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Experimental models in peritoneal dialysis: A European experience

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Background. The development of adequate animal models is important for the *in vivo* study of selected aspects of peritoneal dialysis (PD) that cannot be evaluated by an *in vitro* model, such as peritoneal membrane transport, the influence of local defense mechanisms, and for testing new osmotic agents and their biocompatibilities.

Methods. Our experience with animal models for PD, including the acute Stockholm model in non-uremic rats, the acute and chronic Amsterdam model in non-uremic rats, and the chronic Gent model in uremic rats, is described.

Results. The Stockholm model proved to be useful in understanding the normal physiology of peritoneal transport, and for testing new dialysis solutions and their biocompatibilities. It is a rather simple and inexpensive model, and thus is suitable for screening new solutions and additives. The Amsterdam model permits the study of chemokines and mesothelial cell regeneration *in vivo*, and is applied in a model of chronic peritonitis. The results of the Gent model suggest that chronic peritoneal dialysis in uremic rats is feasible for at least eight weeks. This model is, however, very laborious, time consuming, and expensive.

Conclusion. Further improvement of the technique and increase of the dialysis dose should result in a better and more realistic model for peritoneal dialysis. It is hoped that in the future these models will be useful to test the effects of long-term intraperitoneal application of different dialysis solutions and additives in uremic animals.

Experimental *in vivo* models have been used for many years to study different aspects of peritoneal dialysis. Although experimental lavage in dogs was performed as early as the late 19th century [1], and the effects on intraperitoneal volume of intraperitoneal solutions with different osmolalities were already accurately described in 1894 [2], it was Putnam in 1923 who first characterized the peritoneum as a dialyzing membrane (in the dog) [3]. His work presented convincing evidence that the peritoneal membrane was permeable in two directions, and therefore he established the principles of solute transport and ultrafiltration that are still true today.

In parallel with the development of the clinical CAPD programs in the 1980s, the development of a growing interest in the characteristics of peritoneal transport and permeability stimulated the study of peritoneal physiology in animals.

Studies on the peritoneal circulation [4], peritoneal-plasma transport [5, 6], the kinetics of ultrafiltration, lymphatic absorption [7–9], and the influence of infectious peritonitis on the peritoneal morphology and transport properties [10], have greatly increased our understanding of the peritoneal membrane as a dialyzing organ. Peritoneal pharmacokinetics and the pharmacologic alterations of the peritoneal transport have also been carefully studied in short-term dwells in unanesthetized rabbits [11].

After almost two decades of clinical experience, a number of major concerns remain among the peritoneal dialysis community:

(1.) The viability of the peritoneal membrane during long-term peritoneal dialysis and the ability of this membrane to provide adequate fluid and solute removal in anuric patients [12, 13]. It has now been recognized that conventional CAPD treatment may not provide adequate dialysis in patients without residual renal function [14]. Knowledge of peritoneal fluid and solute transport is therefore fundamental in order to improve peritoneal dialysis efficiency. In Europe, two Swedish groups have extensively used a rat peritoneal transport model to study various transport characteristics of peritoneal dialysis [15–20].

(2.) Despite the advances in peritoneal dialysis technology, bacterial peritonitis remains the most frequent complication of this form of therapy. In preventing peritonitis the local

Key words: Stockholm model, Amsterdam model, Gent model, peritoneal membrane transport, infection, host defense mechanisms, osmotic agents, biocompatibility.

peritoneal defence mechanisms play a key role in the host's resistance to bacterial infection. Increasing our understanding of these mechanisms is essential if we are to reduce the negative consequences of peritoneal inflammation.

(3.) It is now well accepted that the dialysis solutions currently used in clinical practice are not biocompatible [21]. The development of new and more biocompatible solutions has been a focus of research in the peritoneal dialysis field during the past two decades. An ideal solution should have no systemic side effects, should not impair peritoneal host defense, and should preserve long-term peritoneal membrane integrity [22–26].

The majority of biocompatibility studies have used an *in vitro* approach, and although this research has yielded important functional information on the mechanisms of dialysis fluid modulation of cell function, its relevance to the *in vivo* situation is questionable. In particular, *in vitro* systems do not provide information on long-term changes to the peritoneal membrane (that occur over years in humans) nor on peritoneal transport characteristics following exposure to a new dialysis solution. Only with longitudinal clinical studies and relevant *in vivo* models for pre-clinical evaluation of new dialysis solutions will we be able to address this issue [27]. Theoretically, animal models could be used to study many aspects related to peritoneal dialysis, including peritoneal membrane transport physiology, new osmotic agents' solution biocompatibility, the effects of pharmacological manipulation and studies of peritoneal local defense mechanisms against infection.

At present research in this field has been hindered by the lack of suitable animal models for chronic peritoneal dialysis. Most studies using animal models have performed experiments that have lasted only a few hours and have injected the fluids directly into the abdominal cavity rather than delivered them via an implanted catheter. Only rarely has peritoneal dialysis been applied to uremic animals. Only by comparing chronic peritoneal dialysis in uremic animals with non-uremic animals can the effects of uremia *per se* on structural and functional changes of the peritoneal membrane be better delineated from the effects of dialysis itself.

EXPERIMENTAL ANIMAL MODELS USED IN PERITONEAL DIALYSIS

The majority of investigators have used rat or rabbit models of PD. Both animals have their advantages and disadvantages. Rats are easy to maintain, economical, and models of stable chronic renal failure in the rat have been extensively described. On the negative side, rats are small and have a relatively large peritoneal surface area, making infused dialysate difficult to recover, which results in small quantities of the effluent being available for analysis. Moreover, important mesothelial changes may occur after intraperitoneal implantation of biomaterials like silicone or Dacron into the peritoneal cavity [28].

Recently, an experimental model of chronic peritoneal dialysis in normal rats has been described [29]. Peritoneal dialysis was performed in 10 non-uremic rats, dialyzed twice daily for four weeks with a 3.86% glucose-containing fluid. Several effects on the peritoneal membrane were described including: (*a*) healing of the peritoneum after catheter implantation; (*b*) decreased cell count in the drained dialysate; (*c*) decreased permeability of the peritoneum to glucose and total protein; (*e*) increased volume of drained dialysate; (*f*) damage to the peritoneal membrane due to its exposure to peritoneal dialysis solution with concomitant increases in hyaluronic acid levels in dialysate; and (*g*) tendency of the peritoneum to thicken when compared to non-dialyzed animals. To our knowledge, there is only one study describing the effects of "long-term" peritoneal dialysis in uremic rats [35]. Uremia was induced by controlled resection of renal tissue. Animals were dialyzed three weeks with one exchange per day. Stable blood values of creatinine and urea were observed.

