Characterization of experimental *Staphylococcus epidermidis* peritonitis in chronically uremic mice.

by
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Staphylococcus epidermidis peritonitis in chronically uremic mice

Abstract

A mouse model of surgically induced renal failure was utilized to investigate the pathogenesis of Staphylococcus epidermidis peritonitis which is a frequent and serious complication of continuous ambulatory peritoneal dialysis (CAPD). Compared to sham-operated controls, chronically uremic mice were more susceptible to intraperitoneal S. epidermidis inoculation, presenting decreased survival time and survival (10^9 cfu, 10^8 cfu), delayed bacterial clearance and attenuated peritoneal inflammatory response (10^6 cfu). In mice bearing a peritoneal catheter implant, the catheter was a preferred site for peritoneal bacterial persistence up to one month after intracatheter inoculation. Despite in vitro cytotoxicity of commercial peritoneal dialysis solutions toward peritoneal leucocytes, repeated peritoneal instillation of dialysis solutions did not influence S. epidermidis recoveries following inoculation. Although the mouse preparation did not undergo peritoneal dialysis, these studies nevertheless demonstrate that chronic uremia and the peritoneal catheter may be important etiological factors in the development and persistence of CAPD peritonitis.

Résumé

Un modèle d'insuffisance rénale induite chirurgicalement chez la souris a été utilisé pour investiguer la pathogénèse de la péritonite à Staphylococcus epidermidis qui est une complication fréquente et sévère de la dialyse péritonéale ambulatoire continue (DPAC). En comparaison avec les animaux contrôles chirurgicaux, les souris urémiques chroniques étaient plus susceptibles à l'inoculation intrapéritonéale de S. epidermidis, présentant une réduction du temps de survie et de la survie (10^9 cfu, 10^8 cfu), une clairance bactérienne retardée et une réponse inflammatoire péritonéale atténuée (10^6 cfu). Chez les souris porteuses d'un cathéter péritonéal implanté, le cathéter était un site préférentiel pour la persistance bactérienne péritonéale jusqu'à un mois après l'inoculation bactérienne. Malgré la cytotoxicité in vitro des solutions commerciales de dialyse péritonéale envers les leucocytes péritonéaux, l'instillation péritonéale répétée de solutions de dialyse n'a pas influencé le recouvrement de S. epidermidis après inoculation. Bien que la préparation animale ne fut pas soumise à la dialyse péritonéale, ces études démontrent néanmoins que l'urémie chronique et le cathéter péritonéal peuvent être d'importants facteurs étiologiques dans le développement et la persistance de la péritonite de DPAC.

For my grandfather,

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I would like to thank my supervisor, Dr. Raymonde Gagnon for giving me the opportunity to research my thesis subject. During my residency in her laboratory, her guidance, patience and enthusiastic support were sincerely appreciated. I would like to express my sincerest thanks to Dr. Geoffrey Richards under whose direction the microbiological aspects of this study were conducted. I gratefully acknowledge the members of my thesis committee, Drs. N. Christou, M. Levy, G. Richards and E. Skamene, who contributed their time and expertise to the successful completion of this research. I would also like to thank Dr. M. Kaye and Dr. S.I. Vas for their interest and constructive advice during this study.

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counting and *in vitro* assessment of macrophage chemotaxis in the laboratory of Dr. M.M. Stevenson.

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Preface

In accordance with the "Guidelines Concerning Thesis Preparation" of the Faculty of Graduate Studies and Research, papers which have already been published or which have been submitted for publication have been incorporated into the thesis. This format for thesis preparation was approved by the Division of Experimental Medicine, Department of Medicine. The following is quoted directly from "The Guidelines Concerning Thesis Preparation".

> "The Candidate has the option, subject to the approval of the Department, of including as part of the thesis the text of an original paper, or papers, suitable for submission to learned journals for publication. In this case the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation. Additional material (experimental and design data as well as descriptions of equipment) must be provided in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported. Abstract, full introduction and conclusion must be included, and where more than one manuscript appears, connecting texts and common abstracts, introduction and conclusions are required. A mere collection of manuscripts is not acceptable; nor can reprints of published papers be accepted.

While the inclusion of manuscripts co-authored by the Candidate and others is not prohibited by

McGill, the Candidate is warned to make an explicit statement on who contributed to such work and to what extent, and Supervisors and others will have to bear witness to the accuracy of such claims before the Oral committee. It should also be noted that the task of the External Examiner is made much more difficult in such cases, and it is in the Candidate's interest to make authorship responsibilities perfectly clear".

Each paper included in this thesis (Chapters 3, 4, 5, 6, Appendix 2) has its own Abstract, Introduction, Materials and Methods, Results and Discussion. References are included at the end of the papers and are not compiled elsewhere in the thesis. The papers are directly interrelated and were therefore, found not to require connecting texts. The Abstract, General Introduction and General Discussion relate to the combined work presented in all the manuscripts. The references cited in the General Introduction and General Discussion are compiled at the end of the thesis.

I am the first and primary author of the manuscripts represented in Chapters 3 to 6 of this thesis. I designed and executed all experimental assessments and the subsequent discussion of results represents my analysis and interpretation of the experimental outcome relative to the objectives of each phase of this work. Each of the papers prepared for publication is coauthored by my thesis supervisor, Dr. Raymonde F. Gagnon. The manuscript appearing in Section 6.1 is also coauthored by Dr. Mary M. Stevenson in whose laboratory I received instruction in methods for peritoneal leucocyte harvesting, differential counting and in

vitro maintenance. Papers presented in section 3.2, 4.1, 4.2, 5.1, 5.2 and 6.2 are coauthored by Dr. Geoffrey K. Richards in whose laboratory all microbiological evaluations were conducted and who supervised this aspect of the thesis research. I performed all of the procedures described except for hematology assessments which were conducted by Gerry Bibeault and the preparation of histological specimens for light microscopy was conducted by the department of pathology in association with Dr. W. Duguid. I consulted with Dr. J. Lough, also of the pathology department, for interpretation of scanning electron micrographs. On an ad hoc basis, as time and resources permitted, Donna Toman and Maryann van den Hoeven assisted me with animal surgery.

Appendix 1 contains a report entitled "Peritonitis in continuous ambulatory peritoneal dialysis: Retrospective analysis using computerized patient data files" that was prepared for Abbott Laboratories Ltd. (by R.F. Gagnon, B. Gallimore, K. Langer, M. Kaye). My contribution to this report was the collation, tabulation and interpretation of data presented in Tables 1 to 12. My specific interest in the inquiry was the CAPD peritonitis experience of patients of the Montreal General Hospital dialysis unit. Dr. Gagnon is the first author and I am the coauthor of the paper entitled "Characterization of a mouse model of chronic renal failure" which appears as Appendix 2. This paper integrated the results of numerous experiments which were conducted by Dr. Gagnon and/or myself.

Table of Contents

Chapter 1

General Introduction

This thesis includes a number of papers already published or submitted for publication, each of which has addressed a separate, albeit interrelated, aspect of the thesis topic. Therefore an introduction to the overall thesis subject has not been comprehensively presented. The first section of this chapter is presented to familiarize the reader with peritoneal dialysis and the following sections introduce the development of continuous ambulatory peritoneal dialysis (CAPD) and demonstrate the importance of peritonitis as a major treatment complication by reviewing its prevalence, pathogenesis and clinical consequences. Finally, the potential etiological agents in the development and persistence of CAPD peritonitis are introduced and the outcomes of reported clinical and basic experimental investigations of the etiology of CAPD peritonitis are reviewed.

This general introduction is intended to provide the clinical background and rationale for the subsequent experimental assessments of this thesis. The mouse model which was developed and characterized for this investigation was not, in fact, subjected to CAPD. However, the roles of several defined variables, associated with CAPD treatment, in the development and progression of peritoneal infection were evaluated utilizing the described animal preparations.

1. Peritoneal Dialysis

1.1 Peritoneal physiology and solute transfer

The peritoneal membrane covers visceral organs, forms the visceral mesentery that connect loops of bowel (visceral peritoneum) and reflects over the inner surface of the abdominal wall (parietal peritoneum). The peritoneum is a continuous, closed sack that has a total surface area of 1 to 2 m^2 in most adults (1). The surface of the membrane is formed by a thin layer of mesothelial cells which are covered by numerous microvilli. The underlying interstitium contains extracellular fluid, connective tissue, blood vessels and lymphatics (1, 2).

The peritoneum is a semipermeable membrane which is utilized during peritoneal dialysis. A given volume of dialysis solution of known chemical composition and tonicity is instilled into the peritoneal cavity thereby creating a fluid pool into which solutes and body water can move across the peritoneal membrane. In contrast to artificial dialysis membranes which are employed for hemodialysis, the peritoneum is a living membrane across which molecules of varying molecular weight can equilibrate. Solute exchange occurs between the blood of the peritoneal microcirculation and the dialysis solution dwelling in the peritoneal cavity. Solutes diffuse across the membrane at a rate that is dependent on (a) their concentration difference across the peritoneum, (b) the solute permeability characterisitics of the membrane and (c) the rate of dialysate flow. Peritoneal solute transfer can be increased by instilling hypertonic dialysis solutions

which cause solute movement by convection or solute drag (1, 2). Peritoneal ultrafiltration which is accomplished by establishing an osmotic pressure gradient across the peritoneal membrane, provides adequate control of body fluid volume.

Peritoneal dialysis solutions are formulated to regulate body fluid volume, the concentration of electrolytes and endogenous metabolites as well as the acid base balance in end-stage renal disease patients. In order to meet these requirements, peritoneal dialysis solutions contain glucose as an osmotically active agent, the major serum electrolytes in quantities to normalize serum levels and either acetate or lactate buffers. These solutions are manufactured having pH levels between 5.0 and 5.8 in order to reduce the generation of glucose breakdown products during sterilization (3).

Glucose is used exclusively as the osmotically active agent of commercially available peritoneal dialysis solutions. Since glucose is absorbed from the peritoneal pool it is not an ideal osmotic agent. However, other compounds tested for this purpose (eg. fructose, xylitol, dextran) have either been too expensive, toxic or both (2). Dialysis solutions having dextrose concentrations ranging from 0.5 to 4.25% and respective osmolarities from 295 to 500 mOsm/kg H_2O are available. According to the patient's body weight and blood pressure the appropriate dialysis solutions are selected for dialysis.

Dialysis solution electrolytes include sodium, calcium, magnesium and chloride while potassium is absent. Lactate is the buffer most frequently used in peritoneal dialysis

solutions. Bicarbonate containing solutions are not available because of the potential problem of precipitate formation through reaction of bicarbonate with calcium (3).

1.2 Historical development of peritoneal dialysis

The historical development of peritoneal dialysis has been reviewed by several authors (2, 4-6). The evolution of this form of renal replacement therapy is underlined by the mutual contributions of clinical investigation and basic animal research. As early as 1894 the peritoneum was described as a semipermeable membrane across which bidirectional solute exchange between the peritoneal cavity and the blood was demonstrated (7). Subsequent animal studies demonstrated that movement across the peritoneal membrane of dextrose, urea, chloride and various dyes in solution was not accomplished by active transport but rather, diffusion was dependent on concentration gradients and osmotic forces (8).

The first clinical effort to remove uremic substances by peritoneal dialysis was reported in 1923 (9). After a single peritoneal instillation of 1.5 liter physiological saline there was a transient improvement in the symptoms of an acute renal failure patient. Throughout the 1930's a number of unsuccessful attempts of peritoneal dialysis including the first efforts to maintain patients which chronic uremia were reported however, peritoneal dialysis remained a poorly understood experimental treatment of uremia. In 1946 the results of experiments conducted in anephric dogs demonstrated that peritoneal dialysis did have practical clinical applications (10-13). Utilizing this animal model the permeability characteristics of the peritoneal membrane and the factors affecting diffusion across the peritoneum

were determined. Survival of anephric dogs to 10 days post nephrectomy demonstrated that peritoneal dialysis was an efficacious treatment of acute uremia. On the basis of these and previous studies the first patients underwent continuous peritoneal irrigation. Two peritoneal catheters were used, one for inflow and the other outflow, and continuous peritoneal lavage utilized a large volume dialysis solution reservoir. This method of peritoneal dialysis was employed by most investigators from 1938 to 1959 however treatment was frequently complicated by leakage and peritonitis (4).

During the late 1940's insight was gained into the appropriate composition of peritoneal dialysis solutions. Initially unbuffered saline or Ringer's solution, with or without dextrose, was used for peritoneal dialysis (2, 5, 6). These solutions contained excessive concentrations of sodium and chloride while bicarbonate (or acetate, lactate) was absent. As a result, clinical complications of peripheral or pulmonary edema, hypertension, hyperchloremia and metabolic acidosis were reported (6). The concentrations of sodium and chloride were subsequently reduced and bicarbonate was added. Dextrose was used as an osmotic agent and was added in various concentrations.

In the early 1950's peritoneal dialysis was still considered an experimental procedure that was largely limited by equipment and technical difficulties. As a last resort treatment for terminal uremia, its use was frequently complicated by pulmonary edema, peritonitis and death (6). Large volumes of dialysis solution were required for each dialysis which presented technical problems and difficulty

in maintaining sterile technique. In 1959, commercially prepared sterile peritoneal dialysis solution in 1 liter glass bottles became available and intermittent peritoneal dialysis (IPD) became a technically simpler method of treatment (14). Peritoneal dialysis solution contained in two, 1 liter bottles connected by a sterile Y set was instilled into the peritoneal cavity where it would dwell for 30 minutes to 1 hour before drainage and subsequent instillation of fresh dialysis solution.

