J. Pharm. Pharmacol. 1998, 50: 1195–1203
Received April 28, 1998
Accepted June 24, 1998

Development of an In-vitro Model to Study the Growth Characteristics of *Staphylococcus epidermidis* in Continuous Ambulatory Peritoneal Dialysis

JANET A. BRANT, GEOFFREY W. HANLON AND STEPHEN P. DENYER

Microbiology Research Group, Department of Pharmacy, University of Brighton, Moulsecoomb, Brighton, East Sussex BN2 4GJ, UK

Abstract

An in-vitro model of peritonitis in continuous ambulatory peritoneal dialysis (CAPD) has been developed which integrates the parameters of environmental gaseous tension, the changing biochemical profile of the dialysate, and the periodic challenge of fresh fluid.

Dwell times of 4 h during the day and 10 h at night were used to reflect the in-vivo situation. Biochemical analysis of dialysate within the model showed that the profiles for creatinine, glucose and protein approximated those found in patients. *Staphylococcus epidermidis* growing in synthetic dialysis effluent or pooled dialysis fluid were seen to adapt rapidly to the environment after an initial lag phase; a tendency to aggregate together increased over the 50-h period of operation.

In conclusion, the results presented here suggest that this in-vitro model creates an environment which is reflective of the in-vivo situation and therefore has potential for the study of peritonitis in CAPD.

Many characteristics of bacteria, including growth rate, surface properties and expression of envelope proteins are influenced by their environment, and this in turn can influence their survival and virulence (Wilcox et al 1990). In-vitro and in-vivo models have been used to simulate natural growth environments and to establish bacterial cultures that have characteristics close to those found clinically. The use of these systems facilitates understanding of some of the complex processes of pathogenicity.

Continuous ambulatory peritoneal dialysis (CAPD) is an alternative treatment to haemodialysis for patients with end-stage renal failure who are awaiting transplantation. Although it has several advantages for the patient in terms of convenience and increased mobility it carries with it the inherent risk of peritonitis. The primary cause of CAPD peritonitis is coagulase-negative staphylococci derived from the skin microflora (Bint et al 1987).

Animal models of peritoneal dialysis have been used to study the effects of peritonitis on CAPD, in particular the clearance of microorganisms from the peritoneal cavity by defence mechanisms. Calme et al (1992) established a rat model in which a suspension of *Staphylococcus epidermidis* in phosphate-buffered saline was injected into the peritoneal cavity and left for 24 h after which the animals were killed and the surviving bacteria counted. The authors found that both the size and volume of the inoculum affected the number of bacteria recovered from the peritoneal cavity. High initial inocula resulted in animal death and lower concentrations were quickly cleared. It was suggested that volume affected removal rate because of dilution of phagocytes, IgG and fibronectin. The disadvantage of this method is that it requires the killing of the host to enable sampling and does not enable the taking of repeat samples from a single animal, thereby introducing biological variation. It is also difficult to recover bacteria separate from host tissue. Strategies developed to overcome such problems include peritoneal chamber models in the form of perforated centrifuge tubes, practice golf balls and steel chambers (Pike et al 1991). A particularly useful system is the micropore diffusion chamber, a plastic syringe barrel with membrane filters at both ends implanted into mice or rats (Lambert et al 1990). These methods maintain isolation of the bacteria from the tissues but still

Correspondence: G. W. Hanlon, Department of Pharmacy, University of Brighton, Moulsecoomb, Brighton, East Sussex BN2 4GJ, UK.

require killing of the animal for sampling. Pike et al (1991) developed a porous diffusion chamber which was reusable and, most importantly, had an external sampling port which enabled multiple and sequential sampling; this limits the number of animals required for each study. *S. epidermidis* and *S. aureus* were successfully grown in these chambers.

In-vitro models have the disadvantage of not integrating as many parameters of the human peritoneum as do animal models but they have the major benefits of being cheaper, having less variability in performance and enabling the adoption of a minimalist approach. The parameters of an in-vitro model are more readily regulated by the experimenter, giving more freedom to study the individual factors affecting the microorganisms grown within the model.

In an early attempt to study infection in CAPD, a simple in-vitro batch model was developed which involved growing *S. epidermidis* in used pooled dialysis fluid in 5% CO_2–95% air to mimic the growth medium and gaseous tension experienced by the bacteria in the peritoneal cavity (Williams et al 1988; Wilcox et al 1990, 1991; Smith et al 1991; Modun et al 1992). Modun et al (1992) used the peritoneal chamber model of Pike et al (1991) to study the effect of in-vivo growth of *S. epidermidis* on cell wall and cytoplasmic protein expression, and compared this with growth in an in-vitro model. They found similarity between cell envelope protein profiles of cells grown in the two environments, suggesting that the in-vitro model corresponded well to conditions within the dialysed peritoneum. Despite this the model still lacked many characteristics of the in-vivo environment, most particularly the compositional changes occurring in the dialysate during the dwell period (Vonesh et al 1991) and the regular introduction of fresh fluid, which has been shown to have a bacteriostatic effect (MacDonald et al 1986).

The aim of the work described in this paper was to develop an in-vitro model system of CAPD which integrates the important features of the in-vivo environment, such as gaseous tension, changing biochemical status and periodic challenge of fresh fluid.

