiltration volumes were higher in the neostigmine treated group ($p < 0.01$) (Figure 38). Cumulative lymphatic absorption and net transcapillary ultrafiltration at the end of the two hour exchanges in each group of rats are depicted in Figure 39. Although total net transcapillary ultrafiltration did not differ between the two groups, cumulative lymphatic absorption was decreased significantly during the exchanges with intraperitoneal neostigmine
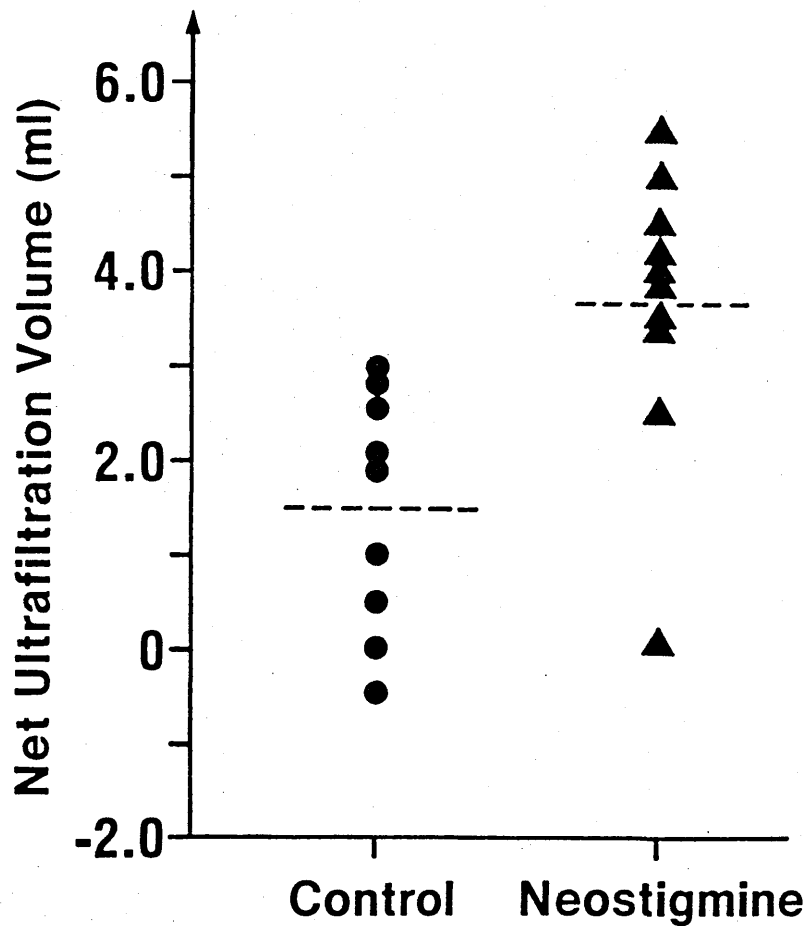
Figure 38:   Net ultrafiltration volumes after two hour exchanges in rats using 20 ml, 2.5% dextrose dialysis solution with and without added neostigmine. The mean of each group is indicated by the broken horizontal line.
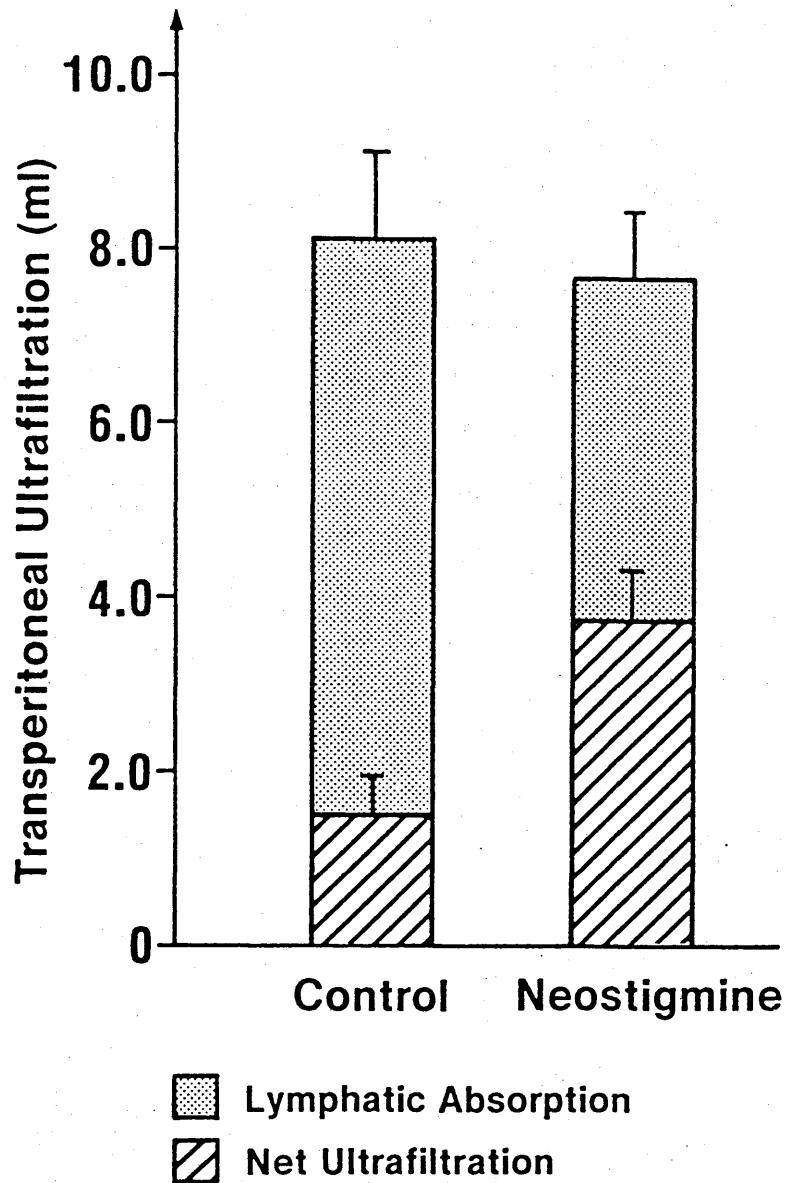
Figure 39: Cumulative net transcapillary ultrafiltration, lymphatic absorption and net ultrafiltration (mean ± SEM) after two hour exchanges in rats using 20 ml, 2.5% dextrose dialysis solution with and without added neostigmine.