By the late 50's and early 60's IPD had become an accepted treatment for acute uremia however complications of peritonitis were common. The frequent connection and disconnection of the Y set for each dialysis exchange was thought to favor peritoneal contamination and led to the development of semiautomatic and automatic cycling machines which were bacteriologically safer (2, 6). The introduction of the reverse osmosis machines which produced sterile non pyrogenic water with a low electrolyte content that was mixed with a dialysate concentrate eliminated the requirement for large volume dialysis solution reservoirs. These machines were costly and more difficult to operate than other cyclers however they did reduce the technical difficulties of IPD and the risk of peritonitis.

Since its inception the bottle neck of peritoneal dialysis has been peritoneal access. During its development an assortment of both rigid and soft peritoneal catheters were tried unsuccessfully resulting in leakage, infection, or obstruction caused by kinking, clots of blood or fibrin and omental wrapping. In 1959 a polyvinyl chloride catheter was

introduced that had multiple side perforations and was transversely ridged to prevent kinking and omental wrapping (15). It was however technically difficult to place intraperitoneally and required a trocar. In 1964 the same catheter with a pointed stylet at the end for piercing the abdominal wall was introduced (16). The stylet catheter (Trocath) is currently the standard catheter for acute dialysis or for temporary peritoneal access in chronic renal failure patients.

The successful application of IPD in acute renal failure led to repeated IPD for the maintenance of chronic renal failure patients however, permanent peritoneal access presented significant problems (4, 6). Initial attempts to leave the Trocath in place for multiple dialysis sessions were unsuccessful. Placement of a plastic conduit for repeated peritoneal access was also unsuccessful and resulted in infection around the conduit, recurrent peritonitis, peritoneal adhesions and death. During the early 1960's long term peritoneal dialysis utilizing a variety of devices for repeated access met with discouraging results. Peritonitis was a major complication and marked protein loss, malnutrition and progressive wasting also complicated treatment (6). Repeated episodes of peritonitis caused peritoneal adhesion formation with partial or more extensive obliteration of the peritoneal cavity thereby decreasing dialysis efficacy. Most patients died within a few months to a year of treatment (6). Because of the difficulties of maintaining a permanent peritoneal access site, repeated abdominal puncture with the Trocath was practiced for long

term IPD.

In 1968 the Tenckhoff catheter became available for chronic peritoneal dialysis (17). With this device long term access to the peritoneal cavity could be maintained and infectious complications were reduced. This silastic catheter had numerous side perforations at the distal end and two Dacron cuffs proximally, one to be sutured just outside the peritoneum and the other in the subcutaneous tunnel before entry into the peritoneal cavity. Tissue grew into the Dacron cuffs thereby fixing the catheter in place. To minimize catheter dislocation and obstruction due to omental wrapping the basic catheter design underwent a number of modifications, one of which included two silastic discs which were placed near the distal end of the catheter (Oreopoulos-Zellerman catheter) (18). In contrast to the Tenckhoff catheter, the Oreopoulos-Zellerman device required surgical implantation. Both of these catheters are currently used for chronic peritoneal dialysis.

Although peritoneal dialysis had evolved into an accepted renal replacement therapy for chronic renal failure patients a major disadvantage was a considerably longer treatment duration compared to hemodialysis (peritoneal dialysis: 3 sessions of 12 to 14 hours per week, hemodialysis: 3 sessions of 6 hours per week) (19). In 1976 continuous ambulatory peritoneal dialysis (CAPD) was introduced by Popovich, Moncrief, Nolph et al (20, 21). Early experience with CAPD utilized 1 to 2 liter glass bottles of sterile dialysis solutions which was technically awkward and the frequent connect/disconnect sequences

contributed to high rates of peritonitis. In 1978 sterile dialysis solutions in polyvinyl chloride bags were introduced and CAPD became technically simpler and rates of peritonitis decreased (2).

2. Continuous Ambulatory Peritoneal Dialysis

2.1 CAPD: Theoretical considerations and practice.

Factors affecting solute transfer during peritoneal dialysis also apply to CAPD however solute transfer characteristics during CAPD require some special consideration due to the longer peritoneal dwell of the dialysis solutions. In contrast to IPD in which dialysis solutions dwell in the peritoneal cavity for 0.5 to 1 hour, peritoneal dwell times of 4 to 8 hours are common during CAPD. When hypertonic solutions are instilled into the peritoneal cavity body fluid and solutes move into the peritoneal pool. These movements will procede with time until fluids and solutes equilibrate across the peritoneal membrane. During the peritoneal dwell dextrose is absorbed from the dialysis solution in the peritoneal cavity and the peritoneal pool gradually loses hypertonicity and therefore ultrafiltration capacity. Dialysis solutions having 1.5% and 4.25% dextrose reach isoosmolarity after 4 and 6 hours of peritoneal dwell respectively (22). With continuing peritoneal dwell accumulating body solutes and metabolic end products will transfer into the peritoneal pool and water and dialysate molecules can efflux from the dialysate into the body pool (3).

Studies investigating the equilibration of peritoneal dialysis solutions during long dwell exchanges have demonstrated that small molecules reach equilibrium within 2 to 3 hours and transfer of larger molecules follows an almost linear peritoneal accumulation for at least 8 hours of peritoneal dwell (22). In comparison to IPD, dialysate flow

rates during CAPD are significantly reduced and solute clearance is decreased however, this is compensated for by increasing the total dialysis time from 36 hours per week for IPD to 168 hours per week for CAPD (9). CAPD thereby effects efficacious clearance of small molecules and clearance of larger molecular weight substances is improved compared to other conventional dialysis treatments.

CAPD is a self treatment, the practice of which entails the introduction of a large volume (usually 2 liters) of sterile dialysis solution contained in a polyvinyl chloride bag into the peritoneal cavity via a transfer set and a permanently dwelling silicone rubber catheter. The dialysis solution which is warmed to body temperature before peritoneal instillation dwells within the peritoneal cavity for 4 to 8 hours and is then drained by gravity into the original dialysis bag. In order to limit the frequency of set connect/disconnect sequences most CAPD programs instruct patients to clamp off the bag after peritoneal instillation of dialysis solution and to roll the empty bag into a pouch that is worn around the waist of the patient. At drainage, the bag is unrolled and the clamp is opened. Drainage is normally completed in 10 to 15 minutes and under aseptic conditions the patient disconnects the transfer set from the used dialysis bag and connects to a fresh bag of dialysis solution. This procedure is repeated three to four times a day, seven days a week.

CAPD has a number of practical advantages that include monetary and geographical factors. CAPD does not require a large initial capital investment and cost per patient is either less than (Can., U.K.) or equal to (U.S.) that of hospital

hemodialysis (2). Furthermore it is practiced by the patient at home and requires a relatively short training period of 1 to 3 weeks. In those areas where dialysis facilities are limited; CAPD offers an alternate therapeutic modality however, a CAPD program must be supported by a hemodialysis facility (23).

CAPD also offers a number of clinical advantages compared to hemodialysis which include improved clearance of larger molecular weight substances, increased hemoglobin levels, improved management of hypertension and improved continuous biochemical control, in spite of fewer fluid and dietary restrictions (23, 24). CAPD is the first choice dialysis therapy for the old or diabetic renal failure patient as well those patients with serious cardiovascular disease or poor vascular access. Small children presenting end-stage renal disease are also good candidates for CAPD (2, 23, 25). Although data is limited, estimates of actuarial survival of CAPD patients were similar to those for conventional hemodialysis patients (26).

Treatment complications encountered during the clinical management of end-stage renal disease patients with CAPD include hyperphosphatemia, hypertriglyceridemia, hyperglycemia in diabetes, continual loss of protein which increases markedly during peritonitis, chronic constipation, hypotension and weight gain (23, 24). Efforts to resolve some of these problems include the use of phosphate binders and dietary management. However a frequent and potentially serious complication of CAPD that is the leading single cause of treatment failure is peritonitis (23, 24, 26-29). All CAPD patients are at risk of developing this complication and future

success of this treatment modality depends on the adequate management and prevention of peritonitis.

2.2 CAPD demographics

Continuous ambulatory peritoneal dialysis is the practiced method of renal replacement therapy for a large proportion of chronic renal failure patients. In Canada in 1985, CAPD was practiced by 1208 end-stage renal disease patients, accounting for 28.4% of all renal replacement therapy (30). Since 1981 the percentage of new patients in Canada who began CAPD as their initial dialysis treatment modality has remained relatively constant and has ranged between 42 and 45%. These statistics exceed the CAPD experience in Europe, where in 1985 8.2% of dialysis patients were practicing CAPD (31). Attitudes toward first choice renal replacement therapy however, vary greatly between and within countries in Europe such that in contrast to the European rate of 8.2%, CAPD accounted for 25 to 30% of dialysis treatment in the United Kingdom (32) and although the overall rate of CAPD treatment was 6% in Italy, some centers reported that more than 50% of new patients' first renal replacement treatment was CAPD (33). The American CAPD statistics lie between the Canadian and European experience and it was estimated that in 1985, 15.6% of U.S. dialysis patients or 11,200 patients practiced CAPD (2).

A large proportion of patients practicing CAPD will be subsequently transferred to hemodialysis for long term maintenance. Of those new end-stage renal disease patients beginning CAPD in the U.S., 66.9% of them will be maintained by this form of renal replacement therapy at the end of their first year of dialysis and this percentage will decrease to

45.4% at the end of their second year (34). The reasons for patient transfer from CAPD to hemodialysis were as follows:

Medical reasons, unrelated to dialysis	39%
Excessive peritonitis	27%
Patient choice	15%
Poor fluid/biochemical control	10%
Socioeconomic factors & other	9%

Similar results were reported in the 1983 Canadian Renal Failure Register (35). Approximately 27% of CAPD treatment failures in Canada and the U.S. were attributed to peritonitis which clearly demonstrates the importance of this treatment complication as the single leading cause for the discontinuation of CAPD. Furthermore, those patients who continue to practice CAPD will go on to present frequent episodes of peritonitis. Examining the U.S. statistics by life table analysis, after 3 months 25% of CAPD patients will have experienced their first episode of peritonitis and by 12 months this statistic will increase to 66%. Estimates of peritonitis rates vary between centers and by year but, national averages from the Canadian (35) and U.S. (34) registry of 1.2 and 1.4 episodes per patient year respectively, have been reported. The results of our analysis of CAPD patients of the Montreal General Hospital practicing CAPD between 1980 and 1984 revealed an average peritonitis rate of 1.4 episodes per patient year, which compares favorably with American and Canadian statistics (Appendix 1, Table 6).

2.3 CAPD peritonitis: Causative microorganism, diagnosis and treatment

The actual source of the bacterial contaminant responsible for a subsequent peritonitis episode is, in most cases unknown (29, 36). However, possible routes of bacterial entry into the peritoneal cavity include a) the peritoneal catheter either via luminal or periluminal contamination with skin commensals, b) transmural migration of enteric microorganisms, c) introduction of endogenous bacteria via the hematogenous route and/or peritoneal lymphatics and d) via the genital tract in female CAPD patients (19, 28). Peritoneal contamination by exogenous bacteria is thought to occur primarily via the peritoneal catheter and is attributed to a break in sterile technique during the patient's dialysis exchange procedure, or to bacterial colonization along the subcutaneous catheter tract to the peritoneal cavity (19, 28).

The proposed sources of peritoneal contamination are supported by the nature of microorganisms recovered from CAPD patients presenting peritonitis (27-29, 36). A majority of CAPD peritonitis episodes are caused by gram positive cocci from the skin and Staphylococcus epidermidis is most frequently isolated in culture and accounts for 50-60% of the microbiologically confirmed cases. Enteric bacteria account for a much smaller proportion (25-30%) of peritonitis events and when mixed organisms are recovered bowel perforation may be responsible. Anaerobic peritonitis is rare and fungal infections account for only a small percentage (1-2%) of peritonitis episodes (28). These statistics compare favorably

with those describing the microbiological distribution of peritonitis episodes presented by CAPD patients of the Montreal General Hospital (Appendix 1, Table 9). Peritonitis caused by skin borne gram positive cocci accounted for 68.3% of cases and 59.3% of episodes were caused by Staphylococcus epidermidis. Enteric microorganisms accounted for a much smaller proportion of the microbiologically confirmed peritonitis episodes.

The signs and symptoms of CAPD peritonitis do not conform to the criteria with which surgical peritonitis is recognized. Secondary bacterial peritonitis patients are considered critically ill and manifestations include hypovolemia which may lead to severe hypotension and death, hypoxia due to upward diaphragmatic displacement and reflex abdominal rigidity, ileus and septic shock (37). Although in some cases peritonitis may be a life threatening complication of CAPD, most episodes of CAPD peritonitis are treated while patients remain ambulatory and symptoms are much less severe than those of secondary bacterial peritonitis. Diagnosis of CAPD peritonitis is based on the presentation of two of the following features: abdominal pain, elevated peritoneal leucocyte count (greater than 100 per mm^3) causing visibly cloudy peritoneal fluid and microbiological culture of the effluent dialysate. Culture positive blood cultures are not usually observed in CAPD peritonitis while 30% of patients with surgical peritonitis present bacteremia (28). The relative severity of symptoms and prognosis of CAPD peritonitis vary with the nature of the infecting organism ranging from symptoms of mild abdominal pain and cloudy fluid with Staphylococcus epidermidis peritonitis to prolonged more severe pain and cloudy fluid with Staphylococcus

aureus (28) or abcess formation and loss of functional peritoneal membrane with Pseudomonas aeruginosa (24).