Materials and Methods

Collection and pooling of used dialysate

Bags of used dialysis fluid were collected from separate patients established on CAPD and who had not received any antibiotic treatment for peritonitis during the previous week. The dialysate was collected after various dwell times (4–12 h) and was derived from fresh fluid with a variety of original glucose concentrations (1·36, 2·27, 3·86%). Collection was made on the day of drainage. The tubes of the collection bag were immediately sealed with clips to maintain the CO_2 tension of the fluid at physiological levels. Each bag of fluid was aseptically drained into a sterile 2-L bottle, sealed, and stored at −20°C until required for use. Before freezing, samples were removed to check for freedom from contamination and residual antibiotic. When a sufficient volume had been collected all fluid was thawed at 37°C under 5% CO_2 (to prevent protein precipitation; Wilcox et al (1990)), and emptied into a 45-L sterilized container. This container was rolled to mix the fluid which was then pumped back into the original bottles. This fluid was subsequently known as pooled dialysis fluid (PDF).

Fresh dialysis fluid was prepared to reflect the composition of commercially available fluid (Dieneal, Travenol) as follows (g L^{-1}): sodium lactate, 5; NaCl, 5·6; $CaCl_2$, 0·26; $MgCl_2.6H_2O$, 0·15; sodium metabisulphite, 0·05; glucose, either 13·6 or 38·6; distilled water to the required volume. The solution of lactate and sodium chloride was autoclaved at 121°C for 15 min. A stock glucose solution was autoclaved separately (115°C, 10 min) before aseptic addition. $CaCl_2$, $MgCl_2$ and sodium metabisulphite stock solutions were filter-sterilized before aseptic addition.

Synthetic dialysis effluent (SDE) was adapted from the formula proposed by Holmes et al (1989) and consisted of (g L^{-1}): nutrient broth No. 2 dried powder, 1·0; NaCl, 8; KH_2PO_4, 0·12; KCl, 0·197; $CaCl_2$, 0·133; $MgCl_2$, 0·81; urea, 1·32; creatinine, 0·083; glucose, 3·37; distilled water to the required volume. The solution, but without $CaCl_2$, $MgCl_2$, urea, creatinine and glucose, was autoclaved at 121°C for 15 min. A stock glucose solution was autoclaved separately (115°C, 10 min) and added aseptically. $CaCl_2$, $MgCl_2$, urea and creatinine stock solutions were also sterilized separately by filtering before aseptic addition. Holmes et al (1989) included foetal calf serum for protein preconditioning of biomaterials before bacterial adhesion. As it was not the intention to perform adhesion studies in the SDE the serum was not included.

Microbial culture

The test microorganism used was a clinical isolate of *S. epidermidis* (designated strain 901) isolated from infected dialysis fluid obtained from a CAPD patient suffering from peritonitis and attending the Renal Unit, The City Hospital, Nottingham, UK. This strain was identified by API Staph (API,

Basingstoke, UK) and its surface characteristics have been documented (Denyer et al 1990). Stocks of cells were kept at −70°C in nutrient broth (No. 2; Oxoid, Basingstoke, UK) supplemented with 10% v/v glycerol. Before use frozen stocks were thawed and grown in 100 mL nutrient broth and their identity confirmed by use of API Staph and Gram staining. Contact angles were routinely determined for reconstituted cultures to check for any significant changes in surface characteristics. These cultures were then maintained as working inocula on agar slopes at 4°C for one month. A separate agar slope was used to inoculate each 10-mL starter culture, by first creating a suspension of cells from the slope in phosphate-buffered saline, adjusting to a concentration of 2×10^8 colony-forming units mL^{-1} and using 0·2 mL to inoculate 10 mL medium contained in a universal jar (either SDE or PDF). These were then incubated statically for 16 h at 37°C in 5% CO_2–95% air.

Viable counts

Viability was assessed by preparing tenfold serial dilutions in sterile PBS (containing 1% Tween 80) and pipetting 0·2 mL of the appropriate dilution, in triplicate, on to over-dried nutrient agar plates and spreading before inversion and incubation at 37°C for 24 h.

In-vitro CAPD model design and operation

When designing the in-vitro model system several factors had to be considered to mimic the in-vivo situation as closely as possible.

The atmosphere in the model chamber was required to be at 5% CO_2 tension, the gaseous tension previously reported to be found in the peritoneum (Denyer et al 1990). To do this the assembled model was placed within a CO_2 incubator set at 5% with air flow through the chamber achieved via filters to prevent possible entry of bacteria from the environment.

When a CAPD patient drains the dialysis fluid from the peritoneal cavity at the end of its dwell time a residual volume of approximately 100 mL remains (Imholtz et al 1992), after which the patient then introduces 2 L fresh fluid. The model was, therefore, designed with a reservoir of 10 mL with a drainage point above this.

Dialysis over the 4-h dwell-time was to be simulated by slowly introducing PDF and mixing with the fresh fluid already in the chamber. This required an overflow to keep the total volume to 200 mL. The PDF was mixed with the contents of the chamber by gentle stirring with a magnetic stirrer. An inlet for the fresh fluid was also included and a separate inlet for the PDF, which included a line break to stop progression of the bacteria back up the pipe with the risk of contaminating the sterile fluid.

These features were incorporated into a glass model constructed according to Figure 1 and connected to silicone rubber tubing (for eventual connection to dialysis fluid containers). The apparatus and tubing were sterilized by dry heat at 175°C for 1 h. Fresh fluid was prepared with glucose concentrations of 1·36 and 3·86%. PDF was defrosted at 37°C in 5% CO_2. The mixing flea was autoclaved at 121°C for 20 min. The model was assembled for at least 12 h before use to enable equilibration of the gaseous environment inside the model with that of the incubator.