Several groups of investigators have used domestic rabbits as a model system. These animals can tolerate a peritoneal catheter for quite long periods of time, and they are rarely subject to peritonitis. Experimental models of peritoneal dialysis in uremic rabbits had already been established in the early 1980s [30], and more recently the effect of the intraperitoneal infusion of dextrose and amino acids on the appetite of both normal and uremic rabbits have been studied [31, 32]. Uremia was induced by subtotal nephrectomy, resulting in blood urea levels between 0.86 and 1.99 g/liter and serum creatinine levels of 2.47 and 8.78 mg%. Only single dwell studies were performed in these uremic animals.

Recently, a non-uremic rabbit model with a PD catheter *in situ* has been used to characterize the peritoneal transport parameters by standard peritoneal permeability analysis [33]. A daily dwell of one hour with a 3.86% glucose solution was used over a four week period. After correction of the solute transport parameters to body surface area, similar values to humans were obtained. In a more recent study [34], the same model was used to investigate the influence of amphotericin B and $HgCl_2$ on peritoneal transport in rabbits.

In the present article, we summarize the efforts of three European research groups to establish rat models for the study of several different aspects of peritoneal dialysis physiology and pathology. The first part describes the experience of the Stockholm group with a model of peritoneal dialysis in non-uremic rats. Short-term peritoneal exchanges were applied to the study of the physiology of small solute and water transport, the effects of non-glucose osmotic agents, the biocompatibility of dialysis solutions, and the effects of addition of pharmacological substances. The second part summarises studies performed in an acute and chronic rat model by the Amsterdam group on the

pathophysiology of peritonitis and of host defense mechanisms in peritoneal dialysis. The third part describes the studies of the Gent group in developing a model for chronic peritoneal dialysis over a prolonged period (8 weeks) in uremic rats. Detailed studies were performed on food and fluid intake of the animals, the daily diuresis, and peritoneal and residual renal fluid and solute removal.

THE ACUTE RAT MODEL TO STUDY PERITONEAL TRANSPORT

The model currently used in Stockholm is a recent modification of a rat peritoneal transport model that was established in the early 1990s [19, 20, 36]. In general, a four hour dwell with frequent dialysate and blood sampling is performed in anesthetized male Sprague-Dawley rats. Intraperitoneal dialysate volume is estimated from the dilution of intraperitoneal ^{131}I-human serum albumin (RISA), with corrections made for the elimination of RISA from the peritoneal cavity and the sample volumes [37]. The peritoneal fluid absorption rate is estimated as the coefficient of RISA elimination from the peritoneal cavity, K_E (ml/min), and the transcapillary ultrafiltration rate is calculated as net volume change plus K_E. The direct lymphatic absorption of fluid from the peritoneal cavity is assessed as the clearance of RISA from the dialysate to the blood, K_{EB} (ml/min). K_{EB} is calculated from the rate of increase of RISA concentration in plasma divided by the average intraperitoneal RISA concentration [38]. The plasma volume is set at 3.6 ml/100 g body wt [38, 39]. The peritoneal solute transport characteristics are evaluated using the dialysate over plasma aqueous concentration ratios (D/P), of the solutes under investigation [40], clearances and the diffusive mass transport coefficients (K_{BD}, ml/min) [41, 42].

The model describes the net change of the solute concentration in peritoneal dialysate over time, which is equal to the rate of solute flow between blood and dialysate due to combined diffusion, convective transport, and peritoneal absorption of the solute. The D/D_0 for glucose [calculated as the dialysate glucose concentration (D) divided by the glucose concentration in the fresh dialysis solution (D_0)] and K_{BD} for glucose are used to evaluate the peritoneal transport of glucose.

In recent years, this transport model has been used to study: (*a*) different osmotic agents and buffers, such as glucose, polyglucose, oligopeptides, albumin and bicarbonate; (*b*) different additives, such as hyaluronan, docusate sodium, atrial natriuretic peptide (ANP), nitroprusside, and amphotericin B; (*c*) different dialysis regimens, such as high dialysate fill volume with 1.36% and 3.86% glucose solutions; and (*d*) biocompatibility of the currently used peritoneal dialysis solutions.

In this article, the effects of different osmotic agents and of selected additives on peritoneal transport kinetics and the potential use of the model for the study of biocompatibility aspects will be discussed.

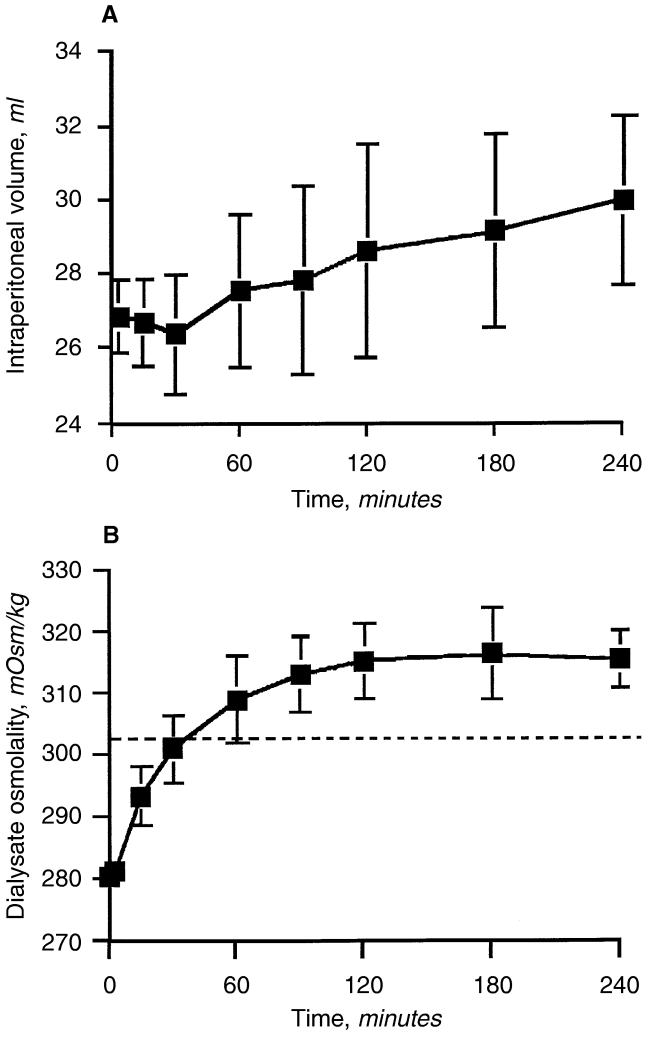

Fig. 1. Intraperitoneal volume (*A*) and dialysate osmolality (*B*) measured immediately after the samples were taken versus dwell time with 7.5% polyglucose solution versus dwell time ($N = 11$) (mean ± SD). The intraperitoneal volume was initially decreased and then started to increase after one hour of the dwell, which was strongly associated with an osmolality gradient between dialysate and plasma. Dashed line represents the plasma osmolality during the dwell.