As clinical experience with CAPD and CAPD peritonitis grows, improved diagnostic methods and treatment strategies continue to evolve. Microbiological diagnosis of CAPD peritonitis relies on culture of the peritoneal dialysate effluent. Initially after the introduction of CAPD there were frequent occurrences of "sterile" peritonitis where patients presented cloudy fluid and abdominal pain yet no bacterial growth was recovered in peritoneal fluid. Diagnostic methods have improved however, and the use of large volume culture and the practice of filtering effluent dialysate to concentrate bacteria and remove antimicrobial compounds have increased the likelihood of bacterial recovery from patients presenting peritonitis (28, 36).

When a CAPD patient presents clinical signs and symptoms of peritonitis antimicrobial therapy commences immediately. Antibiotic administration is frequently intraperitoneal via the inflowing dialysis solution thereby achieving high concentrations of antibiotic at the site of the infection. Broad spectrum antibiotics, (eg. Cephalothin, Tobramycin) are prescribed presumptively and are altered if the results of microbiological sensitivity warrants another antimicrobial therapy. The duration of treatment remains controversial however, in most centers uncomplicated peritonitis is successfully treated in 10 to 14 days which corresponds to a treatment schedule where antibiotics continue for 7 days after the effluent dialysate is clear to visual examination (24, 38). Recently it has been suggested that a longer duration of

treatment may be required to prevent relapses, particularly of those peritonitis episodes caused by S. epidermidis (38). In cases of persistent or recurrent peritonitis and episodes caused by fungal contamination, removal of the peritoneal catheter is indicated after which symptoms most often resolve (24, 28, 36). This treatment strategy requires that patients be at least temporarily maintained with hemodialysis however, recently in a small group of CAPD patients persistent peritonitis was successfully resolved by catheter removal with immediate replacement and resumption of peritoneal dialysis (39). Previously, treatment of peritonitis included repeated peritoneal lavage with large volumes of peritoneal dialysis solution. This approach was discontinued due to the possible loss of immune reactants from the peritoneal cavity as well as the proposed inhibitory effect of low pH and hyperosmolarity of the dialysis solutions on peritoneal host defense and on antibiotic activity (28).

There are numerous serious consequences of CAPD peritonitis. In addition to patient morbidity and hospitalization, CAPD peritonitis may cause significant changes in peritoneal function and morphology and in severe persisting episodes death may ensue (19, 28, 37, 40-42). During peritonitis the ultrafiltration capacity of the peritoneal membrane is temporarily lost as a result of increased vascular permeability and absorption of the osmotic agent from the peritoneal pool. Concurrently protein loss to the dialysis solution is markedly increased frequently leading to a negative nitrogen balance in the patient. Peritoneal infection and inflammation may also cause peritoneal adhesion and abscess

formation which may compromise the dialyzing capacity of the membrane. Recently the development of peritoneal sclerosis has been observed in CAPD patients and proposed etiological factors include discrete episodes of peritonitis and/or smoldering subclinical infections (44-51). This serious, sometimes fatal complication is characterized by sclerotic thickening of the peritoneal membrane causing disruption of the mesothelium which is transformed into a dense layer of fibroconnective tissue infiltrated with focal aggregates of mononuclear and polymorphonuclear leucocytes. As a result, the ultrafiltration capacity of the peritoneal membrane is impaired or may be abolished with subsequent overhydration and increased uremia. In severe cases the small bowel may be totally encapsulated and partial or complete intestinal obstruction as well as fecal peritonitis have been reported (47).

2.4 *Staphylococcus epidermidis* characteristics and host immune response

Historically, coagulase activity of the staphylococci has been associated with virulence. More recently the pathogenicity of coagulase-negative staphylococci has been demonstrated, and while coagulase activity acts as a marker of staphylococcal virulence, it is not an absolute virulence determinant. *S. epidermidis* is a common skin commensal that is currently regarded as the primary pathogen in infections associated with prosthetic devices. Normally of low virulence, prerequisites for the development of *S. epidermidis* infections include immunosuppression or breaks in host defense caused by surgery, catheter placement or prothesis insertion (52).

Difficulties regarding the taxonomic classification of coagulase-negative staphylococci have hampered a better understanding of the pathogenesis of infections caused by these microorganisms. Several different classification schemes have been utilized with varying degrees of practical success (53-55). Phage typing of *S. epidermidis* has been limited by the inability to type all clinical isolates while biotyping may be slow and is limited by a small number of phenotypes and the extent of homogeneity of human strains (53, 54). Antibiogram typing has been utilized and, while multiple antibiotic resistance is a common feature of disease causing strains of *S. epidermidis*, specific antibiotic resistance patterns for classification have not been clearly demonstrated (53, 55-57). Nevertheless, the combination of antibiogram and API

Staph-Ident identification of S. epidermidis may be useful for the classification of clinical isolates (54). Recently, plasmid pattern analysis of coagulase-negative staphylococcal isolates from CAPD patients with peritonitis has been utilized for classification and may provide a useful epidemiological marker for future isolates (58).

Taxonomic classification does not necessarily predict the virulence of a clinical S. epidermidis isolate. Biotyping of clinical isolates of S. epidermidis demonstrated that a large proportion (64%) of disease causing strains belonged to Baird-Parker biotype 1 (59) and extracellular enzyme activity (deoxyribonuclease, lysozyme and several proteases) was greatest for the respective organisms (60). However, no single virulence determinant has been identified that is solely responsible for pathogenicity of S. epidermidis. The possession of multiple toxins, enzymes and cell wall components by S. epidermidis as well as changes in host resistance may collectively determine the virulence of this microorganism.

2.4.1 Staphylococcal armaments

The staphylococci possess numerous characteristics which facilitate colonization of a wide variety of host tissues. The versatility of this microorganism is demonstrated by the utilization of a variety of substrates, during aerobic or anaerobic culture conditions, and growth occurs over a wide range of temperature and pH levels (61, 62). Furthermore, several extracellular products of the staphylococci specifically contribute to successful colonization within the host.

The production of a polysaccharide containing matrix (glycocalyx) which encapsulates the outer cell wall of S. epidermidis may be an important virulence determinant of staphylococci (63-65). In addition to mediating the attachment of the bacteria to smooth surfaces of prosthetic devices (Section 3.3), the glycocalyx may have a direct inhibitory effect on host defense mechanisms. Impaired opsonization and phagocytosis of encapsulated staphylococci has been demonstrated (66, 67) and in vitro exposure of human neutrophils to a S. epidermidis "slime" preparation resulted in a decreased bactericidal activity (68) and lymphocyte mitogen response was inhibited (69). Furthermore, in vitro assessments demonstrated that slime producing S. epidermidis adhering to segments of an intravenous catheter, survived cidal antibiotic concentrations that would have eliminated nonslime-producing strains (70).

The staphylococci produce a variety of extracellular, enzymatically active factors which have potent effects on the

host and which contribute to the infectious process (60, 71-73). For example, the degradation of the intercellular ground substance of connective tissue by staphylococcal hyaluronidase facilitates tissue dispersion. In addition, the extracellular production of staphylococcal catalase may be of particular importance for the inactivation of bactericidal hydrogen peroxide produced by host leucocytes. Studies comparing extracellular enzyme production of clinical S. epidermidis isolates with isolates from healthy individuals have demonstrated that a larger proportion of clinical isolates produced several enzymes including deoxyribonuclease, staphylokinase and lipase-esterases (74). Other exoenzymes which may be important in the pathogenesis of S. epidermidis infections include phosphatase, gelatinase, lysozyme and β-lactamase.

The staphylococci also produce several cytolytic toxins (alpha, beta, delta hemolysins) which have profound effects on host tissue. Delta hemolysin is the most common hemolysin produced by S. epidermidis and has been demonstrated in a large proportion of clinical isolates (60, 75). Hemolytic and leucocidal activities of delta hemolysin have been demonstrated and in vitro tissue culture assessments have revealed cytopathic effects (74, 76). Furthermore, delta hemolysin production by S. epidermidis was associated with the elaboration of several other extracellular enzymes (74).

The variety of staphylococcal extracellular products and the range of their respective effects within the host mediate considerable adaptability to changing environments. In vitro

assessments of factors contributing to the pathogenesis of staphylococcal infections may not necessarily reflect *in vivo* interactive conditions within the host. There is evidence to suggest that the elaboration of several virulence determinants may be regulated by culture media and/or *in vitro*/*in vivo* growth conditions (77-79).

2.4.2 Staphylococcal surface antigens

Peptidoglycans (mucopeptides) and techoic acids are the primary constituents of the cell wall of S. epidermidis and constitute the major antigenic determinants of this microorganism. Peptidoglycan is a primary cell wall component of most gram positive bacteria. It is a macromolecule composed of a network of cross linked, alternating β-1,4-linked N-acetyl-D-glucosamine and N-acetylmuramic acid through which the peptide moiety is linked (80, 81).

The immunogenicity of peptidoglycan has been extensively studied and the induction of both humoral (antipeptidoglycan antibody) and cell-mediated (delayed type hypersensitivity, lymphocyte blastogenesis) immune responses by peptidoglycan have been demonstrated (82-87). Peptidoglycan has demonstrated a wide range of biological properties which include complement activation by both the classical and alternative complement pathways (83, 88), suppression of leucocyte chemotaxis and phagocytosis (89) as well as the induction of histamine release from human basophils (90). Furthermore, staphylococcal peptidoglycan presented endotoxin like characteristics, causing fever, leucopenia, thrombocytopenia and hypovolemia after intravenous injection as well as a local Shwartzman reaction following intradermal injection in rabbits (86).

The cell wall of S. epidermidis is also comprised of α or β-glucosyl glycerol techoic acids which penetrate into the peptidoglycan layer of the cell wall from the cell membrane (91, 92). The extensive structural variety of techoic acids

within the cell wall of staphylococci contributes to their antigenic specificity (91, 93). Highly specific antitechoic acid antibodies have been demonstrated in animal models and clinical conditions of serious staphylococcal infections (91, 94, 95).

Other staphylococcal surface antigens that have been identified include lipotechoic acids of the cell membrane, which extend far enough into the cell wall structure to act as cell surface antigens (91). In addition, encapsulated staphylococci present antiphagocytic surface antigens, the elaboration of which may be regulated by culture conditions (78, 79, 96). Several serologically distinct capsular antigens have been identified in encapsulated S. epidermidis clinical isolates (64).

2.4.3 Host defense against staphylococcal infection

i Phagocytosis and intracellular killing

Once staphylococci have breached the skin and/or mucous membranes of the host, the most important arm of host defense is leucocyte phagocytosis and intracellular killing. Resident tissue macrophages form the first line of this defense which is followed by the rapid influx of polymorphonuclear leucocytes. Although both types of phagocytes contribute to this aspect of host defense, polymorphs are the more effective phagocytic cells demonstrating superior ingestion and intracellular killing of staphylococci (97, 98). Phagocytic leucocytes are chemotactically directed toward the infectious focus from which eminates soluble chemotactic factors. Complement activation generates important neutrophil chemotaxins (C3a, C5a) and activation of complement by cell wall components of the staphylococci as well as by staphylococcal proteases has been demonstrated (83, 88, 89).

Recognition of the bacterium by the phagocyte is mediated by serum opsonins which become firmly fixed to bacterial surface structures, thereby rendering the microorganism susceptible to phagocytic ingestion (100). Both heat stable and heat labile opsonins are crucial for optimal staphylococcal phagocytosis. Heat stable opsonins are comprised of immunoglobulins of the IgG class (101-104), and the heat labile constituent is the C3b component of the complement system (103-106). Heterogeneous opsonic requirements among staphylococcal strains have been reported

(107), however, recent investigation of the opsonic requirements of several S. epidermidis strains, recovered from peritoneal fluid of CAPD patients presenting peritonitis, revealed the consistent requirement of both IgG and C3b opsonins for efficient phagocytosis (108).

The peptidoglycan moity of the staphylococcal cell wall has been revealed as the primary target of the IgG opsonin (66, 109). Although, antitechoic acid antibodies have been demonstrated in sera of patients presenting staphylococcal infections, these constituents do not appear to have a major role in staphylococcal opsonization (110). The generation of the C3b opsonic constituent may proceed by the classical and/or alternative pathways of complement activation. Cell wall peptidoglycan may activate complement by both pathways while techoic acid can do so by the classical pathway only (83, 88, 99, 110). Compared to activation by the alternative pathway alone, opsonization proceeded more rapidly when complement was activated by both pathways (107, 111).

Plasma fibronection may also serve as a staphylococcal opsonin (112-115). Fibronectin in a nonantibody, noncomplement, protein which may become covalently bound to S. aureus (115). However, fibronectin alone is a poor staphylococcal opsonin and its relative importance to staphylococcal opsonization may be minor compared to IgG and C3b opsonins (113). Nevertheless, fibronectin may have an important modulating influence on other opsonic serum factors, thereby functioning as a cofactor for opsonization. Thus fibronectin and complement depleted serum was associated

with a marked reduction in staphylococcal phagocytosis compared to fibronectin or complement serum depletion alone (112, 114).

The attachment of opsonized bacteria to the phagocyte is mediated by cell surface receptors which are specific for either the Fc region of the IgG molecule or the C3b opsonic constituent (100, 107, 116). The interaction between opsonins and surface receptors effects changes in the phagocyte membrane which induce membrane and cytoplasm movements that lead to engulfment of the bacterium. Once staphylococci are ingested and within the cytoplasmic phagolysosome, intracellular killing is accomplished by oxygen dependent and oxygen independent mechanisms. The oxygen dependent bactericidal mechanism is crucial to intracellular staphylococcal killing (117, 118). In response to phagocytosis, leucocyte oxygen uptake increases with a shift in metabolism to the hexose monophosphate shunt which generates staphylocidal hydrogen peroxide, hydroxyl radicals and halide ions (via the halide-myeloperoxidase system).