Calibration of the model

The model was run using a methyl violet solution as a substitute for PDF or SDE and water in place of fresh dialysis fluid. The reservoir was filled with 10 mL dye, to mimic residual dialysate after peritoneal emptying, and water was added to the main chamber to the overflow level. Dye solution was then pumped into the model from the PDF container at a controlled rate over 4 h and samples were removed and the optical density measured at 560 nm. The concentration of dye was calculated as a percentage of the undiluted dye solution. This experiment was repeated at different flow rates and the concentration profile plotted; a typical example is shown in Figure 2. A flow rate of 4·2 mL min^{-1} delivering 1 L dye is adequate to produce a solution equivalent to 95% of the original concentration after 4 h; these flow parameters were used for the delivery of PDF and SDE in the model.

Dilution kinetics

During the running of the model PDF is continuously added, making it necessary to include an overflow in the design to maintain a constant volume of fluid. This means that some bacteria are lost from the chamber causing an overall dilution of numbers. To determine the extent of this dilution the model was operated without inoculation and filled to the overflow level with dye solution of known optical density, to simulate a bacterial suspension at the beginning of a dwell period. Water was used in place of PDF and added at 4·2 mL min^{-1} for 4 h. The optical density was measured every 30 min. Using these results a correction factor was developed to compensate for the effects of this dilution process on cell numbers.

The results can be seen in Figure 3 and show how the bacterial population would be diluted if the

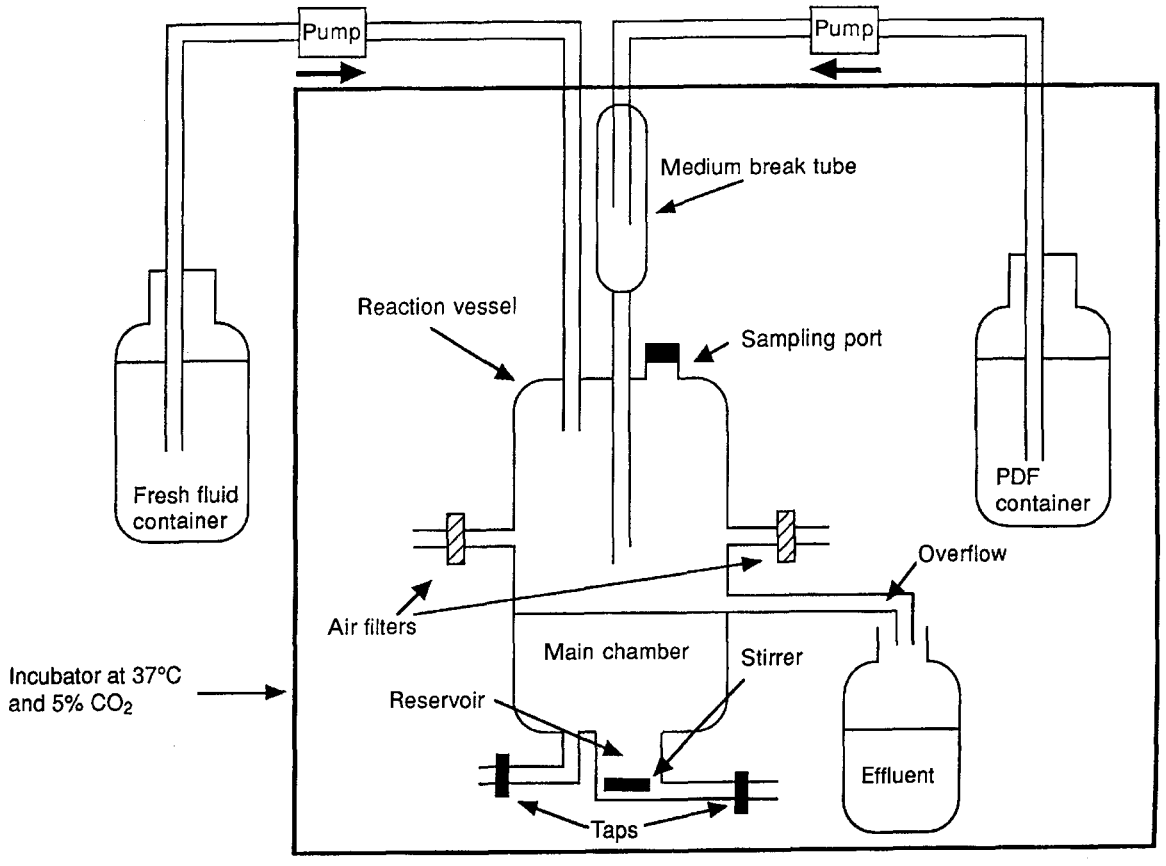

Figure 1. Diagram of in-vitro continuous ambulatory peritoneal dialysis model system.

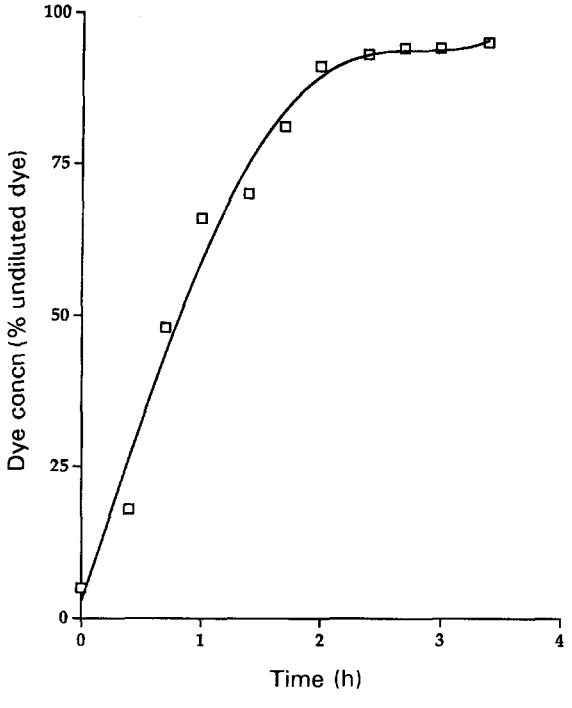

Figure 2. Concentration of methyl violet in the continuous ambulatory peritoneal dialysis model during a 4-h dwell period at a delivery rate of 4.2 L min^{-1}. The dye concentration is expressed as a percentage of the concentration of the undiluted dye.

numbers were to remain static (i.e. cells not growing or dying). The rate of dilution is $10^{-0.44}$. If applied to the analysis of growth curves, plots can be derived which describe the population size that would arise if there were no dilution; the true growth rate at any stage can thus be determined.