(p < 0.01) (Table 21, Figure 39). Calculated net ultrafiltration (cumulative net transcapillary ultrafiltration minus lymphatic absorption) correlated with directly measured net ultrafiltration in both groups (r = 0.90).

Dialysate/serum ratios of urea, creatinine and phosphate after the two hour exchanges were not significantly different between the two groups of rats (Table 22). However, primarily due to the increased drain volume, solute clearances were significantly greater in the neostigmine treated rats (Table 22).

Pre- and post-dialysis body weight, haematocrit and serum and dialysate osmolality were similar in both groups of rats (Table 23).

9.4 DISCUSSION

This study indicates that intraperitoneal neostigmine increases measured net ultrafiltration during peritoneal dialysis in rats by reducing cumulative lymphatic absorption, and without increasing total net transcapillary ultrafiltration during the exchange (Figure 39). Solute clearances are enhanced by the resultant increase in the dialysate drain volume and not by a significant increase in transperitoneal solute transport. That is, both dialysate/serum solute ratios and total net transcapillary ultrafiltration were unaltered by intraperitoneal neostigmine administration (Tables 21 & 22). These data are consistent with the hypothesis that intraperitoneal neostigmine impairs diaphragmatic lymphatic absorption and thus can augment the efficiency of peritoneal dialysis

TABLE 21: INFLUENCE OF NEOSTIGMINE ON ULTRAFILTRATION KINETICS AFTER TWO HOUR EXCHANGES USING 20 ML, 2.5% DEXTROSE DIALYSIS SOLUTION

RAT GROUP	CONTROL	NEOSTIGMINE	*
Net Transcapillary UF (ml)	8.16 ± 0.66	7.74 ± 0.72	
Lymph Flow (ml)	5.58 ± 0.66	3.44 ± 0.51	p < 0.01
Calculated Net UF (ml)	2.58 ± 0.42	4.30 ± 0.48	p < 0.01
Measured Net UF (ml)	1.50 ± 0.44	3.70 ± 0.48	p < 0.01

* Mean ± SEM were compared by Student's t test.

TABLE 22: EFFECT OF NEOSTIGMINE ON SOLUTE TRANSPORT AFTER TWO HOUR EXCHANGES USING 20 ML, 2.5% DEXTROSE DIALYSIS SOLUTION

SOLUTE CLEARANCES (ml/min)	CONTROL	NEOSTIGMINE	*
Urea	0.14 ± 0.03	0.16 ± 0.04	$p < 0.01$
Creatinine	0.09 ± 0.02	0.12 ± 0.05	$p < 0.05$
Phosphate	0.07 ± 0.01	0.08 ± 0.01	$p < 0.05$

DIALYSATE/SERUM SOLUTE RATIOS	CONTROL	NEOSTIGMINE
Urea	0.78 ± 0.08	0.81 ± 0.10
Creatinine	0.50 ± 0.10	0.60 ± 0.20
Phosphate	0.42 ± 0.10	0.42 ± 0.10

* Mean ± SD of solute clearances and dialysate/serum solute ratios in each group of rats were compared by Student's t test.

TABLE 23: PRE- AND POST-DIALYSIS WEIGHT, HAEMATOCRIT AND SERUM AND DIALYSATE OSMOLALITY

	PRE-DIALYSIS		POST-DIALYSIS	
	CONTROL	NEOSTIGMINE	CONTROL	NEOSTIGMINE
Body Weight (g)	400 ± 47	407 ± 35	421 ± 49	441 ± 53
Haematocrit (%)	48 ± 1	48 ± 1	47 ± 1	49 ± 2
Serum Osmolality (mOsm/L)	303 ± 4	303 ± 7	315 ± 12	310 ± 11
Dialysate Osmolality (mOsm/L)	378 ± 8	382 ± 9	306 ± 11	311 ± 5

Mean ± SD values in each group of rats were not significantly different by Student's t test.

without increasing transperitoneal transport of water and solutes into the peritoneal cavity.