Evidence to support the requirement of the oxygen dependent pathway for intracellular staphylococcal killing comes from studies of neutrophil function in chronic granulomatous disease patients (117-119). Leucocytes from such patients do not respond to phagocytosis with increased oxygen uptake or shift in metabolism to the hexose monophosphate shunt and hydrogen peroxide is not produced. Staphylococci are phagocytized normally, but intracellular killing is not effective. Furthermore, catalase production

or failure of the engulfed bacterium to produce hydrogen peroxide promoted survival within the chronic granulomatous disease leucocyte, while hydrogen peroxide producing strains underwent halogenation of the bacterial cell wall. In this latter case, the bacterial hydrogen peroxide acted as substrate for the leucocyte myeloperoxidase system which effected intracellular killing.

Although oxygen independent mechanisms may act in concert with the oxygen dependent bactericidal pathways, these systems alone, are not bactericidal against intact staphylococci (117, 118). Oxygen independent systems include low pH within the phagolysosome, lysozyme which hydrolyzes the cell wall peptidoglycan, lactoferrin which binds iron and prevents its bacterial utilization, and cationic proteins several of which are active against staphylococci.

ii Acquired Immunity

Humoral immune response of the host is the most important arm of acquired immunity and is constituted by antitoxin and antistaphylococcal immunity. The production of toxin-neutralizing antibody readily occurs during staphylococcal infection, however antitoxin antibody does not necessarily change the course of a staphylococcal infection (96).

All species of animals have a high degree of resistance to staphylococcal infection and antistaphylococcal antibody has been revealed in sera of normal subjects (84, 113). Numerous animal studies have demonstrated that staphylococcal vaccines provide protection against rechallenge with the homologuous strains and that type specific immunity can be passively transferred by immune serum. Significantly, the degree of immunity correlated with enhancement of phagocytosis (81, 96, 120, 121). In order to identify the exact nature of the protection inducing antigen, passive immune transfer experiments were conducted in which specific staphylococcal constituents were used to absorb immune sera prior to transfer. Accordingly cell wall components and particularly peptidoglycan, were identfied as important protection inducing antigens (110, 122).

Antibody response to staphylococcal infection has been investigated in animal models as well as during clinical infection and elevated titres of both immunoglobulin classes IgM ad IgG have been reported (77, 84, 95, 123, 124). In a rabbit model of endocarditis, antibody titres were elevated

six days following bacterial challenge and were sustained in culture positive animals to 31 days after inoculation (sacrifice day) (77). Injection of heat killed S. epidermidis invoked elevated antibody titers which peaked five weeks post injection and thereafter declined (123). Subsequently, a booster injection generated a markedly increased and prolonged antibody response.

Delayed type hypersensitivity to staphylococcal antigens has also been demonstrated in animal models (83, 85, 125-127) and in human subjects presenting recurrent infections (128). However, the relative importance of cell-mediated immunity in staphylococcal infection remains unclear since studies of T cell depleted mice did not reveal increased susceptibility to staphylococcal infection (126). It is possible that cell-mediated acquired immunity may have a role in the chronic, smoldering or recurring staphylococcal infections that have been associated with prosthetic devices.

3. Peritoneal Host Defense and the Etiology of CAPD Peritonitis

3.1 Host defense of the peritoneal cavity

Under normal conditions the principal route by which particulate matter, including bacteria, is absorbed from the peritoneal cavity is via the diaphragmatic lymphatics. Bacteria appear in thoracic lymph within minutes of experimental intraperitoneal inoculation (37). Phagocytic and bactericidal functions of fixed tissue macrophages, reticuloendothelial cells of the spleen or liver as well as free macrophages and polymorphs are then directed towards bacteria in the thoracic lymph nodes and the systemic circulation (37, 129). In contrast to secondary bacterial peritonitis, CAPD peritonitis rarely leads to bacteremia and therefore, the importance of lymphatic absorption in bacterial clearance from the peritoneal cavity of CAPD patients is questionable. It has been proposed that mechanical distention and disruption imposed by peritoneal dialysis may interfere with normal mechanisms of lymphatic absorption of bacteria (129).

Host defense mechanisms of the peritoneal cavity invoke specific humoral and cell mediated immune responses as well as non specific phagocytosis and intracellular bacterial killing (37, 129, 130). Resident peritoneal macrophages form the first line of peritoneal defense against bacterial infection. These cells are phagocytic and importantly, play a key role in the initiation and regulation of immune responses. In response to bacterial contamination of the peritoneal cavity, resident

macrophages are stimulated to produce Interleukin-1 which induces lymphocyte proliferation and differentiation and which triggers amplification of the immune response. Alternately, production of prostaglandin by macrophages down regulates further amplification. Concurrently, during an incident of bacterial contamination of the peritoneal cavity the complement cascade is activated and chemotactic, opsonic and vasoactive substances are generated. In response to chemotactic stimuli there is a directed emigration of phagocytic leucocytes into the peritoneal cavity with polymorphs appearing within a few hours of contamination followed by an influx of activated macrophages several hours later (37, 129, 130). Opsonic molecules are produced at the inflammatory site and also accompany the effusion of leucocytes and fluid into the peritoneal cavity. Following phagocytic uptake by host leucocytes, the bacterium is contained within a membrane bound phagosome which fuses with a lysosome containing a variety of bactericidal and bacteriostatic factors (37, 129, 130). Within the polymorph phagolysosome, in addition to other antibacterial substances, the myeloperoxidase system generates a series of potent bactericidal compounds (hydrogen peroxide, superoxide, singlet oxygen and hydroxyl radicals) which effect bacterial killing.

As a consequence of host bacteria interactions inflammatory products are released from phagocytic leucocytes which initiate local serosal injury and increased vascular permeability. Fibrinogen containing plasma is thereby released into the peritoneal cavity where fibrinogen is converted to

fibrin. Since peritoneal inflammation inhibits the normal mesothelial fibrinolytic activity, fibrinous adhesions persist until fibroblasts lay down collagen thereby forming fibrous adhesions which function to localize and minimize the spread of the contamination (37). Consequently peritoneal surfaces may adhere and abscess formation may ensue (37, 129).

In studying the etiology of CAPD peritonitis, both patient characteristics as well as treatment variables should be considered. The practice of CAPD imposes a marked alteration of the peritoneal environment of end-stage renal disease patients such that normal host defenses of the peritoneal cavity may be compromised. Normally the peritoneal cavity contains 50 to 100 ml of fluid which provides lubrication of viscera as they slide over one another (130). Peritoneal fluid cellularity approximates 3000 cells per ml of which the cellular constituents are primarily resident macrophages. With the practice of CAPD, large volumes of hypertonic, acidic dialysis solution are instilled into the peritoneal cavity. While the predominant peritoneal cellular constituent continues to be the macrophage, peritoneal cellularity is markedly reduced to approximately 6 cells per ml in CAPD effluent solution (130). In addition to cellular components, soluble immune reactants may be diluted and lost from the peritoneal cavity as a consequence of repeated instillation and drainage of dialysis solutions. In comparison to serum concentrations, effluent dialysis solutions collected from CAPD patients presented significantly reduced levels of opsonic molecules (130). Furthermore, the CAPD catheter is a

permanently dwelling foreign body which may also contribute to impaired local host immune response (52).

CAPD patients are a highly heterogeneous population and present a variety of characteristics, including chronic renal failure, which may influence immune response. All CAPD patients are at risk of developing peritonitis, however subgroups within this population present higher incidences of peritonitis than do others. The characteristics of this higher risk group of patients have been the subject of several investigations. It has recently been reported that opsonic activity of effluent dialysis solutions of CAPD patients with a history of peritonitis was reduced compared to those recovered from patients that had not experienced peritonitis (131). Also in contrast to CAPD patients with a low rate of peritonitis, peritoneal macrophages of high incidence patients presented increased suppressor activity during in vitro lymphocyte blastogenesis, producing large amounts of prostaglandin E_2 and decreased levels of Interleukin-1. In vitro bactericidal activity against S. epidermidis was also decreased in peritoneal macrophages of high incidence patients (132).

The heterogenous nature of patient characteristics, treatment variables as well as peritonitis experience of CAPD patients complicates the clinical investigation of the pathogenesis of CAPD peritonitis. Thus, the decreased opsonic capacity of effluent dialysis solution and the increased suppressor activity of peritoneal macrophages of high risk CAPD patients may account of their increased incidence of peritonitis. Alternatively, these features may be caused by

anatomical or biological modification of the peritoneal cavity resulting from peritonitis. The further investigation of these and related controversies could be reasonably addressed in controlled experiments utilizing animal models having the appropriate control groups.

3.2 Chronic uremia

Chronic uremia has been described as "nature's immunosuppressive device" (133). An increased prevalence of serious infections has been reported in patients presenting chronic renal insufficiency receiving regular dialysis treatment such that 18-38% of patient fatality is attributed to infection and 60% of surviving patients suffer from serious infections (134-138). Numerous aspects of host defense mechanisms may be impaired in end-stage renal disease patients which, in addition to humoral and cell mediated immunity, include renal failure related alterations of the skin barrier, tenacious mucus and crusting mucous membranes of the tracheobronchial tree, impaired mucociliary clearance mechanisms as well as gastric hypoacidity (134-136).

Clinical investigations of humoral and cell mediated immune responses of chronic dialysis patients have frequently generated conflicting and inconclusive results. The relative influence of chronic uremia and dialysis treatment on host immune response remains unclear. Furthermore, the heterogenous nature of the patient population in terms of underlying disease, duration of renal failure, drug therapy, previous surgery or blood transfusion, and nutritional status may account for some of the existing controversy (138). Studies examining the influence of uremia on antibody synthesis in response to a variety of antigens have generally demonstrated the production of somewhat diminished yet protective antibody titres (136, 138, 139). However, the response of hemodialysis children to live attenuated virus

vaccines was reduced (135). With the exception of decreased circulating levels of IgE, immunoglobulin levels were reported within the normal range in chronic hemodialysis patients in spite of a reduction in the absolute numbers of B lymphocytes (136-138). Diminished levels of complement factor 3 have also been reported in both hemodialysis and to a lesser extent peritoneal dialysis patients while other components of complement were within the normal range (137, 140). In the literature many studies addressing cell mediated immune response in end-stage renal disease patients present evidence of impairment including lymphopenia, with normal relative proportions of B and T lymphocytes, and cutaneous anergy, while in vitro assessments have demonstrated decreased mixed lymphocyte culture reactions and inhibition of the mitogenic response of lymphocytes in the presence of uremic serum (135-138).

Animal models of chronic renal failure have been useful in the investigation of immune response in uremia since clinical variables reflecting the heterogenous nature of patients are eliminated and appropriate control groups can be included in experimental assessments. Numerous studies have been conducted in the rat model of renal failure (5/6 nephrectomy) and impairments of cell mediated immune response have been demonstrated including impaired mitogen response, decreased mixed lymphocyte reaction and reduced graft versus host response (141-152). These impairments were attributed to a suppressor cell, perhaps a macrophage, whose activity may have been increased in renal failure animals. Other studies utilizing intact animals have demonstrated prolonged

allograft survival and impaired delayed type hypersensitivity reactions in chronic renal failure animals (153, 154). Animal models of chronic uremia have been of particular importance in demonstrating the immunosuppressive effect of sham surgery. These observations have clearly defined the necessity of including sham-operated control animals in studies of immune response (138, 143).

Leucocyte phagocytosis and bactericidal function must also be considered in the study of immune response in chronic renal failure. Investigations that have addressed this aspect of host immune response, however, have generated conflicting results which remain inconclusive. Abnormalities of granulocyte function in end-stage renal disease patients on hemodialysis have been demonstrated, however the contribution of mechanical trauma resulting from extracorporeal circulation through hemodialysis machinery to the observed deficit remains unknown (136). Most studies have revealed normal phagocytosis by polymorphs of uremic patients however results describing bactericidal capacity are controversial (136, 155). Furthermore in vivo assessments in renal failure rats have demonstrated that leucocyte mobilization toward an inflammatory focus as well as circulating leucocyte response to bacterial challenge may be impaired in chronic uremia (155, 156).

All of the components of immune response that have been investigated in chronic renal failure patients and animal models may, if impaired, adversely affect the host's ability to resist bacterial and viral infection. Obviously the most direct method of assessing immunocompetence in the

chronically uremic host is by direct challenge with viable microorganisms. Although few such animal studies have been reported, direct bacterial challenge of rats failed to reveal a marked reduction in host resistance to experimentally induced infections with Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Clostridium welchii (138, 157, 158). Thus, although uremia has been associated with an increased prevalence of serious infection, direct experimental evidence identifying uremia as the primary factor increasing host susceptibility to infection does not as yet exist.

3.3 The permanently dwelling CAPD peritoneal catheter

The role of the peritoneal catheter in the etiology of CAPD peritonitis may be twofold. As a foreign body, the catheter may exert on immunosuppressive influence and/or it may provide a smooth surface within the host for bacterial colonization. In cases of persistent or recurrent CAPD peritonitis the infection frequently resolves once the peritoneal catheter is removed from the patient (36, 39). This finding demonstrates the importance of the peritoneal catheter in the persistence, if not in the development, of CAPD peritonitis.