Biochemical changes in dialysis fluid in-vivo

Used dialysate was collected from six patients undergoing CAPD. In the morning a bag of a 2·27% glucose solution was prepared for dialysis and warmed to body temperature. The overnight bag was drained over 20 min and the new solution was infused at a rate of 200 mL min^{-1}. The patient was rolled after every 400 mL to ensure mixing. The dwell time was considered to have started at the moment of completion of the infusion. Dialysate samples were taken at 0, 0·5, 1, 2, and 4 h by draining approximately 200 mL into the attached bag and inverting the bag to mix. Ten millilitres was withdrawn from the disinfected sample port, placed in a sterile tube, sealed, and frozen until analysis. The remaining fluid (190 mL) was re-infused into the patient. The samples were defrosted

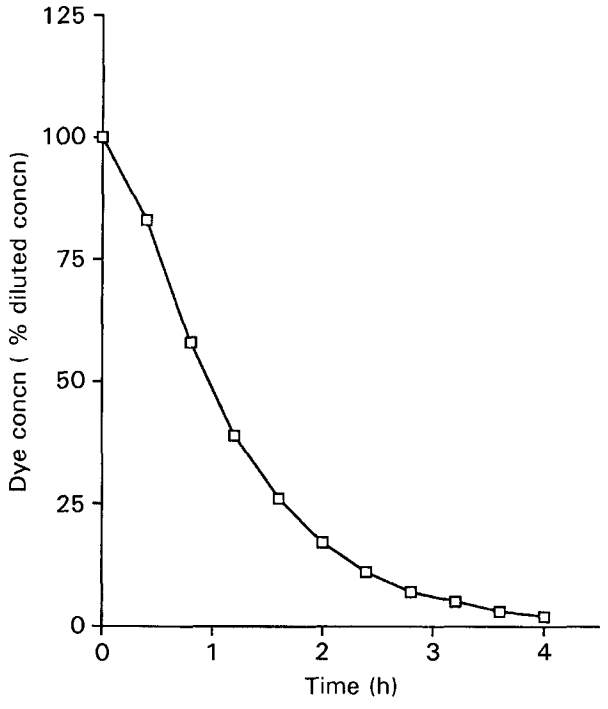

Figure 3. Dilution rate in the continuous ambulatory peritoneal dialysis model using the dye wash-out method.

at 37°C in an atmosphere of 5% CO_2–95% air and left to equilibrate overnight. They were then analysed for their protein, creatinine and glucose concentration. Frozen samples must be adequately vortex-mixed before analysis because more concentrated solutions will thaw first and run to the bottom of the sample tubes; mixing by hand is not adequate (Twardowski et al 1987).

Creatinine concentration was determined by standard colorimetric techniques based upon the Jaffé reaction (Sigma Diagnostics). This reaction is not specific and several substances including protein and glucose (Farrell & Bailey 1991) in PDF will interfere. Addition of acid destroys the colour derived from creatinine but not that derived from interfering chromagens.

Protein concentration was determined by a method based on the Lowry procedure (Sigma Diagnostics). This utilized the indirect method whereby proteins are precipitated before measurement to prevent interference from commonly used chemicals such as Tris, ammonium sulphate, EDTA, sucrose and citrate.

Glucose concentration was determined by using glucose oxidase to produce H_2O_2 which was detected with *o*-dianisidene and peroxidase to form a coloured product (Sigma Diagnostics). Before analysis it was necessary to deproteinate the samples to avoid interference.

Biochemical changes in fluid in-vitro

The model was run aseptically with fresh dialysis fluid (glucose strength 2·27%) and PDF according to the calibrated protocol described earlier. Samples (10 mL) were removed at 0·5-h intervals, placed in sterile tubes, sealed, and frozen until analysis. The samples were defrosted at 37°C in an atmosphere of 5% CO_2–95% air and left to equilibrate overnight. After vortex-mixing they were analysed for protein, creatinine and glucose concentration.

Inoculation and operation of the infection model

A 24-h starter-culture (10 mL) of *S. epidermidis* strain 901 grown in SDE or PDF (5%CO_2–95% air) as appropriate was used to fill the reservoir of the model. The chamber was then filled to overflow level with 1·36% glucose-containing fresh fluid (at room temperature). PDF (1 L) was then pumped in at 4·2 mL min^{-1} to emulate the dialysis process. After 4 h the fluid was drained from the vessel (leaving 10 mL residual fluid in the reservoir), refilled with fresh fluid and a new 1-L container of PDF was attached and the process repeated. Every fourth dwell period, 3·86% glucose-containing fresh fluid was used and the model operated for 10 h (with the PDF still being added during the first 4 h) without fluid change to mimic an overnight dwell period.

Bacterial growth in the CAPD model

Starter cultures of *S. epidermidis* 901 were set up as described above in either SDE or PDF (5%CO_2–95% air) and used to inoculate the model to three different inoculum levels. Samples (1 mL) were removed via the sample port every hour and viable counts measured.