Different osmotic agents: Polyglucose solution, oligopeptides solution

Polyglucose solutions can remove a significant quantity of fluid during long duration dwells despite the low osmolality of the solution (280 mOsm/kg; Fig. 1). During short time dwells, however, there is net fluid absorption. Peritoneal fluid removal is strongly related to the magnitude of increase in dialysate osmolality in the peritoneal cavity. Only when the dialysate osmolality increases to levels higher than that of plasma does the intraperitoneal volume starts to increase. In a parallel *ex vivo* study, there was a marked increase in the osmolality of polyglucose dialysate after 24 hours of storage of the samples *in vitro*, which suggested that this increase was partially due to enzyme

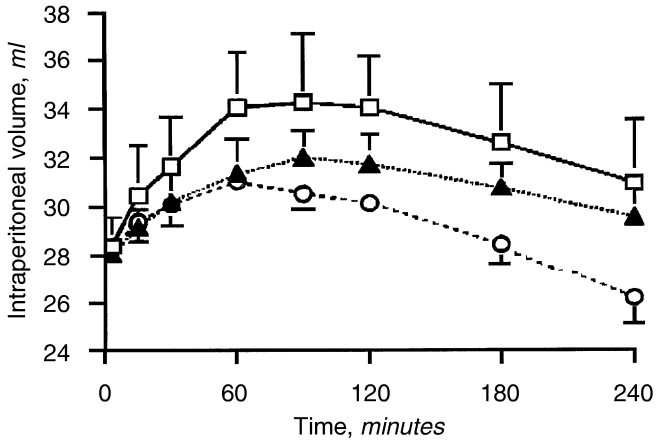

Fig. 2. Intraperitoneal volume versus dwell time for 3.86% glucose dialysate (□, $N = 8$), 2.27% glucose dialysate (○, $N = 8$) and 4% oligopeptides solution (▲, $N = 8$). The intraperitoneal volume at the end of the dwell was significantly higher in the 3.86% glucose dialysate and 4% oligopeptide solution groups as compared to the 2.27% glucose dialysate group (both $P < 0.01$), whereas no significant difference was found between the former two groups.

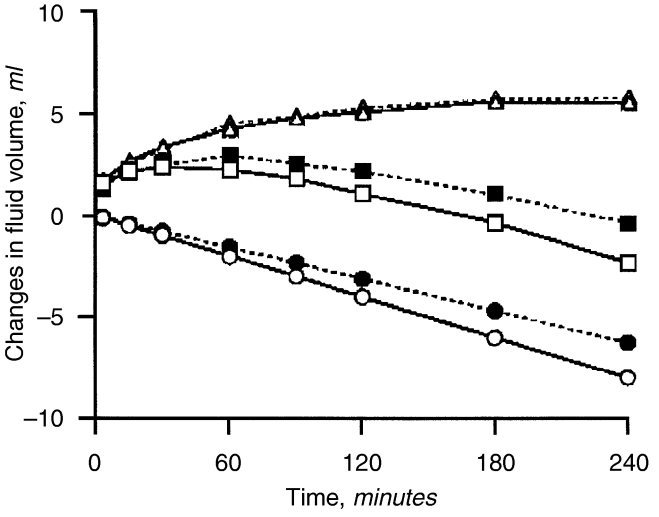

Fig. 3. Changes in fluid volume versus dwell time for 1.36% glucose solution (□, ○ and △; $N = 6$) and 1.36% glucose solution with 0.01% hyaluronan solution (■, ● and ▲; $N = 6$). Open and solid squares represent the net ultrafiltation volume; open and solid circles are the peritoneal total fluid absorption; Open and solid triangles are the transcapillary ultrafiltration volume. The net ultrafiltration was significantly higher in the hyaluronan group as compared to the control group ($P < 0.05$, ANOVA, repeated measurements), which was due to a significantly lower peritoneal fluid absorption. Data are from Wang et al [83]; used with permission.

degradation of the glucose polymer [43]. These results imply that the current combination of concentration and molecular weight of glucose polymer in the polyglucose solution may not be ideal for peritoneal dialysis, especially for shorter dwell times.

It is well known that for high molecular weight solutes the increased osmotic efficiency per molecule is outweighed by the disadvantages associated with the increases in molecular mass required to produce a high osmotic efficiency. In fact, it has been suggested that there is an optimal molecular weight of an osmotic agent (for short and medium term dwell), balancing the benefits of increasing molecular size (and the increased osmotic efficiency associated with this size increment), and the disadvantage of the increases in solute mass per molecule, that is, the increasing concentration in g/liter. According to theoretical calculations this seems to occur at a molecular weight of around 1000 daltons, which is much lower than the average molecular weight of the current formulation of glucose polymer (16800 daltons) [44]. In support of this theoretical calculation, a preliminary study in our rat model has found that a 4% oligopeptide solution (average molecular weight 700 daltons) produces a higher net ultrafiltration than a 2.27% glucose solution and only marginally lower than a 3.86% glucose solution (Fig. 2).

It is perhaps not surprising that several recent studies suggest that polyglucose solutions may result in better ultrafiltration (UF) during peritonitis and in patients with high peritoneal transport rates [45, 46]. The transport rate of amylase (an enzyme capable of degrading glucose polymer) may be considerably higher in patients with high peritoneal transport rates, and the dialysate amylase levels in the dialysate during peritonitis are significantly higher than control effluents [47, 48]. In the present rat model peritonitis does result in increased degradation of glucose polymer, increased dialysate osmolality and better UF [49]. Addition of nitroprusside to polyglucose solutions resulted in an increased peritoneal degradation of glucose polymer [50]. Although there may be some species difference in the peritoneal fluid and solute transport [43], these studies support the previous clinical observations. Further studies are needed, however, to confirm if polyglucose degradation plays a significant role in its ability to alter ultrafiltration *in vivo*.

The study of different additives to the dialysate

Improving the efficiency of the peritoneum as a dialyzing organ is one of the major goals in improving the therapy. Several pharmacological substances have been tested experimentally and clinically [11]. None of them, however, have been accepted into clinical practice. It is known that peritoneal fluid absorption substantially decreases the efficiency of peritoneal dialysis. In CAPD patients peritoneal fluid absorption reduced potential net ultrafiltration by 83.2 ± 10.2%, urea clearance by 16.9 ± 1.9% and creatinine clearance by 16.5 ± 1.9% in one six hour exchange [51]. The contribution of peritoneal fluid absorption to low dialysis efficiency is more significant in high transporters when a 1.36% glucose solution is used [52, 53]. Therefore, reducing the peritoneal fluid absorption should be a effective way to improve PD adequacy (with regards to the

Table 1. Peritoneal fluid and small solute transport parameters after ten days exposure to 1.36% glucose dialysis solution (LGS group) or to 3.86% glucose dialysis solution (HGS group)

	N	Net UF ml	K_E	K_{EB}	$K_{BDglucose}$	K_{BDurea}
			ml/min			
Control	8	3.8 ± 2.1	0.054 ± 0.010	0.008 ± 0.001	0.32 ± 0.006	0.28 ± 0.12
LGS	8	1.3 ± 4.9	0.058 ± 0.024	0.020 ± 0.004[a]	0.43 ± 0.08[a]	0.31 ± 0.10
HGS	8	0.0 ± 1.9[a]	0.072 ± 0.008[b]	0.021 ± 0.004[a]	0.47 ± 0.06[a]	0.51 ± 0.14[b]

Data are mean ± SD. Abbreviations are: net UF, net ultrafiltration volume at 4 hour of the dwell; K_E, total RISA elimination rate representing the fluid absorption rate from the peritoneal cavity; K_{EB}, RISA elimination rate to the blood from the peritoneal cavity representing the peritoneal lymphatic absorption; $K_{BDglucose}$, diffusive mass transfer coefficient for glucose estimated by using modified Babb-Randerson-Farrell model and setting the sieving coefficient equal to 0.55; K_{Bdurea}, diffusive mass transfer coefficient for urea estimated in the same way as for glucose.