The calculated lymphatic flow rates of the control rats in this study (Table 21) are in agreement with directly measured peritoneal lymphatic absorption rates of homologous plasma (Table 1) and confirm that lymphatic drainage significantly reduces measured net ultrafiltration during peritoneal dialysis in the rat (5.5). The close correlation of calculated and directly measured net ultrafiltration volumes further supports the validity of the methods. Linear regression analysis showed that calculated net ultrafiltration = (0.9 x measured net ultrafiltration) + 1.0 ml. This difference was most likely due to unmeasurable residual volumes, since the presence of clinically evident intraperitoneal fluid was excluded by opening the peritoneal cavity after drainage via the peritoneal catheter. The intraperitoneal residual volumes after immediate in and out exchanges in the rat model of peritoneal dialysis average 1.0 ml (5.3.1). Thus the increase in net ultrafiltration in the neostigmine treated rats cannot be attributed to differences in the completeness of drainage of intraperitoneal fluid.

The rats were awake and ambulant during the study exchanges since this model more closely simulates clinical peritoneal dialysis. This is especially important in studies of lymphatic absorption from the peritoneal cavity since the lymphatic flow rate is elevated by a rise in intraperitoneal pressure (112) and reduced by anaesthesia and decreased diaphragmatic movement (113,114). The intraperitoneal pressure was assumed to be similar in both groups of rats since the

same volume of dialysis solution was infused in all rats and both groups of rats were of similar body weight (Table 23) (178). Furthermore, allowing the rats to recover from anaesthesia ensured that differences in the absorption of intraperitoneal fluid by the subdiaphragmic lymphatics were not caused by interindividual variation in the depth of anaesthesia.

The subdiaphragmatic stomata constrict following intraperitoneal carbachol administration and relax when succinylcholine is infused (100). These observations may be explained by the presence of abundant actin filaments in the mesothelial and endothelial cells surrounding the stomata (99,100,211). Neostigmine also ought to constrict the stomata but this has yet to be confirmed by electron microscopy studies. The clinical use of neostigmine is hindered by the risk of muscarinic side effects and the danger of inducing cholinergic crisis in normal patients (210).

Lymphatic drainage from the peritoneal cavity may be amenable to pharmacological manipulation by other drugs. The lymphatic trunks have both innervation by non-myelinated autonomic fibres and inherent contractility (212,213). Although denervation had no effect on spontaneous lymphatic contractility (213), inherent myogenicity was decreased in vitro by calcium antagonists (214). Whether vasoactive drugs in vivo significantly alter lymphatic as well as blood vessel smooth muscle tone and contractility remains unknown. Drugs are delivered to the lymphatic trunks by the vasa lymphorum as well as in lymph (164) and thus oral or parenteral medication may potentially alter lymphatic flow. Review of the lymphatic absorption rates in

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CAPD patients (Chapter 6), however, revealed no influence of concurrent oral vasodilator therapy on lymphatic flow.

Pharmacological reduction of peritoneal lymphatic absorption provides an alternative means of enhancing the efficiency of long-dwell peritoneal dialysis without increasing peritoneal transport of water and solutes into the peritoneal cavity. Investigation of other drugs which may decrease lymphatic drainage is merited.

9.5 CONCLUSIONS

1. Intraperitoneal administration of neostigmine increases measured net ultrafiltration and solute clearances in peritoneal dialysis in the rat.

2. Net ultrafiltration and solute clearances are enhanced in the neostigmine treated group by a reduction in peritoneal lymphatic absorption, and without an increase in transperitoneal transport of water and solutes into the peritoneal cavity.

Chapter 10. INFLUENCE OF PHOSPHATIDYLCHOLINE ON LYMPHATIC

ABSORPTION DURING PERITONEAL DIALYSIS IN THE RAT

10.1 INTRODUCTION

The layer of surface active phospholipids over serous membranes helps to provide mechanical lubrication for the underlying viscera and to repel water (215,216). Peritoneal physiology may be altered in CAPD by the constant removal of phosphatidylcholine and other phospholipids in the dialysate effluent (216). Phospholipid concentrations in dialysate effluent are significantly reduced after long-term CAPD and during episodes of peritonitis, and the poor peritoneal ultrafiltration capacity in such patients is greatly improved with intraperitoneal phosphatidylcholine administration (217). The observed increase in net ultrafiltration and solute clearances with phosphatidylcholine has been attributed to its surface tension lowering properties which result in a reduction in the thickness of the stagnant fluid films over the mesothelium and an increase in transperitoneal water and solute transport (217,218). Since glucose absorption from the dialysate did not increase during exchanges with phosphatidylcholine (217,218), this hypothesis assumes that the thinning of fluid films over the mesothelium only causes unidirectional enhancement of peritoneal transport from the peritoneal vasculature to the dialysis solution (218).

Alternatively, phosphatidylcholine may increase the efficiency of peritoneal dialysis by reducing the reabsorption of water and

solutes during the dwell time. Recent studies in rabbits suggest that phosphatidylcholine increases net ultrafiltration by reducing net fluid absorption late in the exchange (218). This study was performed to evaluate lymphatic absorption, ultrafiltration kinetics and solute transport during hypertonic exchanges with and without phosphatidylcholine in rats.