Growth in PDF over extended periods

To explore population changes over an extended time it was necessary to run the model for longer, but because of the limited amount of dialysis fluid available it was possible to do this on one occasion only. Initial problems with contamination of the PDF in extended runs meant that it was also necessary to use fluid which had not been pooled because reducing the number of manipulations improved the chances of maintaining sterility. Fluid used in this way was analysed for creatinine, protein and glucose to ensure it fell within the normal range. The pattern of fluid changes was altered for experimental convenience, with changes occurring at 5, 10, 14 and 24 h; this still approximates to a typical patient's pattern. One millilitre samples were removed for viable counting.

Results and Discussion

Investigation of biochemical changes in dialysate
The principal feature of any in-vitro CAPD model is to mimic closely the biochemical changes occurring in the dialysate during its dwell in the peritoneal cavity. To achieve this, fresh dialysis fluid was exchanged for used dialysate by continuous addition of pooled dialysis fluid over a 4-h dwell period and at 4·2 mL min^{-1}. This flow rate was derived from preliminary experiments which indicated that 1 L PDF was required to bring about a 95% change in composition from fresh fluid to PDF. The concentration change is illustrated in Figure 2.

The pattern of compositional change created will be uniform for all components; this might not be so during actual dialysis. To establish the true in-vivo situation biochemical analysis was performed of fluid drained from six CAPD patients at various times during a 4-h dwell. The three solutes measured were protein, creatinine and glucose. Protein was chosen because it is a nitrogen source of variable, but large, molecular weight. Creatinine was selected for its low molecular weight and glucose because it is present at large concentrations in fresh dialysis fluid and therefore moves across the membrane in the opposite direction to the other two molecules. Glucose might also be an important energy source for the bacteria.

Figure 4 compares in-vitro creatinine, glucose and protein levels with those found in-vivo. The pattern of creatinine appearance is similar, although in-vivo the concentration continues to increase after 2 h, presumably because the serum concentration is not fully equilibrated within that time (Twardowski et al 1987); despite these different profiles the concentration in the model does fall within the in-vivo range. For glucose there is greater divergence between in-vitro and in-vivo analyses. Both show a reduction in glucose concentration but in-vivo this is in response to a variety of factors, including dilution by fluid entering the cavity by ultrafiltration and glucose active-transport. The appearance of protein in-vivo is constant, in contrast with the in-vitro model; one consequence of this is that significantly more protein will be available to bacteria sooner in the in-vitro model ($P = 0{\cdot}001$). However, Imholtz et al (1993), in their in-vitro studies, have shown that the peritoneal protein clearances are significantly greater ($P = 0{\cdot}005$) in the first hour compared with the next three; this would be reflected in the in-vitro model data.

Variation between patients in in-vivo analyses is large and will serve to minimize the differences in the in-vitro model.

For all three solutes in-vitro there seem to be no dramatic fluctuations in the concentration profiles, which all approximate to that of the dye model. It can therefore be assumed that the PDF is being adequately mixed into the fluid already in the model. Within the limitations of comparison with in-vivo data the model can be considered a good approximation of the in-vivo situation.

The model does not seek to mimic accurately biochemical changes arising during an overnight dwell period (approx 10 h), when a higher concentration of glucose is used (3·86%). In-vivo osmolality balance and glucose equilibrium are reached rapidly (4–5 h; Rubin et al (1979)) through high rates of ultrafiltration, but protein loss continues throughout, although at a progressively slower rate (Rubin et al 1981), and the hypertonic solution encourages faster equilibration of creatinine (Heimbürger et al 1994). The model is unable to mimic changes in intraperitoneal volume consequent upon high glucose concentrations; a 1·36% glucose solution causes only a 5% change in volume over 4 h whereas a 3·86% solution creates up to a 25% fluctuation over a 10 h dwell (Stelin & Rippe 1990; Imholtz et al 1993). A compromise of high glucose concentration and addition of PDF at the normal rate over the first 4 h with no further additions over the next 6 h was employed. In peritonitis, the solute concentrations are even more difficult to predict given the consequential reduction in fluid-removal capacity and increased glucose absorption and transport of low molecular weight compounds and proteins (Krediet et al 1989).

Growth of S. epidermidis *901 in the in-vitro dialysed peritoneal cavity model: growth in SDE*
The preliminary runs of the model were made using SDE to ensure that problems were addressed before moving on to PDF. The model was established and run for progressively longer times, eventually including an overnight dwell; viable counts were made every hour, with one immediately before and after each addition of fresh fluid at 4, 8, 12, 22, 26 and 30 h. The mean results of these counts are given in Figure 5. The cell numbers drop during the first 4 h, probably because of a combination of cell death, the inimical nature of the fresh fluid (MacDonald et al 1986), and the dilution and wash-out effect of the continuous addition of SDE. In the second and third 4-h cycles, however, cell numbers increase such that by 12 h they have risen tenfold over the initial inoculum size. It is during the long simulated night-time dwell, when the cells are left in undiluted SDE for 6 h, that the numbers multiply to levels approaching those found in batch