[a] $P < 0.05$ compared with the control group

[b] $P < 0.05$ compared with the other two groups

removal of both small solutes and fluid), especially when high dialysate fill volumes are used.

Using the rat model, we have observed that peritoneal absorption can be significantly reduced and peritoneal small solute clearance increased by adding 0.01% hyaluronan, a long polysaccharide chain made up of repeating disaccharide units of N-acetylglucosamine and glucuronic acid to peritoneal dialysis solutions (Fig. 3) [54]. The effect of hyaluronan is potentially due to the accumulation of a restrictive filter "cake" of hyaluronan chains at the tissue-cavity interface [54]. Hyaluronan plays an important role in tissue hydraulic conductivity and has been shown to exhibit a high resistance to water flow. It can thus act in tissue as a barrier against rapid changes in tissue water content [55]. These promising results now need to be confirmed in clinical studies.

Atrial natriuretic peptide (ANP), a hormone with well known diuretic and vasodilating properties, has recently been reported to increase peritoneal fluid formation and increase peritoneal solute clearance. The addition of ANP to peritoneal dialysis solutions significantly increases peritoneal fluid removal in the rat by decreasing the peritoneal fluid absorption rate (by 51%). It also decreases the direct lymphatic absorption by 43% [56]. Its mechanism of action is still, however, not clear.

The effects of dioctyl sodium sulphosuccinate (DSS), amphotericin B, and nitroprusside have also been investigated in this rat model. DSS is a surfactant and has been shown to increase peritoneal small solute clearance. DSS, similar to another surfactant, phosphatidylcholine was found to increase peritoneal fluid and small solute removal whereas the peritoneal solute transport rate did not change [57]. Intraperitoneal use of amphotericin B has been reported to increase ultrafiltration during short peritoneal dwell in rabbits [58]. However, in the rat model, amphotericin B did not increase peritoneal fluid removal after four hours of dwell. Higher D/P and K_{BD} values as well as higher clearances for potassium were observed, suggesting a possible local release of potassium due to a cytoxic effect of amphotericin B. It was therefore concluded that amphotericin B would not be useful for the improvement of peritoneal dialysis efficiency [59].

Biocompatibility of peritoneal dialysis solutions

The bioincompatibility of dialysis solutions has been suggested as one of the reasons for the changes in the structure and function of the peritoneum during long-term peritoneal dialysis [60]. There is still, however, little direct evidence that more biocompatible dialysis solutions would preserve the function of the peritoneum.

Recently, the biocompatibility of the dialysis solution has been explored using a rat model [19]. Ten days exposure to 3.86% glucose dialysis fluid reduced the peritoneal fluid removal, which was partially due to the fluid absorption rate being increased, whereas the small solute transport rate was increased (Table 1). Net fluid removal and small solute transport rate (except glucose) did not change significantly following exposure to 1.36% glucose solution. The peritoneal lymphatic absorption rate and glucose absorption rate, however, were significantly increased in both solutions, suggesting a possible physiological adaptation to the infused glucose containing dialysate. It should be noted that the diffusive mass transfer coefficient for glucose ($K_{BD\ glucose}$) was equal to or even higher than the mass transfer coefficient for urea, which is in contrast with results obtained in patients, as such this may be a species-specific phenomenon [36, 37].

This study suggests that daily exposure to currently used glucose-based peritoneal dialysis solutions may result in functional changes to the peritoneum. The decreased fluid removal in the 3.86% glucose solution group, due to increased fluid reabsorption, results in decreased clearances for small solutes as well as increased glucose absorption, and represents changes that in the human may prevent the peritoneum from long-term use for peritoneal dialysis. These results indicate that functional studies of peritoneal transport characteristics after daily infusion of dialysis fluid may be a useful model to assess the *in vivo* effects of peritoneal dialysis solutions.

In summary, this acute peritoneal transport model in the

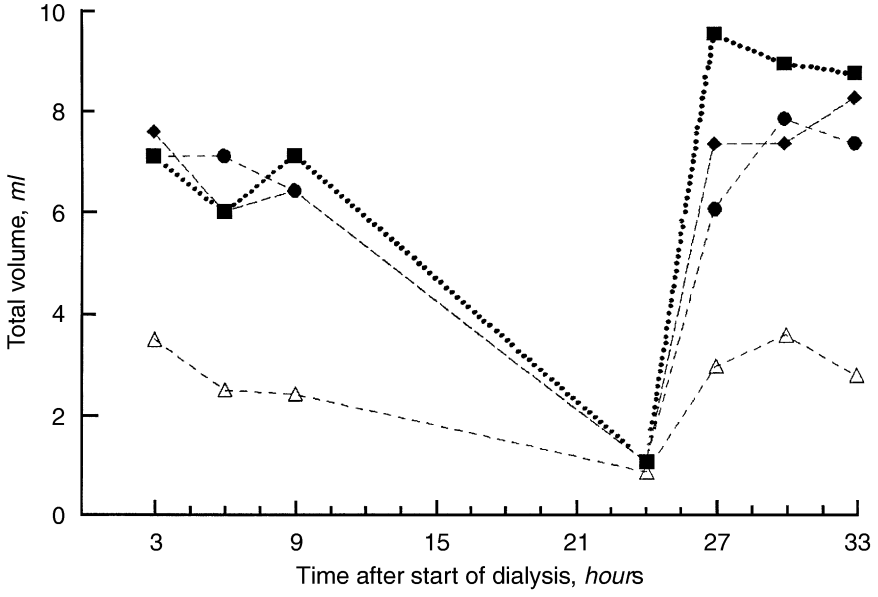

Fig. 4. Total volume of peritoneal fluid collected at various intervals after the start of dialysis, with physiological saline (△), fluid with 1.36% glucose (○), fluid with 2.27% glucose (♦) or fluid with 3.86% glucose (■).

normal rat could help us understand the physiology of peritoneal transport. It can be used to study various aspects of peritoneal dialysis, including new dialysis solutions with different osmotic agents and additives, and may prove useful in testing the *in vivo* effect of the dialysis solutions.