10.2 MATERIALS AND METHODS

10.2.1   STUDY PROTOCOL

Four hour exchanges with 20 ml of 4.25% dextrose dialysis solution were performed in four groups of ambulant, male Sprague-Dawley rats. The study dialysis solution in each group of six rats was as follows:

Group 1 = 4.25% dextrose + 1.5% albumin

Group 2 = 4.25% dextrose + 1.5% albumin + 50 mg/L phosphatidylcholine

Group 3 = 4.25% dextrose

Group 4 = 4.25% dextrose + 50mg/L phosphatidylcholine

This dosage of phosphatidylcholine (50 mg/L) has been shown to increase net ultrafiltration during peritoneal dialysis in man and rabbits (217,218). Groups 1 and 3 served as control groups for the phosphatidylcholine treated rats with and without albumin (25% human serum albumin) added to the dialysis solution. Exchanges in groups

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3 and 4 were performed without albumin since it is anionic at physiological pH and may interfere with the mechanism of action of phosphatidylcholine (218).

Following ether anaesthesia, the abdominal area of the animals was shaved and scrubbed with 10% povidone-iodine solution for five minutes. The study dialysis solution was then injected intraperitoneally using a 22 gauge needle. The rats were allowed to recover and permitted free access to water and rat chow throughout the dwell time. Ten minutes before the end of the four hour exchanges, the rats were again anaesthetised, a peritoneal catheter was inserted in the midline (5.2.1) and the dialysate drained completely.

Pre-dialysis blood was obtained from the tail vein and post-dialysis blood was collected during sacrifice of the rats. The peritoneal cavity was then opened to ensure complete drainage of dialysate, and the drain volume was measured in a graduated cylinder.

10.2.2   LABORATORY METHODS AND CALCULATIONS

The osmolality and glucose, albumin, urea and phosphate concentrations of the pre- and post-dialysis serum and dialysate samples were measured as described previously (5.2.4 and 9.2.1). Cumulative lymphatic absorption and net transcapillary ultrafiltration were calculated as before (5.2.5 and 6.2.4). Dialysate albumin concentrations at the end of the exchanges with 1.5% added albumin were corrected for albumin influx during the dwell

time by subtracting the mean dialysate albumin concentration of the corresponding rat group without albumin added to the dialysis solution. Net ultrafiltration was calculated as the drain volume minus the infusion volume. Solute clearances and dialysate/serum solute ratios were determined (9.2.2) to assess solute transport during the exchanges in each group of rats.

Results in groups 1 and 2 with albumin added to the dialysis solution and groups 3 and 4 without added albumin in the dialysate were compared by Student's non-paired t test.

10.3 RESULTS

The dialysate drain volumes were significantly higher during the exchanges with phosphatidylcholine. The drain volumes in group 1 averaged 24.2 ± 1.3 (SEM) ml compared with 30.8 ± 0.9 ml in group 2 (p < 0.005). In group 3 the drain volumes averaged 27.5 ± 1.2 ml compared with 32.8 ± 0.3 ml in group 4 (p < 0.005). Consequently net ultrafiltration volumes were higher during the exchanges with phosphatidylcholine (groups 2 and 4 in Figures 40 and 41, respectively).