The increased risk of bacterial infection in the vicinity of foreign implants has been well documented however, the pathogenesis of foreign body infection is not well understood (159). Such infections are characterized by (a) the increased infectivity of a small inoculum, frequently staphylococci, which in the absence of the foreign body would be effectively eliminated by host defense mechanisms, (b) their slow, prolonged evolution, (c) their site specific infection, exclusively in the vicinity of the foreign body and (d) their persistence without removal of the prosthesis (160). In a series of key experiments conducted in an animal model, the underlying mechanism of defective host defense at the site of a foreign implant was extensively investigated (159-161). *In vitro* phagocytosis and bactericidal function of polymorphs harvested from sterile tissue cage implants of guinea pigs was reduced compared to that of peritoneal or circulating polymorphs. This impairment was attributed to the continuous

interaction of polymorphs with a nonphagocytosable foreign body. Constant phagocyte stimulation and degranulation would eventually exhaust phagocytic and bactericidal activity of the polymorphs. When fresh blood polymorphs were introduced into the tissue cage implants, at the time of Staphylococcus aureus inoculation, there was a significant reduction in the rate of infection. Furthermore, in contrast to polymorphs harvested from sterile tissue cages, those recovered from infected tissue cages presented normal phagocytosis and bactericidal activity, perhaps reflecting their emigration from the peripheral blood. Nevertheless, the lag time between bacterial contamination and polymorph infiltration into the infected site may be sufficient to allow other pathogenic factors to effect bacterial infection.

Adhesion of bacteria on to mammalian tissue surfaces is recognized as an important initial step in the pathogenesis of an infectious process and may be similarly important in the development of infections associated with prosthetic implants (162, 163). S. epidermidis which is notoriously associated with foreign body infections, including those of intravenous (164-167) and peritoneal (168, 169) catheters, may be uniquely adapted to adhere to smooth surfaces. In contrast to routine laboratory cultures or contaminants, clinical isolates of S. epidermidis from catheter associated infections frequently produced an extracellular polysaccharide (slime) (52). Slime production by bacteria enhances adherence to smooth surfaces and may be a critical factor in the pathogenesis of S. epidermidis infections of medical implants (52, 166, 170). In an animal model of S. epidermidis foreign body infection, slime-

production was demonstrated to be an important virulence determinant while slime-producing and non slime-producing strains had similar virulence in the absence of a prosthetic implant (65).

The kinetics of intravenous catheter colonization with slime-producing S. epidermidis has been examined by scanning electron microscopy (171, 172). Initially (5 to 30 minutes after exposure) single cocci adhered preferentially to any surface irregularities that were present on the otherwise smooth catheter. Microcolonies of cocci, in a single layer on the catheter surface, were seen 1 hour after exposure and heavily colonized surfaces with multiple layers of cocci embedded in a "slimy" material are observed after 6 to 12 hours. The amount of this amorphous cementing material clearly increased with prolonged incubation time (171). Recently, naturally infected intravenous (165, 166) and peritoneal catheters (168, 169) have also been examined by scanning electron microscopy and the existence of S. epidermidis embedded in a cementing "slimy" matrix, referred to as biofilm has been documented.

In addition to enhancing adherence to smooth surfaces, staphylococcal slime production may also directly interfere with host defense mechanisms (66-70). The combination of slime production by S. epidermidis and immunosuppression in the vicinity of the foreign implant may generate favorable conditions for bacterial colonization of implanted prosthetic devices. Host defenses and antimicrobial therapy may be ineffective once the bacterial biofilm has been established on the prosthesis surface and resolution of the infection may then require removal of the medical implant.

3.4 Peritoneal dialysis solutions

The non physiological characteristics of peritoneal dialysis solutions as well as the prolonged exposure of the peritoneal cavity to large volumes of these solutions during CAPD has led to the investigation of their influence on peritoneal host defense. Following peritoneal instillation the pH of the peritoneal dialysis solution pool reached physiological levels after approximately 30 minutes of peritoneal dwell while isoosmolarity was achieved after 4 to 6 hours (22, 173). In addition to being hypertonic and having low pH, dialysis solutions may contain unidentified constituents generated during sterilization or compounds leached out of the plastic bag. Therapeutic agents (eg. heparin, insulin) are also frequently added to the inflowing dialysis solution. All of these substances may affect peritoneal immune responses to varying degrees.

Results of <u>in vitro</u> assessments have demonstrated that exposure of circulating and peritoneal leucocytes to peritoneal dialysis solutions inhibited phagocytosis and bactericidal activity (173, 174). Furthermore, leucocytes were rendered non viable after incubation, ranging from 30 minutes to 3 hours, in fresh dialysis solution (174, 175). The deleterious effect of dialysis solutions decreased with increasing peritoneal dwell time however, dialysate effluents collected up to two hours after peritoneal instillation significantly impaired leucocyte phagocytosis (174). After dwelling in the peritoneal cavity of a CAPD patient for 4

hours, peritoneal dialysis solutions had achieved physiologic osmolarity and pH and did not subsequently impair leucocyte function (174, 175).

Mechanical disruption of the peritoneal environment resulting from the peritoneal instillation of large fluid volumes may also interfere with host defense against bacterial infection. Presumably, increasing peritoneal volume dilutes soluble immunoreactive substances and reduces the frequency of bacteria phagocyte interactions. In vitro bactericidal activity of peripheral blood leucocytes against Escherichia coli decreased with increasing fluid volume (176). Furthermore, in rats subjected to intraperitonel E. coli inoculation, the LD_{50} decreased with increasing inoculation volume (176, 177) as did the rate of clearance of a non lethal inoculum (176).

The ability of peritoneal dialysis solutions to support bacterial growth and the efficacy of antimicrobial agents in the presence of dialysis solutions has also been investigated. Fresh dialysis solution was not a satisfactory medium for microbiological culture (178-180) however conditions for growth improved markedly with peritoneal dwell time (180). Antimicrobial activity was impaired in the presence of dialysis solution such that Tobramycin's capacity to reduce bacterial colony forming units was only 10% of its bactericidal capacity in standard nutrient broth (180). Furthermore, dialysis solutions severely compromised the opsonic and bacteriolytic functions of serum complement (181).

The non physiologic nature of dialysis solutions introduced in large volumes into the peritoneal cavity may generate an environment in which immune reactants are diluted, leucocyte viability and functions are challenged and antibiotic, opsonic as well as complement functions are impaired. Yet, only one study has been reported in the literature that has addressed the influence of dialysis solutions dwelling in the peritoneal cavity on the outcome of intraperitoneal bacterial inoculation in chronic renal failure animals. Assessments were conducted in the rat model of renal failure 24 hours after inoculation of 2×10^7 cfu *E. coli* and a moderate elevation in bacterial recovery from the spleen of those animals that had undergone peritoneal infusion of dialysis solution was demonstrated (158). Further more comprehensive investigations including differing microorganisms, inocula size and dialysis solution infusion volumes may be required to fully explore the *in vivo* implications of the results of *in vitro* assessments.

4. Efforts to Resolve CAPD Peritonitis

4.1 Technical advances in ancillary equipment

Since the introduction of CAPD, consistent efforts to resolve peritonitis have been successful in reducing the incidence of this complication. Factors which may have contributed to the achieved reduction in peritonitis rates include improved training programs, better equipment (particularly the introduction of plastic bags containing dialysis solutions), a better understanding of the sources of infection and improved therapeutic methods (182). Despite these improvements CAPD peritonitis has not been eliminated and remains a serious clinical complication.

The etiology of CAPD peritonitis and the factors that might contribute to its prevention remain largely unknown. Considerable efforts have focused on patient compliance to the sterile technique required for the dialysis solution exchange procedure (183). A number of different approaches to improve the connection between the tubing set and the dialysis bag have been devised and range from simple manually operated connections to more complex semiautomatic devices. The common objective of all designs has been to limit touch contamination of the tubing set during dialysis solution exchange.

Technically simple devices employ a shield around the junction between the tubing set and the dialysis bag which protects against touch contamination (184-186). Incorporated into some of these devices is a disinfectant, proviodine iodine, which is either applied to the open connection before

peritoneal instillation or to the closed junction with a sponge and clamp device. Of particular interest is a double bag system with a Y connecting set in which a disinfecting solution (25 to 50% hypochlorite) resides. This device has had more clinical success than the others tested (187-190). Clinical experience has been limited yet a small prospective controlled study has demonstrated a significant reduction of peritonitis in the group of patients using the Y connector compared to those practicing standard methods of CAPD. However, peritonitis was not eliminated and the prevalent causative organism in both groups of patients was Staphylococcus epidermidis (187). More complex devices combine a mechanical connector with an active sterilization process which have included thermal sterilization with electrical, flame or microwave heating (191-193) or sterilization with an ultraviolet sterilization system (194). In addition, unidirectional bacteria retentive filters in the tubing set have also been evaluated in attempts to improve peritonitis rates (195, 196).

Considerable effort has been expended on the development of new connection systems for CAPD and the results of in vitro assessments of these devices show that they may have an effective clinical application. However, to date, there is limited clinical experience with these devices and the lack of prospective randomized clinical trials makes evaluation difficult. Preliminary results suggest that although improved technology reduced the incidence of peritonitis, it may not eliminate this serious complication of CAPD.

4.2 Animal models

Animal models have a fundamental importance in medical research, offering *in vivo* systems for experimental investigations that could not be otherwise conducted. A primary objective of animal modelling is to mimic, as closely as possible, the pertinent clinical condition in a well defined and controlled experimental setting. In contrast to the heterogeneous nature of clinical conditions, animal model characteristics are less variable and the use of inbred animal strains further reduces interindividual variation.

Small rodents are frequently utilized for animal models in experimental medicine. Rats or mice are relatively inexpensive, are easily maintained, and animals of the same age and sex can be readily acquired in quantities sufficient for statistical evaluation. Inbred strains are genetically and immunologically well defined; characteristics which are of particular importance in animal models addressing host immune response. The scope of experiments based on inbred animals may be increased by utilizing different inbred strains for several distinct models.

In spite of these advantages, one must carefully scrutinize the extrapolation of results based on animal model experimentation to clinical situations. The homogenous nature of animal models clearly does not reflect the clinical setting and therefore, by design, the model does not exactly parallel clinical conditions. Nevertheless, animal models provide a simplified *in vivo* system which accommodates controlled and reproducible experiments and interpretation of results is not impeded by confounding clinical variables.

Several animal models of peritonitis have been described in which peritoneal infection has been induced by devascularization of a segment of intestine, intraperitoneal administration of fecal material or inoculation of pure bacterial culture (197). Clearly the development of an animal model of CAPD in which to investigate the development and persistence of peritonitis would be extremely useful. In addition to variables associated with patient management, the heterogenous nature of the dialysis patient population has hinded the interpretation of clinical investigations. An animal model would allow investigations to be conducted under controlled experimental conditions however, attempts to establish an infection free and otherwise thriving animal model of CAPD have been unsuccessful.

The primary obstacle in developing an animal model of CAPD in normal and chronic renal failure animals has been peritoneal access. In rabbits having normal kidney function spontaneous bacterial colonization and occlusion of the catheter was observed during the three weeks following peritoneal catheter implantation (198-199). Rats and guinea pigs having normal kidney function underwent short term peritoneal dialysis (48 hours) however hemoconcentration developed and there was a marked protein loss to the dialysis solution (200). Chronic renal failure rabbits were maintained on CAPD for 14 days during which time total plasma protein and body weight decreased significantly (201). Omental wrapping of the catheter and obstruction by fibrin clotting also complicated animal management. Anephric dogs survived 37 to 83 days with CAPD as renal replacement therapy however animals developed

peritonitis which required daily antibiotic administration and all dogs presented significant weight loss (202). In chronic renal failure rats CAPD was limited to 48 hours due to one way catheter obstruction such that peritoneal instillation of dialysis solution was accomplished but drainage was not possible (158).

Although chronic renal failure animals were successfully maintained with CAPD for varying periods of time, high concentrations of heparin and/or antibiotics were added to the dialysis solution in order to maintain a patent catheter and an infection free host. Furthermore spontaneous bacterial contamination of the peritoneal catheter was common. Although the developed animal models may be useful for certain investigations the addition of therapeutic agents to the dialysis solution and the development of spontaneous peritonitis render these animal preparations unacceptable for studies of controlled peritoneal bacterial challenge.

Chapter 2

Aims of the present study.

In spite of efforts to prevent the development of peritonitis it remains a major clinical complication of CAPD. Furthermore, clinical investigations have failed to clearly define the etiological agents which contribute to the development, persistence and recurrence of CAPD peritonitis. The development of an animal model in which to evaluate variables that may predispose to CAPD peritonitis in a controlled experimental system would be of obvious value. Although the animal preparation utilized for the subsequent investigation was not a model of CAPD, several variables associated with CAPD treatment were, nevertheless, represented.

The overall objective of the current study was to investigate the relative roles of uremia and CAPD treatment variables in the pathogenesis of peritoneal Staphylococcus epidermidis infection in a mouse preparation of chronic renal failure. The aims of each of the subsequent sections presented in the thesis are as follows:

1. To further characterize the mouse model of chronic renal failure and to expand the mouse model to include an infection free permanently dwelling peritoneal catheter implant.
2. To investigate the influence of chronic renal failure on response of mice to intraperitoneal S. epidermidis challenge. Response parameters include (a) survival time following large inocula as well as (b) the kinetics of bacterial clearance following a smaller

inoculum and (c) the peritoneal and systemic inflammatory response of mice following intraperitoneal inoculation.

3. To investigate the role of a permanently dwelling peritoneal catheter in the development and persistence of peritoneal S. epidermidis infection in mice and to study the influence of chronic renal failure on response of mice bearing a peritoneal catheter to peritoneal S. epidermidis challenge.
4. To investigate the ability of commercially available peritoneal dialysis solutions to maintain mouse peritoneal leucocytes (resident and elicited) during in vitro incubation.
5. To investigate the influence of repeated intracatheter peritoneal instillation of dialysis solutions on the response of sham-operated and renal failure mice to peritoneal S. epidermidis challenge.