A RAT MODEL FOR THE STUDY OF PERITONEAL DEFENSE MECHANISMS

The macrophage is the predominant cell type in the peritoneal cavity of healthy animals as well as in patients undergoing peritoneal dialysis, and is thought to play a key role in the local host defense against infection. Phagocytosis and killing of microorganisms by peritoneal cells seem to be essential in an early stage of contamination. Studies of macrophages in CAPD patients suggested that a chronic sterile inflammation exists in the peritoneal cavity. Characterization of the immuno-phenotypes and immuno-effectors of the peritoneal cavity have confirmed these observations [61–63]. Analysis of the peritoneal cellular immune system shortly before a clinical peritonitis showed a decreased phagocytic capacity by peritoneal macrophages [64].

The continuous exposure of the peritoneal cavity to commercial dialysis fluid itself might reduce the local antibacterial defence mechanisms. A rat model was therefore developed to investigate the influence of PD fluids on the function of peritoneal cells and the structure of the peritoneal membrane. Previous studies revealed that a single intraperitoneal (i.p.) administration of peritoneal dialysis fluid induced an acute exudate, compared to that observed after saline infusion [65]. A rapid influx of neutrophilic granulocytes and exudate macrophages occurred into the peritoneal cavity. These findings strongly suggested that frequent i.p. injections of PD fluid might lead to a chronic inflammation, comparable with the situation present in CAPD patients.

An experimental infection model in the rat was established in which the effect of inoculum and volume on bacterial killing was investigated [66]. A suspension of 0.5 ml, containing 3×10^8 *Staphylococcus aureus* (ATCC 25923) was injected into the peritoneal cavity of the rat. These organisms were cleared in a time-dependent fashion over a three to five day period.

In the PD setting, short dwell times reduced the peritoneal defence mechanisms, including bacterial killing [67]. The effect of prior injection of PD fluid on bacterial clearing was then studied in an animal infection model. The data clearly showed that prior injection of PD fluid compromised peritoneal antibacterial defence. It was found that the glucose in PD fluid was responsible for the impairment of antibacterial defence [68, 69].

A major drawback of this infection model was that only the effect of a single administration of PD fluid was explored. The main aim of later studies was to develop a peritoneal dialysis model in the rat. Initially, an experimental model was established lasting 33 hours, with exchanges of fluids every three hours during the day and an overnight dwell of 15 hours. After the first three hour exchange, bacteria were administered in the infected animal group. In this approach, a silicone tube of ±15 cm length was placed and secured in the peritoneal cavity and subcutaneously tunneled to the neck region. The end of the canula was exposed through an incision in the skin and plugged. As described in other comparable models, prophylactic antibiotics were administered via a feeding tube. After the animals had recovered, the exchange of fluids (Dianeal, glucose 1.36 to 3.86% solutions, or physiological saline, respectively) was performed by administration of 10 ml of

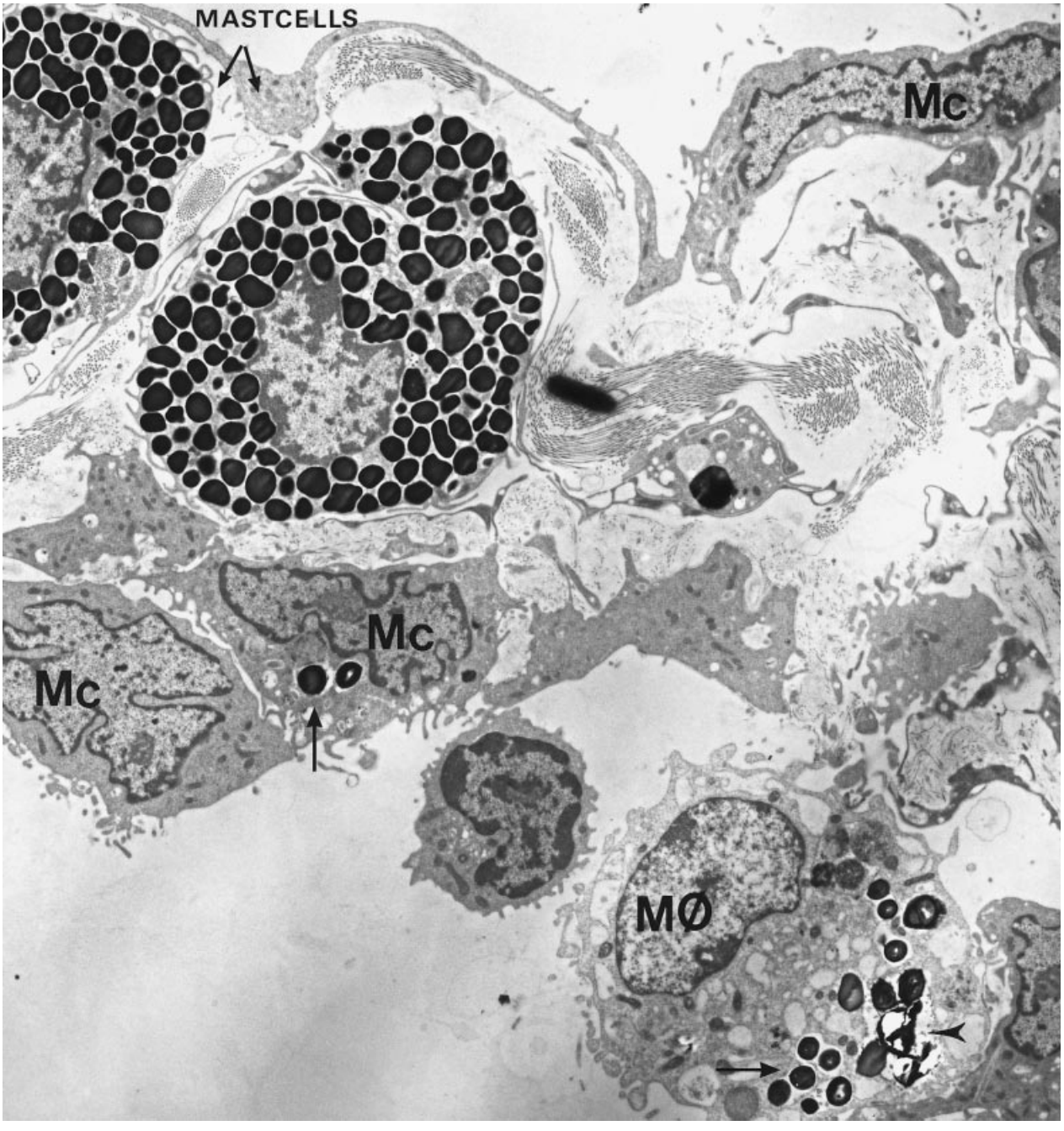

Fig. 5. Ultrathin section of omentum tissue six hours after injection of *Staphylococcus aureus*. Abbreviations and symbols are: MC, mesothelial cell; MØ, macrophage; arrows, *S. aureus*; arrowheads, digested *S. aureus*.