Cumulative lymphatic absorption after the four hour dwell time was reduced during exchanges with phosphatidylcholine (8.0 ± 1.4 ml in group 2 and 14.3 ± 1.2 ml in group 1; p < 0.01). Cumulative net transcapillary ultrafiltration during the dwell time was calculated by adding measured net ultrafiltration and lymphatic absorption (5.2.5). The calculated net transcapillary ultrafiltration volumes
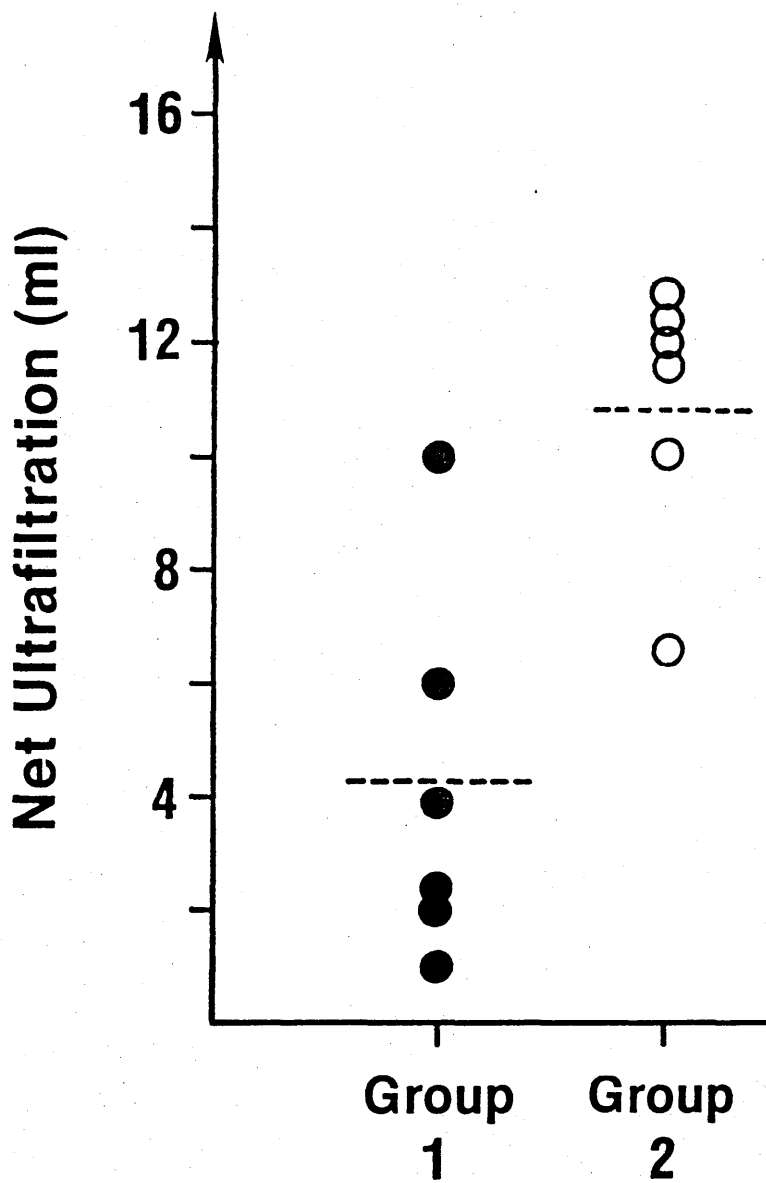
Figure 40:   Net ultrafiltration   volumes after four hour exchanges in rats using 20 ml of 1.5% albumin, 4.25% dextrose dialysis solution with (group 2) and without (group 1) added phosphatidylcholine. The mean of each group is indicated by the horizontal broken line.
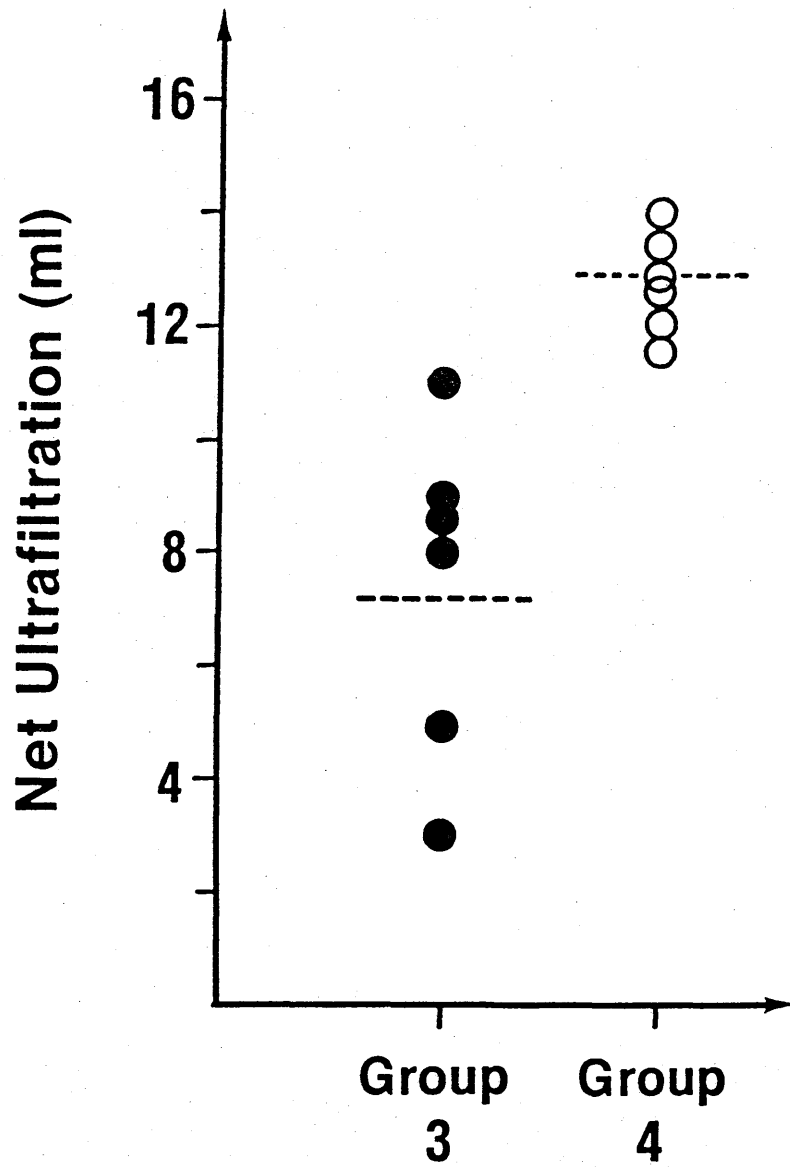
Figure 41: Net ultrafiltration volumes after four hour exchanges in rats using 20 ml, 4.25% dextrose dialysis solution with (group 4) and without (group 3) addition of phosphatidyl-choline.

in group 1 (18.4 ± 1.0 ml) were similar to group 2 (18.8 ± 1.2 ml). Estimation of cumulative net transcapillary ultrafiltration from the dilution of the initial dialysate albumin concentrations (6.2.4) showed similar results (20.0 ± 1.4 ml in group 1 and 17.5 ± 1.6 ml in group 2; N.S.). Thus the increase in net ultrafiltration during exchanges with phosphatidylcholine is due to a reduction in lymphatic absorption during the dwell time rather than enhanced net transcapillary ultrafiltration (Figure 42).