Chapter 3

Establishing a mouse model of chronic renal failure in which to characterize response to intraperitoneal *Staphylococcus epidermidis* challenge

Section 3.1

Description and characterization of a mouse model for the investigation of the etiology of CAPD peritonitis

Presented in part at the 55th Annual Meeting of the Royal College of Physicians and Surgeons of Canada, Toronto, Canada, September 21-25, 1986

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Abstract

Controversy exists over the mechanism(s) by which continuous ambulatory peritoneal dialysis (CAPD) is complicated by peritonitis. This animal model was developed to characterize the microbiological, inflammatory and morphological responses of mice to peritoneal bacterial challenge and thereby provide a basis for the evaluation of factors that may predispose to peritonitis. The mouse model of renal failure was prepared by a two step surgical procedure beginning with electrocoagulation of the right renal cortex and followed two weeks later by left nephrectomy (renal failure) or surgical exploration of the left renal area (sham-operated). Two weeks after the second surgical procedure, a segment of a CAPD catheter was implanted entirely within the confines of the peritoneal cavity of mice and experimental assessments were conducted one month after catheter implantation. This animal preparation has demonstrated (1) marked retention of nitrogenous compounds, severe anemia and growth retardation which are prominent features of chronic renal failure, (2) that the preparatory surgical procedures as well as the intracatheter peritoneal instillation of dialysis solution was well tolerated and did not lead to contamination of the peritoneal cavity and (3) that repeated peritoneal instillation of dialysis solution did, however, produce a local inflammatory reaction. Having characterized the mouse model, further investigation of the mechanisms that initiate and contribute to the progression of CAPD peritoneal infection, and evaluations of antimicrobial treatment strategies can now be conducted.

Introduction

The management of end stage renal disease patients with continuous ambulatory peritoneal dialysis is frequently complicated by peritonitis (1,2), however the relative roles of renal failure, CAPD treatment variables and virulence determinants of the infecting microorganism in the pathogenesis of this serious complication remain to be defined. The common skin commensal, Staphylococcus epidermidis, causes a majority of CAPD peritonitis episodes (3-5) and is thought to gain access into the peritoneal cavity either by touch contamination when the permanent peritoneal catheter is open during dialysate exchange or by colonization from the skin along the subcutaneous catheter tract to the peritoneal cavity. Host defenses against bacterial infection which are impaired in the vicinity of foreign implants (6) may be further compromised in the presence of peritoneal dialysis solutions. Slime production by certain strains of S. epidermidis favors colonization of CAPD catheters by enhancing bacterial attachment to the catheter surface and interfering with host defense mechanisms (7,8).

Models of peritoneal dialysis in chronic renal failure and normal animals have been limited by uncontrolled peritoneal infection, obstruction of the peritoneal catheter and failure of experimental animals to thrive (9-12). In the current mouse model of renal failure, a segment of a CAPD catheter was implanted completely within the confines of the peritoneal cavity and fixed to the lateral abdominal wall. There was no

exit site of the catheter through the abdominal skin thereby maintaining the integrity of the peritoneal cavity. Transcutaneous injection into the catheter lumen was a simple procedure requiring light ether anesthesia of mice. This animal model was developed to investigate the role of a peritoneal catheter implant and repeated intraperitoneal instillation of peritoneal dialysis solution without peritoneal drainage, in the pathogenesis of peritoneal Staphylococcus epidermidis infections in renal failure mice. Although the peritoneal cavity of mice was repeatedly exposed to dialysis solution, CAPD was not actually conducted.

Materials and Methods

Preparation of animals

Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY, USA) at five weeks of age. The animals were allowed to acclimatize in holding facilities for one week prior to use. All animals had free access to water and mouse chow.

Renal failure:

The preparation of renal failure and sham-operated mice was accomplished by a two step surgical procedure which has been previously described (13). Briefly, six week old mice were anesthetized with ether and a small flank incision was made through which the right kidney was separated from the adrenal gland and freed of perirenal fat. Particular caution was taken to avoid trauma to the ureter. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, Calif., USA) and excluding a 2mm margin around the

renal pelvis, the exposed surface of the kidney was electrocoagulated. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Two weeks after right renal electrocoagulation, a left nephrectomy was done in those animals that would constitute the renal failure group and surgical exploration of the left renal area was conducted in sham-operated controls. In studies characterizing the mouse model of renal failure, normal control mice were also included in our assessments.

Catheter implantation:

The mouse model of chronic renal failure was expanded to include a permanently dwelling peritoneal catheter. Two weeks elapsed between nephrectomy or sham surgery and peritoneal implantation of a custom made segment (length: 12 mm, outer diameter: 5 mm) of an Oreopoulos-Zellerman CAPD catheter (Accurate Surgical Instruments Co., Toronto, Canada). Both ends of the segment were bevelled and six perforations were regularly spaced along the catheter length (Figure 1). According to the manufacturer's specifications, catheter segments were washed in mild detergent, rinsed several times in distilled water and gas sterilized. Through a left flank incision the catheter segment was placed entirely within the confines of the peritoneal cavity and was secured to the lateral abdominal wall by a single anchoring suture passing through one of the side perforations. The free end of the catheter was directed towards the left lower quadrant of the abdominal cavity. The incision was closed with a running suture through the deep layers and surgical clips were applied

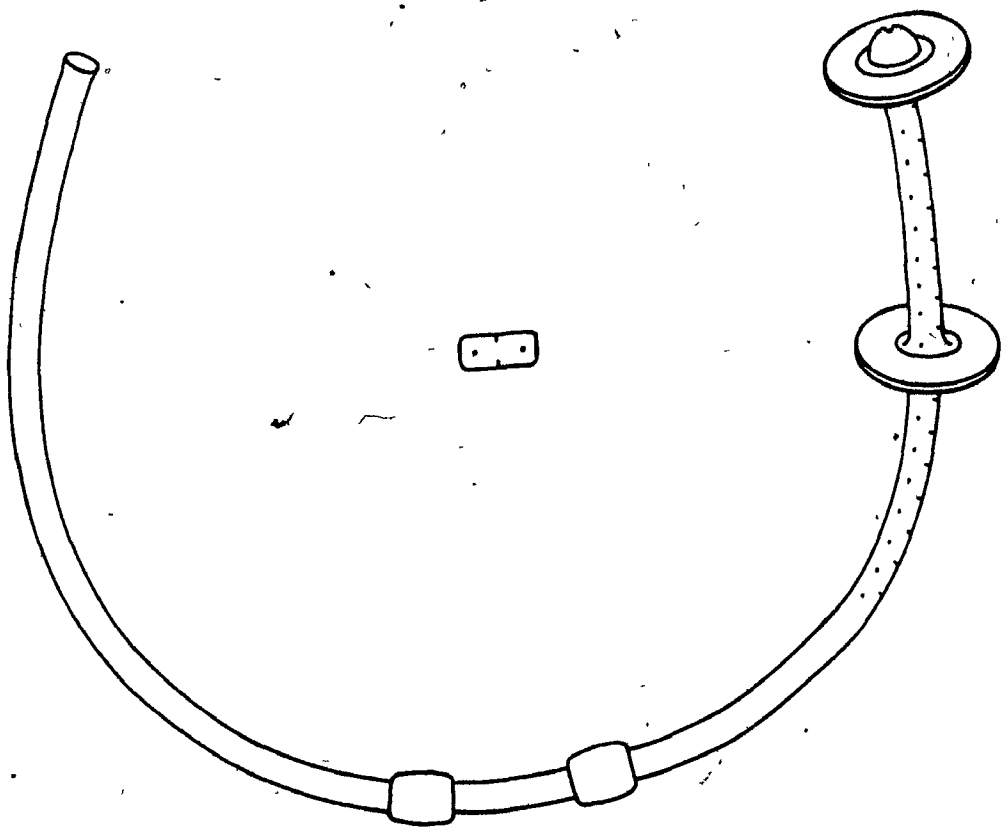

Figure 1. A schematic representation demonstrating the relative size of the catheter segment for mice and the Oreopoulos-Zellerman peritoneal dialysis catheter used for CAPD.

to the skin. Four to six weeks later animals were used for experimentation.

Access to the peritoneal cavity:

Under light ether anesthesia, the catheter segment was manipulated to palpate the lumen cavity. The abdomen was washed with alcohol and a 25 gauge needle was passed through the abdominal wall into the lumen of the free end of the peritoneal catheter (Figure 2). During bacterial challenge experiments a 1 ml inoculation volume was thus injected transcutaneously into the catheter lumen. Peritoneal instillation of larger volumes of peritoneal dialysis solution was similarly administered through the catheter lumen and was conducted daily for periods of up to two weeks. Dialysis solution containing 4.25% dextrose (Abbott Laboratories Ltd, Montreal, Canada) was prewarmed to 37°C before peritoneal infusion and a total daily instilled volume of 3 ml was administered once or devided into two equal infusions, morning and evening. Body weight of mice was determined daily throughout the infusion procedure.

Blood analysis

At sacrifice, mice were anesthetized with ether, weighed and exsanguinated by cardiac puncture. Blood was collected in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for biochemistry and hematology analysis. Blood urea nitrogen (BUN) levels were determined with an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass., USA). Routine hematology was done utilizing a Coulter counter (Model 2B1 Coulter Electronics Inc., Hialeah, Fla.,

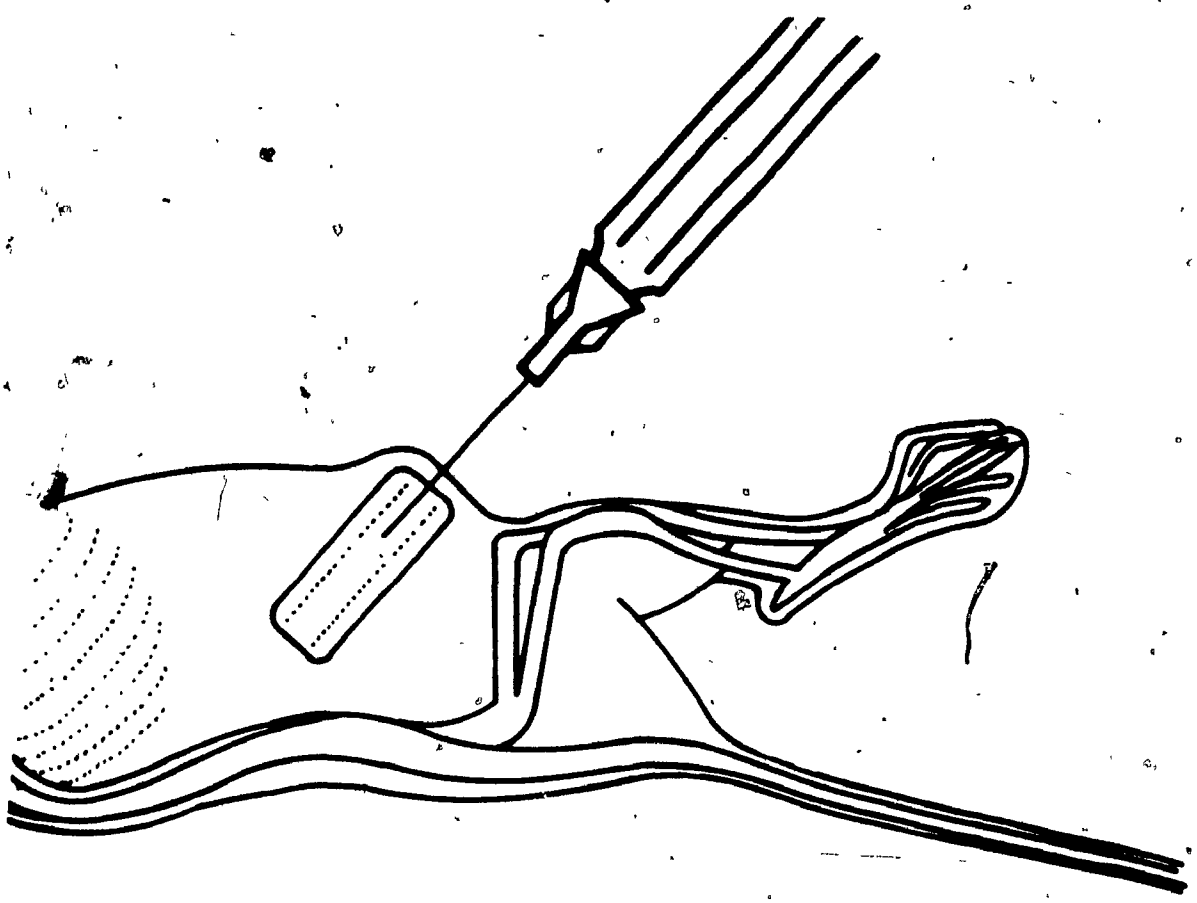

Figure 2. A schematic representation of transcutaneous injection into the lumen of the peritoneal catheter segment in mice. Drawing made from a lateral decubitus X-ray.

USA) and differential cell counts were performed on Wright-stained blood smear preparations.

Collection and processing of specimens for microbiological assessment

The abdomen of each animal was washed with alcohol and the skin and loose connective tissue were retracted aseptically to expose an intact translucent abdominal wall (parietal peritoneum constituting the inner lining). Ten ml of minimum essential medium (Eagle Modified) containing 10% heat inactivated fetal calf serum and 20 mM HEPES buffer was injected through the abdominal wall directly into the peritoneal cavity and the peritoneal washout was slowly aspirated. Cellularity of each peritoneal washing was enumerated in a Neubauer counting chamber and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, Ill., USA). An aliquot of each sample was quantitatively cultured onto 5% horse blood Columbia agar for bacterial content and identity.