PD fluid via the cannula and collection of the effluent three hours later, followed by the administration of fresh PD fluid. In summary, in the PD group effluents varying from 6 to 9 ml were collected at all of the different time-points (except for physiological saline), whereas the overnight dwell resulted in volumes of 1 ml or less (Fig. 4). In the infection group, the volumes of the collected fluids were lower, especially between 24 to 33 hours. Moreover, in the PD group, the number of cells collected ranged from 1 to 3×10^7, and consisted for about 40% of neutrophilic granulocytes. In contrast, higher cell numbers (3 to 8×10^7), with a higher percentage of neutrophilic granulocytes were found in the infection group.

This model has allowed the evaluation of immunological events occurring during short-term peritoneal dialysis. However, several problems appeared, such as the necessity for continuous antibiotic therapy, and the recovery of high percentages of neutrophilic granulocytes in the control

Table 2. Comparison of cellular composition of effluents of CAPD patients and PD rats

	Macrophages	Lymphocytes	Neutrophils
CAPD patients[a]	70–75%	15–20%	2–5%
PD rats 12 weeks	65–75%	10–25%	1–7%

[a] Betjes et al, *Kidney Int* 43:641–648, 1993

group, indicating ongoing inflammation in the infection group. In addition, compartimentalization of the peritoneal cavity due to fibrin and adhesion formation occurred that complicated fluid collection. These problems made the model unsuitable for studying long-term effects of exposure to PD fluids.

One of the possible solutions to the "mechanical" catheter obstruction could be the performance of an omentectomy. However, the omentum (and especially the milky spots) is an important source of both mesothelial cells and macrophages [70, 71]. Omentectomy impairs antibacterial defence [70, and unpublished observations]. Despite the fact that others have been successful in establishing a continuous rat PD model after omentectomy [29], we have avoided this procedure, since this may have implications for the immune status of the animal.

Chemokines produced by mesothelial cells and macrophages play a central role in the regulation of inflammatory cell influxes in CAPD patients, as they are important for bacterial killing [72–77]. We have recently established in a rat model that after an i.p injection, *Staphylococcus aureus* bacteria were clearly visible within mesothelial cells *in vivo* (Fig. 5) [78]. These data suggest a potentially important role of the mesothelium in bacterial clearance and cell activation *in vivo*, and confirm *in vitro* findings [74]. Whether different PD fluids influence the mesothelial handling of bacteria and thereby regulate the inflammatory response *in vivo* upon bacterial challenge has yet to be investigated.

To study the role of mesothelial cells *in vivo* during PD and to start possible intervention studies on chemokines and mesothelial cell regeneration, an appropriate animal model was required. We therefore developed a rat model to determine the effects of PD fluid on the morphology of the peritoneum and on local host defence mechanisms [79].

In this model a peritoneal catheter was implanted in the peritoneal cavity. This catheter (~15 cm in length), connected to a mini-vascular access port, was inserted into the peritoneal cavity and the port was tunneled subcutaneously to the neck of the rat. After the implantation of the catheter, rats were given 2 ml of saline with 1 U/ml heparin daily to allow healing of the incision in the abdomen. Six days later, PD fluid instillation was commenced. The six days period was implemented because direct installation of 10 ml PD fluid immediately after surgery often resulted in subcutaneous leakage of the PD solution, resulting in inflammation. The animals continued to gain weight during the experiments. About 20% of the animals were excluded from the experiments because outgrowth by the omentum obstructed the catheter. Using this approach, daily instillation of PD fluid in the rat peritoneal cavity can be performed for at least 20 weeks without significant complications. This technique makes it possible to compare the cellular composition of these animals at several time intervals and to analyze whether this cellular composition is related to the intraperitoneal chemokine secretion profile. In addition, changes in the mesothelial monolayer at several time points as well as other morphological changes in the peritoneum can be studied. The results in this model show that four weeks after fluid instillation an initial increase in total cells is followed by a decline with a lower cell count after 12 weeks. The cellular composition 12 weeks after daily PD-fluid installation closely resembles that found in PD patients (Table 2). The number of neutrophilic granulocytes ranges from 1 to 7%, indicating that no infection or acute inflammation are present. The percentages of lymphocytes at 12 weeks range between 10 to 25% with about 70% macrophages, resembling a chronic sterile inflammation as described above. Morphological changes of the mesothelial cell monolayer are detected in this model. A higher density of cells is observed after four weeks, and foci of young mesothelial cells within activated mesothelium are found after 12 weeks. Twelve weeks after daily PD-fluid installation appears to be a good time to observe mesothelial damage and regeneration.

This latter model clearly does not completely mimic the PD situation, as no exchanges are carried out and the animal is not uremic. Advantages of this model are due to the use of a mini vascular access port, which removes the necessity for the administration of antibiotics and provides access for the performance of an omentomectomy. In addition, the cellular composition resembles the human situation (low percentages of neutrophilic granulocytes) and the experimental treatment is very well tolerated by the animals. Finally, this model can be combined with the previous infection model to study whether long-time exposure to PD fluid further impairs peritoneal host defence and to determine what effects different PD fluids have on these different parameters. Also, the role of the mesothelial cell *in vivo* in this infection model can now be fully investigated.

A MODEL OF CHRONIC PERITONEAL DIALYSIS IN UREMIC RATS

Chronic renal failure was surgically induced by partial nephrectomy in male adult Wistar rats. Under general anesthesia with pentobarbital 50 mg/kg, the left kidney was exposed through a flank incision, and both the anterior and posterior poles and approximately one third of the remaining cortical tissue from the exterior lateral part of the kidney were removed. This resulted in a total resection of >85% of the renal tissue. Bleeding was controlled by the

Table 3. Evolution of body weight, daily food and water intake and daily urinary volume in dialyzed and non-dialyzed uremic animals

Weeks		Before	1	2	3	4	5	6	7	8
Body weight *g*	PD	320 ± 45.5	320 ± 32.7	316 ± 39.0	313 ± 32.9	326 ± 34.1	313 ± 39.7	306 ± 34.1	311 ± 37.2	318 ± 14.1
	non-PD	316 ± 43.0	305 ± 31.2	302 ± 39.6	282 ± 40.5	295 ± 55.0	307 ± 49.5	301	316 ± 36.8	235
Daily food intake *g*	PD	18.3 ± 5.1	20.2 ± 4.5	20.0 ± 2.8	19.0 ± 4.8	16.7 ± 2.9	14.3 ± 7.5	17.2 ± 3.6	16.3 ± 2.6	17.8 ± 3.3
	non-PD	18.6 ± 3.5	19.1 ± 3.5	17.5 ± 4.7	18.7 ± 3.33	20.1 ± 3.3	18.0 ± 1.9	21.7	20.2	16
Daily fluid volume *ml*	PD	63.9 ± 22.8	71.1 ± 15.1	67.5 ± 14.2	65.2 ± 20.6	57.8 ± 13.9	48.5 ± 25.3	62.8 ± 10.4	60.5 ± 18.0	59.6 ± 12.1
	non-PD	60.5 ± 8.7	57.2 ± 9.1	55.1 ± 8.9	52.7 ± 6.92	59.0 ± 6.0	58.5 ± 8.0	67.7	50.3	50
Daily urine volume *ml*	PD	38.3 ± 9.3	49.0 ± 15.8	52.1 ± 14.6	51.2 ± 19.0	55.5 ± 19.2	39.5 ± 16.3	56.5 ± 15.3	58.1 ± 20.6	50.5 ± 6.2
	non-PD	36.9 ± 7.4	36.3 ± 4	37.7 ± 8	34.1 ± 7.3	37.5 ± 11.6	36.6 ± 11.5	33.4	34.9	36.5