Peritoneal clearances of urea and phosphate were also increased during the exchanges with phosphatidylcholine (Table 24). Dialysate/serum solute ratios after the four hour dwell time were similar in all groups of rats (Table 24) and thus the augmented solute clearances in groups 2 and 4 are mainly due to the increased dialysate drain volume. Drain dialysate albumin concentrations did not differ significantly in groups 3 and 4 (0.11 ± 0.02 and 0.09 ± 0.01 g/dl, respectively), and further suggest that peritoneal permeability x area is unchanged by adding phosphatidylcholine to the dialysis solution.

Initial/drain dialysate glucose ratios were similar in each rat group (Table 24). Nevertheless, due to the higher drain volumes, glucose absorption from the dialysate was lower after the exchanges with phosphatidylcholine (p < 0.01). The addition of albumin to the study dialysis solution, however, significantly lowered the initial dialysate glucose concentrations in groups 1 and 2 compared with groups 3 and 4 (p < 0.05) (Table 25). Consequently ultrafiltration kinetics and solute clearances in the first two groups of rats were
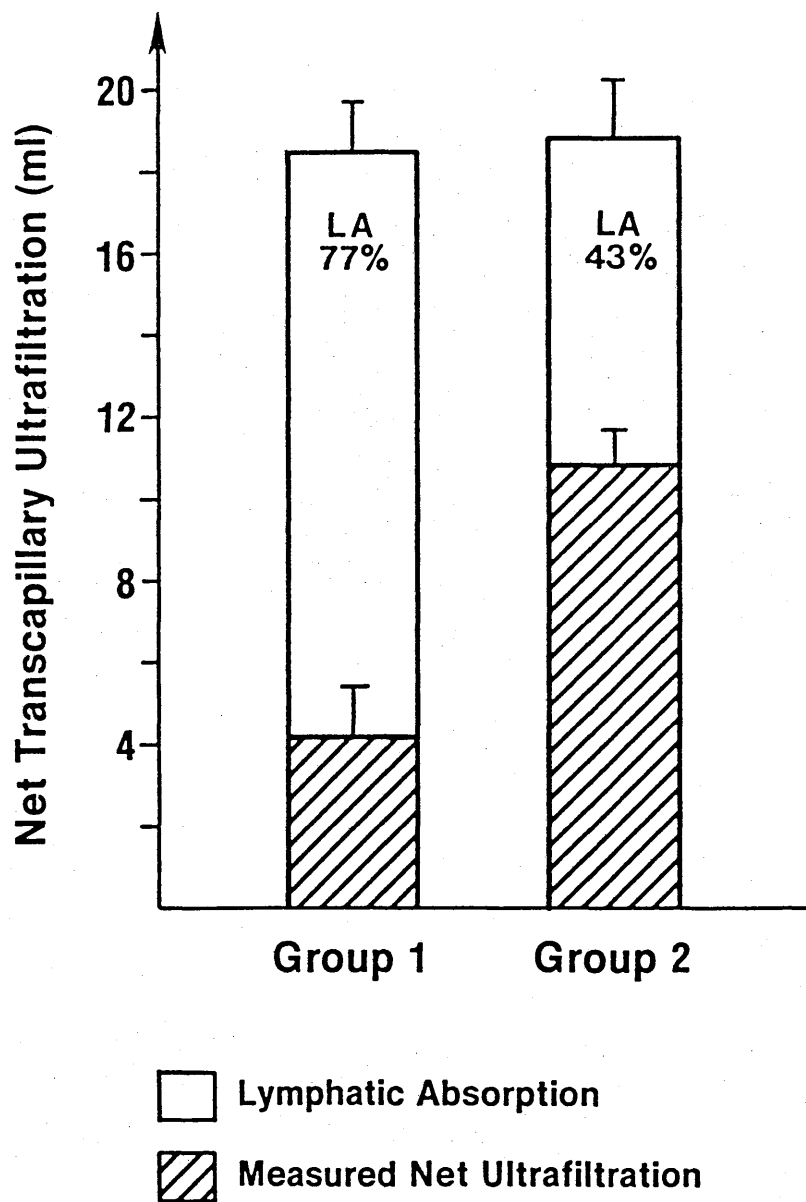
Figure 42:   Cumulative   net   transcapillary   ultrafiltration, lymphatic absorption and net ultrafiltration (mean ± SEM) after four hour exchanges in rats using 20 ml, 4.25% dextrose dialysis solution with and without added phosphatidylcholine.