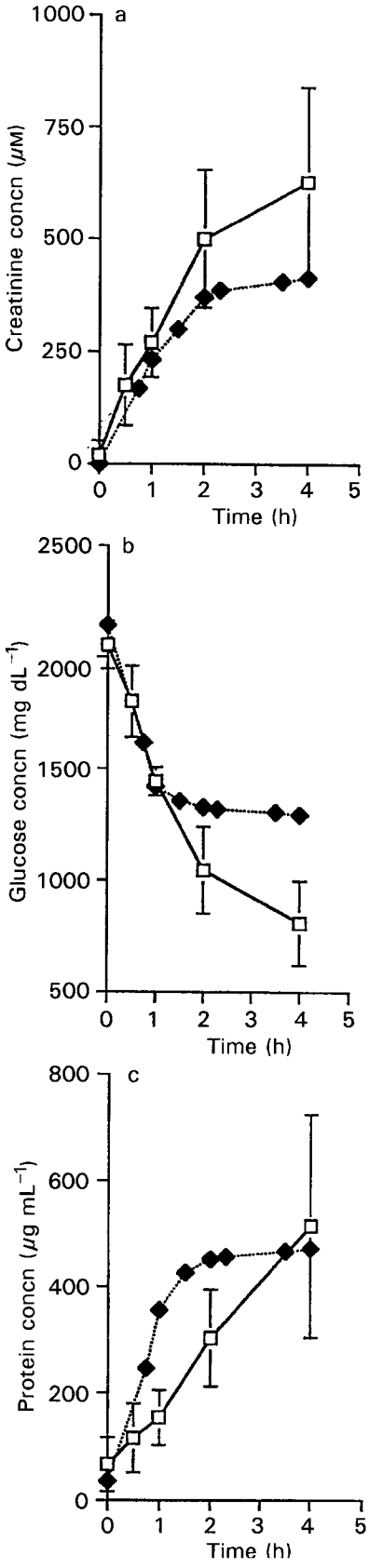

Figure 4. Comparison of (a) creatinine, (b) glucose and (c) protein concentrations during a 4-h dwell period in the continuous ambulatory peritoneal dialysis model (◆) and in patients (□) (n = 6; ± s.d.).

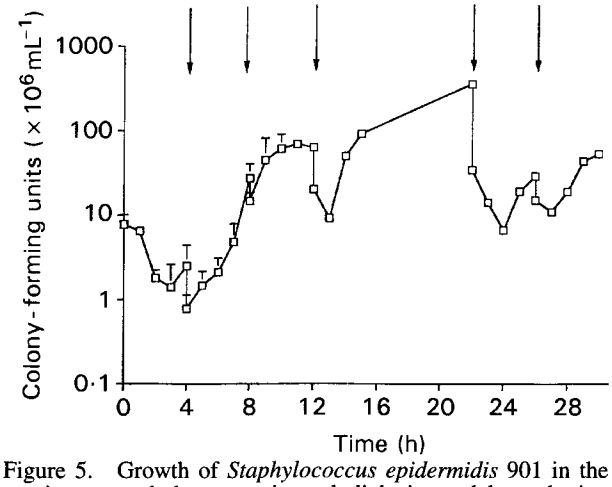

Figure 5. Growth of *Staphylococcus epidermidis* 901 in the continuous ambulatory peritoneal dialysis model employing SDE (+ s.d.). Arrows indicate times of fluid changes.

stationary phase ($7{\cdot}5 \times 10^8$ mL^{-1}). During the two cycles of the second day, cell numbers continue to follow a similar cyclical pattern but do not experience the same dramatic drop seen initially on the first day.

Growth in PDF

The model was run with PDF for up to 50 h but because of the limited amount of dialysis fluid available it was only possible to do this on one occasion. The growth profile over 50 h can be seen in Figure 6. The initial pattern was similar to that seen for SDE—a lag phase in the first 4 h with growth occurring in subsequent dwell periods. Next day the initial drop in cell numbers was smaller and the cells began to grow sooner.

At each fluid exchange a twentyfold dilution of the bacteria remaining in the reservoir (10 mL reservoir in 200 mL) should have been observed, but the actual reduction in numbers was much less than expected. This might have been because of

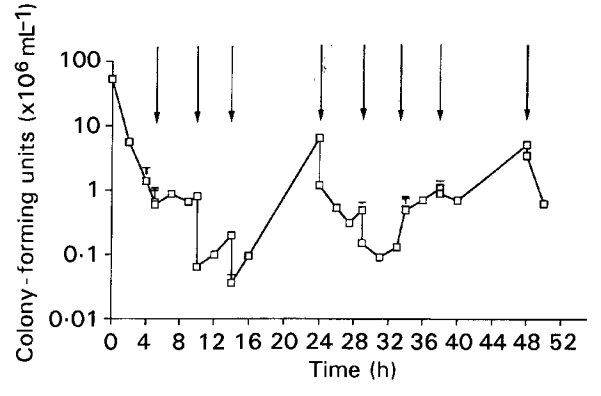

Figure 6. Growth of *Staphylococcus epidermidis* 901 in the continuous ambulatory peritoneal dialysis model growing in PDF (+ s.d.). Arrows indicate times of fluid changes.