A specimen of the exposed abdominal wall (approximately 2 x 2 cm) was excised aseptically and was homogenized in 2 ml phosphate buffered saline (PBS). The peritoneal catheter segment was removed by careful dissection of any adhering tissue and by cutting the anchoring suture to the lateral abdominal wall. Fluid within the catheter lumen was aspirated and total cellularity determinations as well as cytocentrifuge preparations for differential cell counts were conducted as for peritoneal washings. Granulation tissue always formed a

continuous sheath around the catheter segment. This tissue was aseptically removed and homogenized in 1 ml PBS. Homogenized tissue specimens were inoculated onto 5% horse blood Columbia agar.

Recovered catheter segments were assesed microbiologically following a modification of a previously described method (14). Using light downward pressure the catheter segment was streaked across a blood agar plate ten times. The catheter was rotated 90° and again streaked ten times across the agar plate. This process was repeated four times and the catheter was then incubated in trypticase soy broth.

Histological assessment

A 2 mm transverse section of the catheter associated tissue was peeled away from the anchoring end of each catheter segment and was reserved for light microscopy. Following standard techniques, the tissue was fixed in 10% buffered formalin and stained with hematoxylin and eosin.

Experimental protocol

The protocol for the preparation of the mouse model including peritoneal catheter implantation and peritoneal instillation of peritoneal dialysis solution is summarized in Table 1.

Statistical analysis

All results are expressed as mean±SD. Unpaired data from sham-operated and renal failure mice were compared by Student's t test and techniques for analysis of variance were used for multiple comparisons between experimental conditions of infusion and no infusion.

Table 1: Animal preparation protocol

Week	Procedure
0	Electrocoagulation of right kidney surface (sham-operated and renal failure mice).
2	Left nephrectomy (renal failure mice) or surgical exploration of left renal area (sham-operated mice).
4	Implantation of the CAPD catheter segment within the peritoneal cavity of mice.
8+	Daily intracatheter peritoneal instillation of peritoneal dialysis solution begins and continues for two weeks.
10	Peritoneal infusion of peritoneal dialysis solutions ends and mice are sacrificed for assessment.

+ Experimental assessment was conducted six to eight weeks after the induction of renal failure or sham surgery.

Renal failure

All sham-operated and renal failure mice tolerated right renal electrocoagulation and two weeks later underwent a second surgical procedure. Following the surgical exploration of the left renal area, all sham-operated mice survived indefinitely. Approximately 20% of renal failure mice died within four days of nephrectomy as a result of excessive renal electrocoagulation and the remaining 80% survived until their designated experimental sacrifice date 6 to 8 weeks after the induction of renal failure. Normal control littermates were also included in experiments conducted to characterize the mouse model of chronic renal failure.

Our assessment of serum creatinine in normal C57BL/6 mice demonstrated a level of 0.1 mg/dl which is at the lower end of the range of published normal values for mice (15-17) and is approximately one tenth the normal level in man. Routinely blood urea nitrogen (BUN) served as the key biochemical parameter for the measurement of the degree of renal failure in the mouse model. Mice present normal values for several other parameters that differ from those of man (15-17), including the smaller size of red blood cells, the larger proportion of lymphocytes in the differential count of circulating leucocytes (over 90% lymphocytes, less than 10% polymorphs) and higher platelet counts. However none of these species differences precluded the development of the present animal model.

At the time of our assessment, six weeks after the induction of renal failure, mice presented markedly elevated

levels of BUN, significant anemia and growth retardation compared to sham-operated controls (Table 2). Microbiological assessment of peritoneal washings and parietal peritoneum of sham-operated and renal failure mice did not reveal evidence of bacterial contamination. Peritoneal and circulating leucocyte populations harvested from mice six weeks after nephrectomy or sham surgery were similar in cellularity and differential nature to those of normal control mice (Table 3).

Catheter implantation

Initial attempts to implant the peritoneal catheter segment at either the first (kidney electrocoagulation) or second (nephrectomy or sham surgery) surgical procedure were not tolerated by renal failure animals. Catheter implantation was however well tolerated by all mice when a third surgical procedure for this purpose was performed two weeks after nephrectomy or sham surgery. Subsequent investigation of this phenomenon demonstrated that catheter implantation could be successfully accomplished at the time of the first or second surgery if the interval between these procedures was extended. Nevertheless the current animal model is based on three separate preparatory surgical procedures conducted over a six week period.

Within 48 hours of catheter implantation, the formation of granulation tissue around the catheter segment was evident. After one month the implant was completely surrounded by a fibrous tissue sheath. Immediate peritoneal distribution of a 1 ml intracatheter injection volume was not, however, impeded by the catheter associated granulation tissue as demonstrated

Table 2: Characteristics of sham-operated and renal failure mice harbouring peritoneal catheter segments[a,b]

Animal status:	Sham-operated (54)	Renal failure (57)
Blood urea nitrogen (mg/dl)	21.4±4.7	85.8±25.4[c]
Hemoglobin (g/dl)	13.1±1.0	9.0±1.4[c]
Body weight (g)	20.7±1.9	19.6±2.0[c]

[a]Results are expressed as mean±SD.
[b]Sample size is indicated in parenthesis.
[c]Significant difference ($p<0.05$) between the two groups of mice.

Table 3. Peritoneal and circulating leucocytes of normal, sham-operated and renal failure mice[a,b]

Animal status:	Normal	Sham-operated	Renal failure
Peritoneal leucocytes	(24)	(12)	(15)
Leucocytes/ml ($x10^6$)	0.8±0.3	0.6±0.2	0.8±0.4
Mononuclear cells (%)	95.8±2.8	97.2±3.4	98.1±1.5
Polymorphs (%)	2.0±2.4	2.5±3.4	1.4±1.4
Circulating leucocytes	(13)	(10)	(15)
Leucocytes/ml ($x10^3$)	4.4±1.4	5.7±2.3	5.8±3.4
Mononuclear cells (%)	96.1±4.1	94.1±4.8	92.0±5.4
Polymorphs (%)	3.9±4.1	5.9±4.8	8.0±5.4

[a]Results are expressed as mean±SD.
[b]Sample size is indicated in parenthesis.
[c]Mononuclear cells refer to lymphocytes and macrophages.

by intracatheter injection of Renographin-60 (Figure 3). Histological examination of the granulation tissue revealed a poorly vascularized well established tissue which consisted primarily of fibroblasts and lymphocytes (Figure 4). The cellular constituents of fluid aspirated from the catheter lumen were 15% erythrocytes and 85% leucocytes of which approximately 28% were polymorphs.

Experimental bacterial challenge and intracatheter infusion of peritoneal dialysis solution

Mice underwent peritoneal bacterial challenge by transcutaneous injection through the abdominal wall either directly into the peritoneal cavity or into the catheter lumen. Microbiological assessment of peritoneal structures and recovered catheter segments did not reveal evidence of bacterial contamination and the only positive bacterial recoveries were those of the experimental challenge bacterium, S. epidermidis.

During control studies, under conditions of twice daily intracatheter instillation of peritoneal dialysis solution for a period of two weeks, mice did not present evidence of peritoneal contamination. No bacterial growth was recovered from peritoneal structures or catheter segments of five renal failure and five sham-operated mice subjected to this repeated infusion schedule. There was, however, evidence of local inflammation measured by elevated peritoneal polymorph counts in renal failure and sham-operated mice receiving daily peritoneal infusion compared to non infused controls (Table 4). Inflammation was most severe in renal failure mice in which

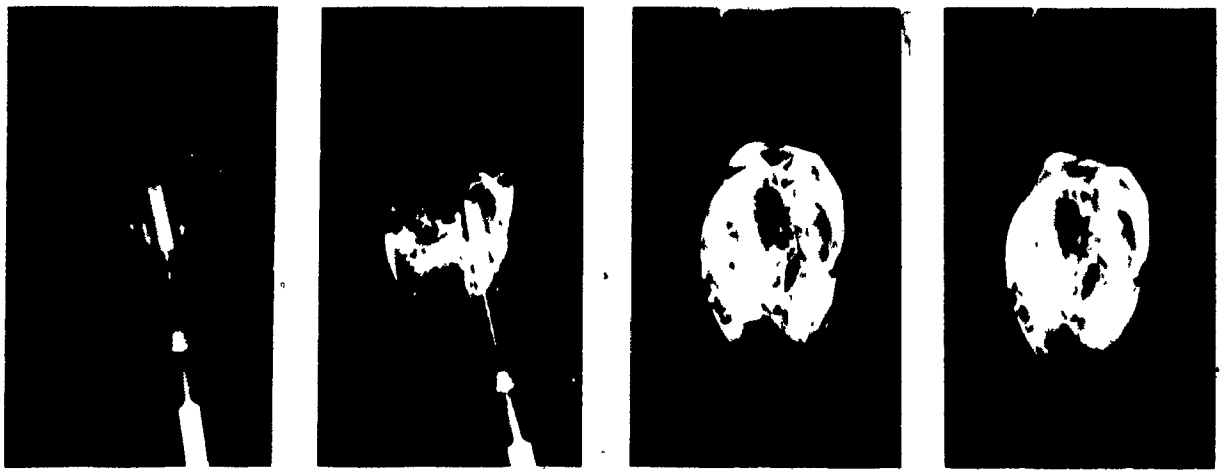

Figure 3. Time sequence of peritoneal distribution of Renographin-60 at (from left to right) 10, 20, 30 and 40 seconds after intracatheter injection of a 1 ml volume.

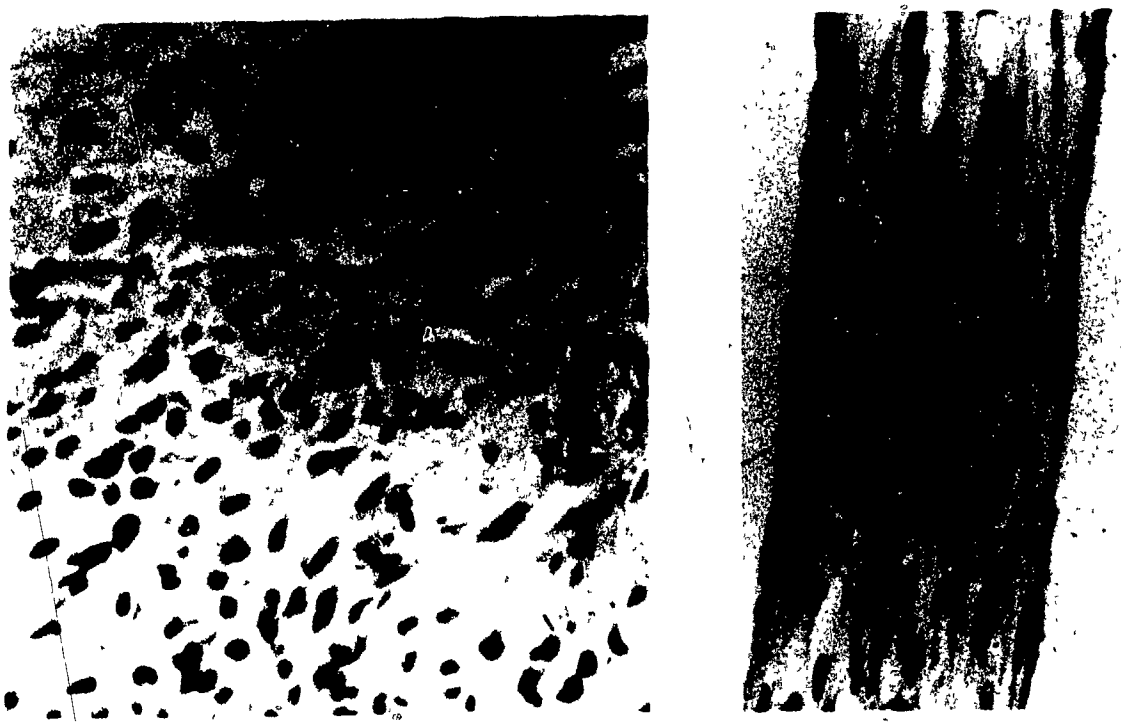

Figure 4. Histological sections of granulation tissue associated with the peritoneal catheter segment one month after implantation. Cellular constituents of tissue are predominantly fibroblasts and mononuclear leucocytes (Hematoxylin and eosin x 400).
Left panel: A thicker tissue is associated with the end of the catheter segment. Fibroblasts and mononuclear leucocytes are evident.
Right panel: A thinner fibrous sheath, consisting predominantly of fibroblasts is associated with the length of the catheter segment.

Table 4: Peritoneal and circulating leucocytes of mice subjected to twice daily peritoneal infusion of 4.25% dextrose peritoneal dialysis solution[a,b]

Animal Status:	Sham-Operated		Renal Failure	
Condition	No Infusion (12)	Infusion (5)	No Infusion (16)	Infusion (5)
Peritoneal leucocytes				
Leucocytes/ml ($x10^6$)	0.6±0.2	0.1±0.1[c]	0.8±0.4	1.5±0.7[c,d]
Polymorphs (%)	2.5±3.4	15.6±16.1[c]	1.4±1.4	41.0±15.9[c,d]
Circulating leucocytes				
Leucocytes/mm ($x10^3$)	5.7±2.3	5.5±2.9	5.8±3.4	6.1±2.7
Polymorphs (%)	5.9±4.8	14.0±12.3	8.0±5.4	6.6±4.7

[a]Results are expressed as mean±SD.
[b]Sample size is indicated in parenthesis.
[c]Significant difference ($p<0.05$) between infused and non infused mice.
[d]Significant difference ($p<0.05$) between renal failure and respective sham-operated mice.

peritoneal leucocyte counts doubled and polymorphs increased dramatically during the infusion procedure. Sham-operated mice receiving daily infusion presented a more moderate elevation of peritoneal polymorphs and an unexplained fall in the levels of peritoneal leucocytes compared to non infused mice. There was no significant systemic inflammation as a consequence of daily infusion in either renal failure or sham-operated mice. Body weight of mice remained constant throughout the period of peritoneal instillation of dialysis solution and was similar to preinfusion measurements.