application of thrombin (2500 U/ml) to the cut surfaces. One week later, the right kidney was removed after ligaton of the blood vessels and ureter under general anesthesia. Three weeks later, a silicone catheter (CH15; International Medical) was introduced into the abdominal cavity, strictly following the protocol as described by Miller, Findon and Rowe [35]. The catheter was pushed over a trocar that was then used to create a subcutaneous track ending at the back of the neck of the animal. Omentectomy was not performed. After carefully securing the catheter and closure of the surgical wounds, the silicone catheter was closed by a Luer-Lock adaptor.

Chronic peritoneal dialysis was started at least three weeks after the insertion of the catheter. Each morning, the Luer-Lock adaptor was removed, a polyethylene, gas-sterilized catheter (PE 240; Beckton- Dickinson, USA) was passed down the permanent indwelling catheter and rinsed with 15 ml of pre-warmed saline. After draining this fluid, 15 ml pre-warmed dialysis fluid, either containing 1.36% or 2.27% glucose (Bieffe Company, Italy) was instilled by gravity for a dwell time of three hours in the unanesthetized animal. Ceftazidim 125 mg/liter, gentamicin 8 mg/liter, and ciprofloxacine 25 mg/liter were added to every dialysate exchange.

After the first dwell of three hours, the effluent was drained as completely as possible and a second dwell with 15 ml of dialysis fluid was begun for another three hours. At the end of the second dwell, the fluid was drained, and the peritoneal cavity was rinsed with 15 ml of saline. After draining this rinse, the Luer-Lock adaptor was closed and the animal returned to its cage. On Monday and Friday of each week, blood was sampled by puncture of the tail vein. Creatinine, urea, electrolytes, osmolality, total protein, and glucose were measured in each effluent and in the 24-hour urine volumes, collected on Monday and Friday of each week. The blood samples were assayed for creatinine, urea, osmolality, total protein, and hematocrit. Each animal was housed in a metabolic cage and daily food and fluid intake, 24-hour urine volumes and quantities of feces were measured. Body weight was followed for the entire duration of the experiment. Urea and creatinine were enzymatically measured [80, 81]. Electrolytes were assayed by ion-selective electrodes, glucose by the hexokinase method [82], total protein by the Biuret method and osmolality by standard osmometry.

In 13 animals, chronic renal failure was induced by the same surgical method and a peritoneal catheter was implanted in 4 of them. These animals were housed in metabolic cages but peritoneal dialysis was not performed, and they served as time controls for the dialyzed animals.

The clinical and biological parameters that are presented in the dialyzed group are from the 12 animals that were dialyzed for at least four weeks. These data are compared with the results obtained in the 13 non-dialyzed uremic animals. The evolution of body weight, daily food and water intake, and daily urinary volume in both dialyzed and non-dialyzed uremic animals are given in Table 3.

The mean body weight in the PD group before the start of PD was 320 ± 45.5 g and remained stable. In the non-PD animals, a tendency for a decreasing body weight was observed. Although peritoneal dialysis had apparently no impact on either food or fluid intake, the daily food intake of non-uremic animals of the same body weight ($N = 25$) and housed under the same circumstances, fluctuated around 30 ± 4.2 g. Over the same period (8 weeks), the non-uremic animal's body weight increased by 150 g.

In non-uremic rats of the same body weight, ($N = 25$), the daily fluid intake was 40 ± 6.7 ml. It should be noted that the fluid intake of the uremic rats was much higher than in non-uremic animals, but not different between the dialyzed and non-dialyzed animals. It is remarkable to note that in contrast to the non-dialyzed animals, the daily urine output dramatically increased after the start of peritoneal dialysis. The evolution of the serum creatinine, hematocrit values, residual urine and peritoneal creatinine clearances, drain volumes, D/P creatinine values, the percent glucose absorption per dwell of three hours, and the leukocyte counts in effluent are presented in Table 4.

The starting serum creatinine values were significantly higher compared to non-uremic animals; in our laboratory, the blood urea and serum creatinine levels, the latter determined with the enzymatic assay, in non-uremic rats ($N = 25$), were 0.22 ± 0.035 mg% and 26 ± 4 mg%, respectively. In contrast with non-dialyzed animals, both blood urea and serum creatinine values significantly decreased during the first two to three weeks of peritoneal

Table 4. Evolution of the biological data, residual urine and peritoneal function in uremic animals

Weeks		Before	1	2	3	4	5	6	7	8
Blood creatinine *mg%*	PD	1.72 ± 0.5	1.33 ± 0.4	1.36 ± 0.4	1.47 ± 0.4	1.84 ± 0.4	1.70 ± 0.2	1.72 ± 0.2	1.79 ± 0.4	1.69 ± 0.4
	non-PD	2.07 ± 0.82	1.97 ± 0.65	2.18 ± 0.8	2.1 ± 0.6	1.95 ± 0.95	2.22 ± 1.2	1.90	2.11	2.2
Hematocrit %	PD	34.0 ± 3.6	31.0 ± 3.9	32.0 ± 3.3	32.0 ± 5.7	34.0 ± 4.3	34.0 ± 5.7	33.0 ± 4.8	30.0 ± 4.8	30.0 ± 3.2
	non-PD	37.0 ± 7.3	33.0 ± 6.1	32.0 ± 6.5	30.0 ± 7.4	26.0 ± 10.3	30.0 ± 9.6		37.0	31.0
Renal creatinine clearance *ml/min*	PD	0.29 ± 0.1	0.39 ± 0.2	0.41 ± 0.2	0.33 ± 0.1	0.24 ± 0.1	0.20 ± 0.1	0.29 ± 0.1	0.25 ± 0.1	0.24 ± 0.1
	non-PD	0.29 ± 0.1	0.28 ± 0.14	0.24 ± 0.11	0.27 ± 0.19	0.29 ± 0.2	0.27 ± 0.3	0.19	0.11	0.16
Peritoneal creatinine clearance *ml/min*	PD	—	0.05 ± 0.013	0.05 ± 0.016	0.05 ± 0.019	0.05 ± 0.022	0.05 ± 0.025	0.06 ± 0.030	0.05 ± 0.038	0.05 ± 0.021
Drain volume *ml* (3 hrs)	PD	—	12.67 ± 0.46	9.68 ± 0.19	10.11 ± 1.25	9.54 ± 0.97	11.41 ± 1.86	9.89 ± 2.70	10.12 ± 1.66	12.56 ± 1.06
D/P creatinine *3 hrs*	PD	—	0.28 ± 0.03	0.91 ± 0.03	0.96 ± 0.03	0.90 ± 0.03	0.97 ± 0.03	1.0 ± 0.06	0.97 ± 0.03	0.91 ± 0.15
Percent glucose absorption % (over 3 hrs)	PD	—	—	92.7 ± 1.35	92.4 ± 0.43	93.4 ± 0.46	88.7 ± 1.73	92.3 ± 1.53	92.8 ± 1.24	92.0