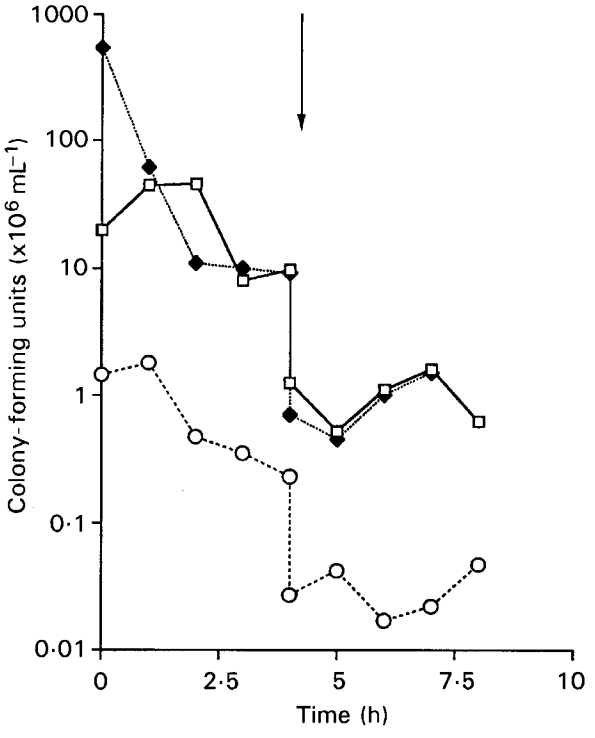

Figure 7. Effect of starter culture concentration on the growth pattern of *Staphylococcus epidermidis* 901 in the continuous ambulatory peritoneal dialysis model. Growth medium PDF. Starter culture concentrations: (◆) 5×10^9 colony-forming units mL^{-1}; (□) 5×10^8 colony-forming units mL^{-1}; (○) 5×10^7 colony-forming units mL^{-1}. Arrow indicates time of fluid change.

clumping of the bacteria resulting in artificially low viable counts at the end of each dwell period. When samples of the cells were studied by phase-contrast microscopy an increase in clumping was observed as each dwell period progressed, even though the cell concentration was not thought high enough to promote heavy clumping. It was also noticed that addition of fresh fluid broke up any large clumps present in the fluid therefore producing a higher viable count after dilution. After 48 h the clumps of cells were of sufficient size to be visible to the eye, with the largest settling out. So many of the cells had clumped that the suspending fluid was almost clear. The experiment was terminated at this stage.

Effect of inoculum size on growth in the peritoneal cavity model

Three inoculum sizes were examined, 5×10^7, 5×10^8 and 5×10^9 cells mL^{-1}, for their effect on growth in the model (Figure 7). Reproducible growth patterns comparable with those seen in Figures 5 and 6 were observed for the two lower inocula. The potential for early growth does not seem to be limited by nutrient availability at these inoculum sizes. For the highest inoculum a significant early drop in cell numbers occurred which cannot be explained by dilution alone. This implies some cell death, possibly as a result of limited nutrition in an inimical environment, or possibly cell aggregation. Interestingly, as soon as the inoculum drops to that comparable with the 5×10^8 starter culture, a normal pattern of survival and growth is apparent.

Determination of growth rate of S. epidermidis *in the peritoneal model*

The addition of PDF to the model causes a reduction in cell numbers. To establish a true growth rate at any given time it is necessary to take into account the dilution effect. True growth rate was therefore calculated by subtracting the dilution rate from the apparent growth rate, which was determined from the growth plots. No dilution occurred during the last 6 h of the night dwell, when the pump was switched off, and so no correction was needed over this period. Growth rates can be compared against cell numbers (Figure 8) so that an assessment of growth phase can be made.

On addition of fresh fluid to the model cell death occurs for only a brief period (maximum 2 h), this is then very quickly reversed to growth, with the rate of growth increasing until by 7 h it has reached 0·5 s^{-1} (equivalent to a doubling time of 1·4 h). The rate then stays at approximately this level for a further 9 h and then falls during overnight growth, but does not reach zero, indicating that the cells are entering into, but do not quite reach, stationary phase. On the 2nd day the cells reach their maximum growth rate within 5 h, implying adaptation to their new environment. Once established in the model the growth rate of the cell is generally 0·5 s^{-1} (doubling time 1·4 h), compared with 0·2 s^{-1} (doubling time 3·45 h) which is found when *S. epidermidis* is grown in PDF in batch culture and stirred at the same rate as in the model. There might be several reasons why

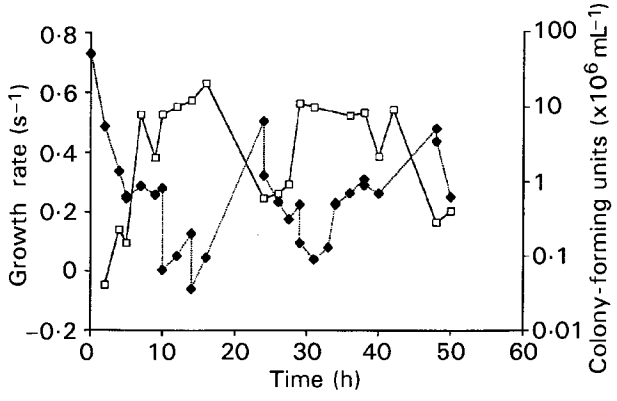

Figure 8. Calculated growth rates of *Staphylococcus epidermidis* 901 (□) compared with uncorrected viable counts (◆) in the continuous ambulatory peritoneal dialysis model using PDF.

the cells grow faster in the model than in batch culture, but one explanation could be that the dilution and fluid drainage processes both result in the selection of faster growing cells. This phenomenon might also arise in-vivo to a certain extent, because of the fluid exchanges, but the continual dilution occurring in the model will accelerate this process. There is no indication from these plots that the growth rate is affected by the fluid changes, implying that fresh fluid has no significant effect on cell viability.

This new model reveals several characteristics of *S. epidermidis* which could not have been readily predicted by other in-vitro techniques: the tenacity of the cells in a new and changing environment; the minimal effect of fluid changes on growth; and, perhaps most significantly, the extent of cell clumping. The growth environment is very important in dictating microbial characteristics, therefore a good in-vitro model will incorporate as many conditions found in-vivo as possible. This model has the potential to be used for investigating the behaviour of contaminating microorganisms in the dialysed peritoneum and provides the opportunity to harvest sufficient cells for the examination of surface characteristics.

Acknowledgements

This work was supported by the Biotechnology and Biological Sciences Research Council which provided a studentship to J. A. Brant (Reference 92300259).