TABLE 24: INFLUENCE OF PHOSPHATIDYLCHOLINE ON SOLUTE CLEARANCES AND DIALYSATE/SERUM SOLUTE RATIOS AFTER FOUR HOUR EXCHANGES USING 20 ML, 4.25% DEXTROSE DIALYSIS SOLUTION

RAT GROUP	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Urea Clearance (ml/min)	0.08 ± 0.01	0.10 ± 0.01**	0.10 ± 0.02	0.10 ± 0.01
Phosphate Clearance (ml/min)	0.05 ± 0.01	0.07 ± 0.01*	0.06 ± 0.01	0.07 ± 0.02*
Dialysate/Serum Urea Ratio (4 hrs)	0.88 ± 0.05	0.84 ± 0.05	0.82 ± 0.12	0.72 ± 0.09
Dialysate/Serum Phosphate Ratio (4 hrs)	0.54 ± 0.06	0.53 ± 0.04	0.50 ± 0.11	0.55 ± 0.14
Initial/Drain Dialysate Glucose Ratio	0.15 ± 0.03	0.17 ± 0.01	0.18 ± 0.03	0.19 ± 0.01

Mean $\pm$ SD values of groups 1 versus 2 and groups 3 versus 4 were compared by Student's t test ; * $p < 0.05$, ** $p < 0.01$.

TABLE 25: PRE- AND POST-DIALYSIS WEIGHT, HAEMATOCRIT AND SERUM AND DIALYSATE OSMOLALITY AND GLUCOSE CONCENTRATIONS

PRE-DIALYSIS DATA	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Weight (g)	470 ± 27	458 ± 22	443 ± 22	419 ± 23
Haematocrit (%)	47 ± 2	48 ± 2	47 ± 2	48 ± 2
Serum Osmolality (mOsm/L))	305 ± 5	297 ± 5	299 ± 8	304 ± 10
Dialysate Osmolality (mOsm/L)	457 ± 5	456 ± 8	462 ± 5	462 ± 5
* Dialysate Glucose (mg/dl)	4482 ± 272	4466 ± 258	4751 ± 100	4751 ± 100

POST-DIALYSIS DATA	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Weight (g)	464 ± 28	454 ± 20	437 ± 22	414 ± 22
Haematocrit (%)	48 ± 3	50 ± 1	50 ± 2	50 ± 2
Serum Osmolality (mOsm/L)	307 ± 9	302 ± 6	310 ± 11	307 ± 10
Dialysate Osmolality (mOsm/L)	288 ± 4	288 ± 4	282 ± 13	288 ± 8
Dialysate Glucose (mg/dl)	672 ± 152	751 ± 53	862 ± 150	902 ± 44

* The initial dialysate glucose concentrations (mean ± SD) were lower in groups 1 and 2 due to dilution with added albumin ($p < 0.05$).

not compared with groups 3 and 4. The drain dialysate was hyposmolar to post-dialysis serum in all of the rats (Table 25) and accords with prior observations during long-dwell exchanges in rats (Chapter 5). Body weight, haematocrit and serum osmolality were similar in each group of rats (Table 25).

10.4 DISCUSSION

This study confirms that the addition of phosphatidylcholine to the dialysis solution significantly increases net ultrafiltration and solute clearances in peritoneal dialysis (217,218). The enhanced efficiency of peritoneal dialysis with phosphatidylcholine has been postulated to result from a reduction in surface tension, a decrease in the thickness of stagnant fluid films over the mesothelium and a subsequent rise in transperitoneal transport of water and solutes into the peritoneal cavity (217). Chlorpromazine is presumed to increase net ultrafiltration and solute clearances by a similar mechanism (219).

The data reported herein, however, indicate that net ultrafiltration and drain volumes are increased during exchanges with added phosphatidylcholine without a concurrent rise in total net transcapillary ultrafiltration (Figure 42). Likewise, since drain dialysate/serum solute ratios were unchanged in exchanges with phosphatidylcholine, transperitoneal solute transport rates were not increased and the enhanced solute clearances were related primarily to the rise in dialysate drain volume. Dialysate/serum solute ratios

during exchanges with identical dialysis mechanics (dwell time, osmolality and volume of dialysis solution) are a useful index of peritoneal permeability x area (189). Thus, phosphatidylcholine increases net ultrafiltration and solute clearances during exchanges with the same dialysis mechanics without altering peritoneal permeability x area and without increasing transperitoneal transport of water and solutes from the peritoneal microcirculation.

Net ultrafiltration and solute clearances are augmented during exchanges with phosphatidylcholine due to a reduction in cumulative lymphatic absorption during the dwell time (Figure 42). This mechanism of action accords with earlier studies in rabbits which suggested that phosphatidylcholine reduced net fluid absorption late in the dwell time (218). Intraperitoneal volumes in the phosphatidylcholine treated and control rabbits were similar after one and two hour dwell times, yet the drain volumes in the phosphatidylcholine exchanges were greater than twice control values after five hours. The similar intraperitoneal volumes in the first two hours and the unchanged rates of glucose absorption throughout the five hour dwell time suggest that both net transcapillary ultrafiltration early in the exchanges and the transperitoneal osmotic gradient during the exchanges are unaltered by the addition of phosphatidylcholine in rabbits (218). Short-dwell peritoneal exchanges with and without phosphatidylcholine would help to confirm these observations.