dialysis. Thereafter, values comparable with those obtained at start of PD were obtained. The renal creatinine clearance was lowered to 0.29 ± 0.1 ml/min in both groups of rats at the start of dialysis, which is approximately 12% of the normal creatinine clearance in non-uremic animals of the same strain and body weight in our laboratory (2.47 ± 0.72 ml/min). There was a tendency of the residual creatinine clearance to decrease with time on dialysis.

The peritoneal data were calculated on 2 × 3-hour exchanges on two days per week. The instilled volume being 15 ml, it is clear that the drained volume was always lower. This explains the relatively low calculated peritoneal creatinine clearances, which represent only approximately 20% of the renal clearances. The D/P creatinine values remained high, with a calculated transperioneal glucose absorption ranging around 90 to 93% over three hours. This should be compared with a percent glucose absorption of 85 to 91% after four hours in single dwell studies with a 2.27% glucose solution in non-uremic animals in our laboratory. From the first week on, a dramatic increase in total leukocyte count in the drained dialysate was observed. Differentiation of these leukocytes by FAC scan analysis revealed the majority to be lymphocytes and macrophages. Based on the relative increase of the granulocytes in the dialysate, 16 episodes of peritonitis were observed in a total number of 307 dialysis weeks. Cultures of the dialysate yielded coagulase-negative staphylococci on 7 occasions, *Streptococcus agalactiae* on 3 occasions, and *Escherichia coli* in 2 and *Corynebacterium spp*. on 1 occasion.

There was no difference in the cumulative survival rate of the PD animals after the start of dialysis compared with the survival of the non-dialyzed uremic animals over the total of eight weeks. Twenty-nine uremic animals were successfully prepared and started peritoneal dialysis. Twelve animals were dialyzed for at least four weeks, eight for six weeks, and five for eight weeks. When the calculation of the eight-week survival rate was limited to the dialyzed or non-dialyzed animals surviving at least four weeks, a statistically greater survival of the dialyzed animals was observed (Fig. 6).

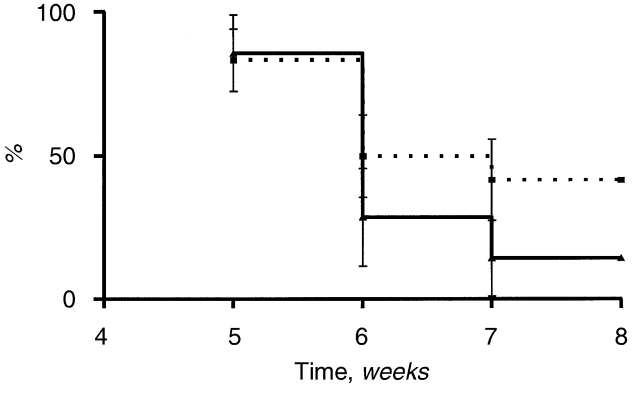

Fig. 6. Cumulative survival dialyzed (dotted line) versus non-dialyzed (solid line) uremic animals surviving at least four weeks.

These preliminary results on "chronic" peritoneal dialysis in uremic animals deserve comment. At first glance it is disappointing that the cumulative survival of the uremic animals was only minimally increased by peritoneal dialysis, compared to the non-dialyzed uremic animals. This lack in overall improved survival is explained by the relatively high mortality of the dialyzed animals occurring in the first three to four weeks after the initiation of peritoneal dialysis. In the animals surviving these first weeks, however, the mortality during the next four weeks was substantially lower than in the uremic, non-dialyzed animals. These results are therefore encouraging and justify in our opinion to continue these studies. It is clear that this model should be improved further to better imitate the clinical peritoneal dialysis setting.

Two major problems remain still unsolved. The first is

the relatively small contribution of the peritoneal clearance to the overall clearance. Based on the creatinine clearances, it can be calculated that the peritoneal clearance in our experimental group contributes only 10% of the total creatinine clearance. One of the reasons for this low peritoneal clearance is the continuously negative peritoneal ultrafiltration that was obtained with the daily use of one dwell with 1.36% and one dwell with a 2.27% glucose dialysate. The poor outflow was most certainly due to either catheter malposition or ongoing peritonitis. In infection, fluid drainage becomes difficult because the peritoneal cavity becomes compartimentalized due to fibrin and adhesions formation, resulting in the blocking of the peritoneal catheter by the omentum. It should be stressed that no omentectomy in these animals was performed. The second reason for the low peritoneal clearances is the relatively small dialysate volumes used in the two dwells (15 ml) and the fact that peritoneal dialysis was only performed during one quarter of the working day, with only one dwell of three hours on Saturday and no exhange on Sunday. The peritoneal clearance, however, remained constant. The peritoneal membrane of these animals can be interpreted as being hyperpermeable, as judged by both the high D/P creatinine and high glucose absorption. This hyperpermeability can also be the consequence of the state of "chronic" peritonitis that was present in all animals. A future treatment schedule should consist of dialysate volumes of at least 20 ml of glucose 3.86% and an additional long overnight dwell preferably with a polyglucose solution.

After only two days of dialysis, the peritoneal leukocyte count was elevated in the dialysate. Differential counts revealed that most of these cells were macrophages and monocytes. Granulocytosis of the effluent only appeared in the presence of confirmed bacterial peritonitis. Further refinements of our technique will hopefully solve some of these problems, allowing the utilization of this model for the study of "chronic peritoneal dialysis" in an uremic animal.

CONCLUSIONS

The present article describes the experience of three European research groups using different animal models of peritoneal dialysis. The acute model developed in Stockholm is appropriate for the study of the normal physiology of peritoneal transport and for a "first screening" of new dialysis fluids. It has the advantage of being relatively simple and cheap, but it does not allow conclusions on the effects of long-term dialysis in uremia.

The Amsterdam model explores the mechanisms of peritoneal defense against peritonitis and provides long-term information on the cellular influx and the behavior of the mesothelial cells during infection. The disadvantage is that no dialysis is performed and that non-uremic animals are studied.

The Gent model is by far the most complex, since it tries to submit uremic animals to long-term peritoneal dialysis. A number of difficulties are yet to be overcome before this model will be working satisfactorily. Some of these models will also have to be adapted to new findings.

It is hoped that in the future some of the European laboratories will be able to combine their experience for establishing a suitable animal model for long-term peritoneal dialysis.

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