References

Bint, A. J., Finch, R. G., Gokal, R., Goldsmith, H. J., Junor, B., Oliver, D. (1987) Diagnosis and management of peritonitis in CAPD. Report of a working party of the British Society for Antimicrobial Chemotherapy. Lancet 1: 845–849

Calme, W., Afram, C., Blÿleven, N., Hendrickx, R. J. B. M., Namavar, F., Beelan, R. H. J. (1992) Establishing an experimental infection model for peritoneal dialysis: effect of inoculum and volume. Peritoneal Dial. Int. 13: 579–581

Denyer, S. P., Davies, M. C., Evans, J. A., Finch, R. G., Smith, D. G. E., Wilcox, M. H., Williams, P. (1990) Phenotypic changes in staphylococcal cell surface characteristics associated with growth in human peritoneal dialysis fluid. In: Wadstrom, T., Eliasson, I., Holder, I., Ljungh, A. (eds) Pathogenesis of Wound and Biomaterial-associated Infections. Springer, London. pp 233–244

Farrell, S. C., Bailey, M. P. (1991) Measurement of creatinine in peritoneal dialysis fluid. Ann. Clin. Biochem. 28: 624–625

Heimbürger, O., Waniewski, J., Werynski, A., Sun Park, M., Lindholm, B. (1994) Dialysate to plasma solute concentration (D/P) versus peritoneal transport parameters in CAPD. Nephrol. Dial. Transplant 9: 47–59

Holmes, C. J., Evans, R. C., Vonesh, E. (1989) Application of an empirically derived growth curve model to characterize *Staphylococcus epidermidis* biofilm development on silicone elastomer. Biomaterials 10: 625–629

Imholtz, A. L. T., Koomen, G. C. M., Struijk, D. G., Arisz, L., Krediet, R. T. (1992) Residual volume measurements in CAPD patients with exogenous and endogenous solutes. Adv. Perit. Dial. 8: 33–38

Imholtz, A. L. T., Koomen, G. C. M., Struijk, D. G., Arisz, L., Krediet, R. T. (1993) Effect of dialysate osmolarity on the transport of low-molecular weight solutes and proteins during CAPD. Kidney Int. 43: 1339–1346

Krediet, R. T., Struijk, D. G., Boeschoten, E. W., Koomen, G. C. M., Stouthard, J. M. L., Hoek, F. J., Arisz, L. (1989) The time course of peritoneal kinetics in continuous ambulatory peritoneal dialysis patients who develop sclerosing peritonitis. Am. J. Kidney Dial. 8: 299–307

Lambert, P. A., Shorrock, P. J., Aitchison, E. J., Domingue, P. A., Power, M. E., Costerton, J. W. (1990) Effect of in vivo growth conditions upon expression of surface protein antigens in *Enterococcus faecalis*. FEMS Microbiol. Immun. 2: 51–54

MacDonald, W. A., Watts, J., Bowner, M. I. (1986) Factors affecting *Staphylococcus epidermidis* growth in peritoneal dialysis fluid. J. Clin. Microbiol. 24: 104–107

Modun, B., Williams, P., Pike, W. J., Cockayne, A., Arbuthnott, J. P., Finch, R., Denyer, S. P. (1992) Cell envelope proteins of *Staphylococcus epidermidis* grown in vivo in a peritoneal chamber implant. Infect. Immun. 60: 2551–2553

Pike, W. A., Cockayne, A., Webster, C. A., Slack, R. C. B., Shelton, A. P., Arbuthnott, J. P. (1991) Development and design of a novel in vivo chamber implant for the analysis of microbial virulence and assessment of antimicrobial therapy. Microb. Pathog. 10: 443–450

Rubin, J., Nolph, K. D., Popovich, R. P., Moncrief, J. W., Prowant, B. (1979) Drainage volumes during CAPD. ASAIO J 52: 54–60

Rubin, J., Nolph, K. D., Arfania, D., Prawart, B., Brown, P., Moore, H. (1981) Protein losses in continuous ambulatory peritoneal dialysis. Nephron 28: 218–221

Smith, D. G. E., Wilcox, M. H., Williams, P., Finch, R. G., Denyer, S. P. (1991) Characterization of cell envelope proteins of *Staphylococcus epidermidis* cultured in human peritoneal dialysate. Infect. Immun. 59: 617–669

Stelin, G., Rippe, B. (1990) A phenomenological interpretation of the variation in dialysate volume with dwell time in CAPD. Kidney Int. 38: 465–472

Twardowski, Z. J., Nolph, K. D., Prowant, B., Ryan, L., Moore, H., Nielsen, M. (1987) Peritoneal equilibration test. Perit. Dial. Bull. 7: 138–147

Vonesh, E. F., Lysagh, M. J., Moran, J., Farrell, P. (1991) Kinetic modelling as a prescription aid in peritoneal dialysis. Blood Purif. 9: 246–270

Wilcox, M. H., Smith, D. G. E., Evans, J. A., Denyer, S. P., Finch, R. G., Williams, P. (1990) Influence of CO_2 on growth and antibiotic susceptibility of coagulase-negative staphylococci cultured in human peritoneal dialysate. J. Clin. Microbiol. 28: 2183–2186

Wilcox, M. H., Finch, R. G., Smith, D. G. E., Williams, P., Denyer, S. P. (1991) Effects of CO_2 and sub-lethal levels of antibiotics on adherence of coagulase-negative staphylococci to polystyrene and silicone rubber. J. Antimicrob. Chemother. 27: 577–587

Williams, P., Denyer, S. P., Finch, R. G. (1988) Protein antigens of *Staphylococcus epidermidis* grown under iron-restricted conditions in human peritoneal dialysate. FEMS Microbiol. Lett. 50: 29–33