The site of action of phosphatidylcholine remains presumptive. The high density anionic charge on the lymphatic endothelium and, to

a lesser extent, the microvilli and intercellular clefts of the subdiaphragmatic mesothelium may play a role in the resorption of intraperitoneal fluid into the interstitium or lymphatics (220). Positively charged choline may bind to and negate the surface anionic charge and thereby reduce the functional patency of the intercellular channels between mesothelial and lymphatic endothelial cells (221). This hypothesis is supported by the observation that the cationic dye, alcian blue, abolishes the effect of phosphatidylcholine _in vitro_ (218). In this study, however, net ultrafiltration and solute clearances were still increased with phosphatidylcholine when anionic albumin was also added to the infused dialysis solution. Alternatively, the surface active properties of phosphatidylcholine may form a water repellent layer which limits fluid contact with the resorptive peritoneal surface.

Phosphatidylcholine is a normal constituent of dialysate effluent and fluid in the serous cavities (215-217). The prominence of intracellular synthetic organelles in the mesothelium from CAPD patients may reflect increased production of phosphatidylcholine in an attempt to compensate for constant losses in the dialysate (16,217). Phospholipid levels in the drain dialysate are decreased after long-term CAPD, especially in patients with poor ultrafiltration capacity and during episodes of peritonitis (217). The addition of 50 mg/L of phosphatidylcholine to the infused dialysis solution significantly improved ultrafiltration in a small series of such patients despite the likelihood of co-existing alterations in the integrity, and perhaps surface anionic charge, of

the mesothelium (15-17). Further clinical studies are required to confirm these encouraging preliminary observations.

Reduction in peritoneal ultrafiltration capacity is not infrequent in CAPD patients (40-42) and has raised concerns regarding the long-term durability and viability of the peritoneum as a dialysing membrane when it is almost continuously exposed to non-physiological hypertonic dextrose dialysis solutions (222-224). Sustained net transcapillary ultrafiltration can be achieved with isosmolar glucose polymer dialysis solutions (225). However the clinical use of glucose polymer as an osmotic agent has so far been limited by its systemic absorption and subsequent accumulation of slowly metabolised and potentially toxic disaccharides.

Reduction of cumulative lymphatic absorption by phosphatidylcholine provides an alternative means of enhancing net ultrafiltration without increasing the osmolality of the dialysis solution. Phosphatidylcholine offers several potential advantages. Firstly, phosphatidylcholine is a normal component of peritoneal fluid and its addition to the dialysis solution may help restore and maintain normal peritoneal physiology. Secondly, by reducing intraperitoneal fluid absorption, phosphatidylcholine may decrease dialysate glucose absorption and allow a decrease in the osmolality of the dialysis solution. Thirdly, initial clinical studies indicate that phosphatidylcholine can greatly improve net ultrafiltration in patients with poor peritoneal ultrafiltration. Although injection of drugs into the infused dialysis solution may increase the risk of peritonitis, intraperitoneal administration of insulin in diabetic

CAPD patients has not been found to increase the incidence of peritonitis (10).

Phosphatidylcholine may have considerable clinical benefits in long-dwell peritoneal dialysis, and merits more extensive clinical evaluation.

10.5 CONCLUSIONS

1. Phosphatidylcholine increases net ultrafiltration and solute clearances after four hour exchanges in rats.

2. Phosphatidylcholine increases the efficiency of long-dwell peritoneal dialysis by reducing cumulative lymphatic absorption and without increasing transperitoneal transport of water or solutes into the peritoneal cavity.

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SCIENTIFIC PRESENTATIONS AND PUBLICATIONS

Parts of this work have been presented at scientific meetings and accepted for peer review publication during the past two years.

SCIENTIFIC PRESENTATIONS

1. Mactier RA, Nolph KD, Khanna R, Twardowski ZJ, Moore H, McGary T. Lymphatic absorption in peritoneal dialysis in the rat. Lymphology 1987; 20: 47.

   Presented at the North American Society of Lymphology, St. Louis, Oct. 1986.

2. Mactier RA, Khanna R, Twardowski Z, Nolph KD. Lymphatic absorption in CAPD. Kidney Int 1987; 31: 252.

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3. Mactier RA, Khanna R, Nolph KD, Twardowski Z, Moore H. Neostigmine increases ultrafiltration and solute clearances in peritoneal dialysis by reducing lymphatic drainage. Perit Dial Bull 1987; 7: S50.

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4. Mactier RA, Khanna R, Moore H, Twardowski Z, Nolph K. Phosphatidylcholine enhances the efficiency of peritoneal dialysis by reducing lymphatic reabsorption. Kidney Int (in press).

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1. Mactier RA, Khanna R, Twardowski Z, Nolph KD.   Role of
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2. Nolph KD, Mactier R, Khanna R, Twardowski ZJ, Moore H, McGary
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3. Mactier RA, Khanna R, Twardowski Z, Moore H, Nolph KD.
    Contribution   of   lymphatic   absorption   to   loss   of
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    J Clin Invest (in press).

4. Mactier RA, Khanna R, Twardowski Z, Nolph KD. Failure of
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