Discussion

We have used this mouse model to study the mechanisms by which patients treated with continuous ambulatory peritoneal dialysis develop peritonitis. The development of this model and the characteristics of the resulting animal preparation, however, have not been previously reported. Implantation of the catheter entirely within the confines of the peritoneal cavity and aseptic transcutaneous injection into the catheter lumen provided peritoneal access without risking bacterial contamination of an open exit site to the skin. Repeated daily peritoneal instillation of dialysis solution was successfully conducted however drainage of the instilled volume was not achieved. In spite of this obvious difference between our experimental preparation and the practice of CAPD, this animal model has been successfully used in the investigation of the response of chronically uremic mice to peritoneal challenge with S. epidermidis (18-21).

The mouse model of renal failure was originally developed

to study the influence of severe and chronic uremia on immune responses. In comparison to other animals, inbred strains of mice have been well defined immunologically and therefore the mouse is a preferred experimental species for studies of host defense mechanisms. Characteristics of the mouse model of renal failure have been previously described (22). Six weeks after the onset of renal failure, mice were azotemic and presented significant anemia and growth retardation. Microbiological assessment of peritoneal structures did not present evidence of peritoneal contamination resulting from preparatory surgical procedures and the nature of peritoneal and circulating leucocytes was similar to that of normal control mice. For the investigation of CAPD associated peritonitis, the mouse model of renal failure was expanded to include a permanently dwelling peritoneal catheter. The nature of these studies required an infection free host in which controlled experimental bacterial challenges could be conducted. Catheter implantation was never complicated by bacterial contamination of the peritoneum or the catheter site. One month after catheter implantation, at the time of experimentation, the only culture positive recoveries from peritoneal structures of mice were those of the experimental inoculum.

Granulation tissue invariably surrounded the peritoneal catheter segment of mice one month after implantation. Encapsulation of the peritoneal catheter in the rat model of CAPD has been previously reported (12). In the current study, histological assessment of the fibrous sheath encompassing the

catheter revealed a poorly vascularized tissue which consisted predominantly of fibroblasts and mononuclear leucocytes and was characteristic of low grade chronic inflammatory response to the foreign implant (23,24). Catheter segments for mice were manufactured from the same silicone rubber tubing used for the Oreopoulos-Zellerman CAPD catheter. Although any foreign insoluble material can provoke chronic inflammatory responses, silicones are chemically inert and induce little inflammatory or immunological response (25-27). In order to minimize the peritoneal response to the catheter implant both ends of the catheter segment were bevelled smooth (23,28). In contrast to observations in rats and mice, a similar granulation tissue reaction is not apparent when peritoneal catheters are surgically removed from CAPD patients (29,30). These differences may be explained by the varying lengths of time that the peritoneal catheters reside in the peritoneal cavity (one month in mice, varying longer periods in man) or by the size and shape of the catheter segment.

Repeated daily peritoneal instillation of peritoneal dialysis solution without peritoneal drainage was successfully conducted in mice for periods of up to two weeks. Characterization of the mouse model has demonstrated that renal failure mice frequently produce large volumes of dilute urine (22), which presumably allowed the renal failure mice in the current preparation to maintain constant body weight levels during the period of peritoneal infusion. In establishing the instillation volume of dialysis solution for mice, ratios of body surface area or body weight of mice and man resulted in

inappropriately large or small volumes. Finally, visual abdominal distension of the mouse following peritoneal instillation was the selection criteria for the infusion volume such that the abdomen was moderately distended but animal mobility was normal. Even under extreme conditions of twice daily intracatheter infusion, microbiological assessment of peritoneal structures were always culture negative. There was, however, demonstrable local inflammation in response to the infusion procedure which was most apparent in renal failure mice. Although peritoneal dialysis per se was not accomplished, studies addressing the influence of repeated exposure of the peritoneal cavity to peritoneal dialysis solution on the response of mice to peritoneal bacterial challenge can be accommodated with this animal preparation.

The development of models of long term peritoneal dialysis in animals having functional peritoneal catheters has been hampered by numerous obstacles (9-12). Peritoneal catheters were frequently obstructed by fibrin clots as well as omental wrapping and uncontrolled peritoneal infection was observed. Severe malnutrition and failure of uremic animals to thrive also contributed to the serious complications encountered in previously described animal preparations. Therapeutic intervention to inhibit fibrin clot formation and prevent peritoneal infection may resolve the immediate problems presented by these animal models, however this would disallow their use in the study of host defense mechanisms to experimental bacterial challenge. In the current mouse model, implanting the peritoneal catheter segment entirely within the

confines of the peritoneal cavity and limiting the model to peritoneal instillation of dialysis solution has overcome previous limitations. Controlled destruction of renal parenchyma produced chronic renal failure mice that survived indefinitely without dialysis support and therefore large numbers of animals could be prepared for experimentation without unrealistic technical requirements for their maintenance.

In conclusion, the detailed characterization of the mouse model presented in this report provides a firm basis for designing additional studies to investigate the factors and mechanisms promoting the development of peritoneal infection and for assessing preventive and therapeutic measures.

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Chapter 4

Response of renal failure mice to intraperitoneal *Staphylococcus epidermidis* challenge

Section 4.1

Intraperitoneal challenge with *Staphylococcus epidermidis* in chronically uremic mice: effect of inoculum size

Presented in part at the 15th Annual Scientific Meeting of the National Kidney Foundation Inc., New Orleans, Louisiana, December 14-15, 1985 and the 6th National Conference on CAPD, Kansas City, Missouri, February 5-7, 1986.

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Abstract

Response to the intraperitoneal injection of Staphylococcus epidermidis in terms of animal survival and bacterial growth from peritoneal samples was measured in chronically uremic mice and their sham-operated controls. Renal failure was induced surgically by sequential electrocoagulation of the right kidney surface and left nephrectomy and bacterial challenge was performed 6 to 9 weeks after the second surgery. Over a wide range of inocula (10^6 to 10^9 colony forming units), susceptibility to S. epidermidis was greater in uremic than in control mice. Renal failure was associated with: 1) higher and accelerated lethality after large inocula (10^8, 10^9), and 2) delayed bacterial clearance after a smaller inoculum (10^6) or after a large inoculum (10^8) in surviving animals. Thus renal failure-related impairment of host defenses to infection must be considered in the pathogenesis of peritonitis during continuous ambulatory peritoneal dialysis.

Introduction

Staphylococcus epidermidis peritonitis is an important clinical complication of continuous ambulatory peritoneal dialysis (CAPD). This infection might be attributed to a breakdown of local defenses in an already immunocompromised host (1-3). Recent studies utilizing rat models of chronic uremia have failed to demonstrate increased susceptibility to intraperitoneal (ip) challenge with Escherichia coli and Pseudomonas aeruginosa (4,5). In view of the multiplicity of

factors operating in CAPD patients, the significance of these observations remained inconclusive.

To further clarify the pathophysiology of peritonitis in CAPD patients, in the current report we have studied the response to ip challenge with S. epidermidis in mice made uremic by a combination of thermal injury and ablation of renal parenchyma. Studies were carried out 6 to 9 weeks after the surgical induction of renal failure, when the uremic state was well established. Increased susceptibility to the bacterial challenge, in terms of decreased animal survival following large ip inocula and delayed peritoneal clearance of smaller inocula, was evident in uremic mice compared to controls.

Materials and Methods

Six week old female C57BL/6 inbred mice (Charles River Breeding Laboratories, Kingston, NY, USA) underwent electrocoagulation of the surface of the right kidney as previously described (6). Either contralateral nephrectomy (uremic mice) or surgical exploration of the left renal area (sham-operated controls) was performed two weeks later. Animals had free access to water and standard chow.

Six to nine weeks after the second surgery, mice received intraperitoneally either 10^9, 10^8 or 10^6 cfu S. epidermidis. Aliquots of bacteria (a clinical isolate from peritoneal fluid reference number 29260 kindly provided by Dr. S.I. Vas, Toronto Western Hospital, Ontario, Canada) were frozen in glycerol broth and were grown overnight on Columbia agar prior to challenge. Serial dilutions were made in

phosphate buffered saline (PBS) and the ip inocula were injected in a one ml volume into the right lower quadrant under light ether anesthesia. To exclude the possibility that the two surgical procedures used to induce renal failure might alter local host defenses, the response to bacterial challenge was always evaluated in mice made uremic by electrocoagulation of the right renal cortex and left nephrectomy and in sham-operated controls. In some experiments normal mice were also included.

Following bacterial challenge survival was recorded and at selected times surviving mice were anesthetized with ether, weighed and blood was collected by cardiac puncture. Under aseptic conditions peritoneal washings were performed and samples of anterior parietal peritoneum were obtained according to previously reported methods (7). Aliquots of peritoneal washings and homogenized peritoneum were inoculated onto blood agar and incubated for 24 hours. Recovered bacteria were enumerated and positive identification was ascertained by gram stain, catalase production and the characteristic antibiotic sensitivity profile. Blood urea nitrogen (BUN) and hematology assessment of blood samples were determined by an IL-9 autoanalyzer and a Coulter counter, respectively.

Results

Renal failure model

Levels of BUN, hemoglobin concentrations and body weights are presented in Table 1.

The values for uremic mice are contrasted to those observed in normal and sham-operated littermates. Marked azotemia was present in the uremic mice 6 weeks after the onset of renal failure. At sacrifice, body weight was decreased in uremic mice and hemoglobin concentrations were markedly reduced in the uremic animals compared to controls.

Table 1. Characteristics of normal, sham-operated and chronically uremic mice[a]

	Normal	Sham	Uremia
Blood urea nitrogen (mg/dl)	21.9±4.5 (35)	25.1±5.3 (54)	116.3±42.3[b] (57)
Hemoglobin (g/dl)	13.8±0.7 (36)	13.5±0.7 (42)	8.1±1.6[b] (33)
Body weight (g)	22.2±2.5 (38)	21.0±1.6 (54)	19.3±2.9[b] (54)

[a]Results are expressed as mean±SD with number of mice indicated in parentheses.

[b]Significant difference ($p<0.05$) between uremic and control mice.

Survival studies

Survival of sham-operated and uremic mice following ip inoculation of 10^9 and 10^8 cfu S. epidermidis is illustrated in Figure 1. All mice succumbed within 24 hours of the 10^9 cfu ip challenge. Time to 50% mortality for uremic and sham controls was 3.5 and 16.5 hours, respectively. No mortality occurred in control mice following a 10^8 cfu inoculum however the uremic group suffered a 25% animal loss within the first 96 hours after challenge. No subsequent mortality was recorded, to one week follow up. Challenge with a smaller inoculum, 10^6 cfu, caused no mortality in uremic, sham-operated or normal mice.

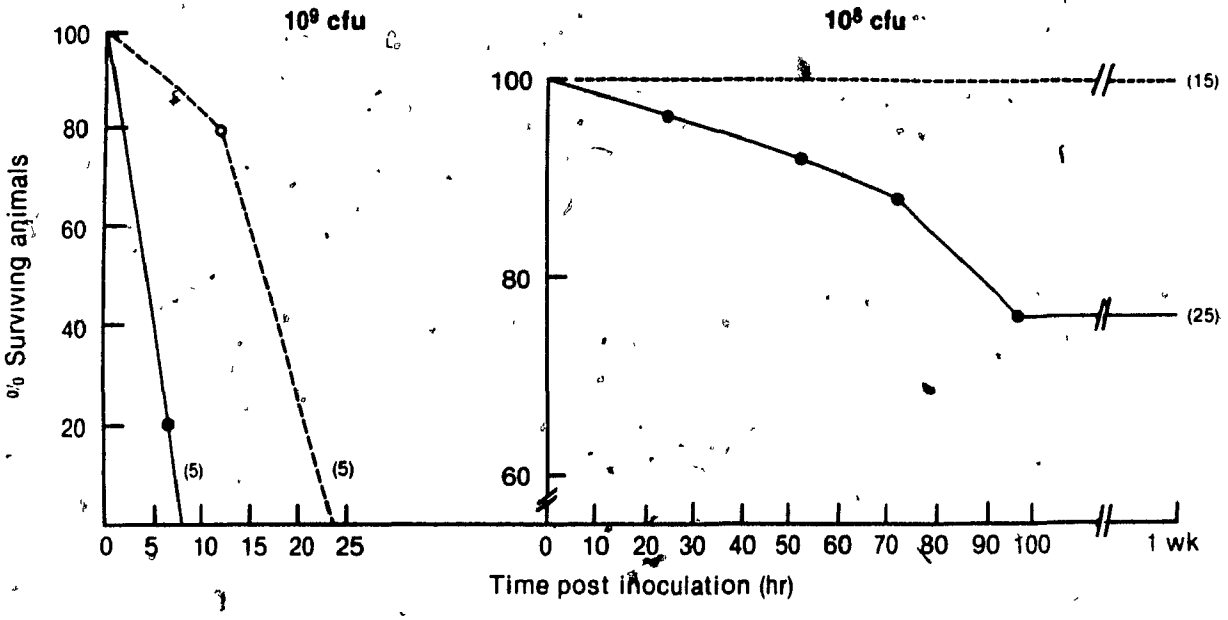


Figure 1. Survival curves of mice following the ip inoculation of 10^9 (left panel) or 10^8 (right panel) colony forming units of Staphylococcus epidermidis. The solid and dashed lines on each panel represent chronically uremic mice and sham-operated controls, respectively. Numbers of animals in each group at the time of bacterial challenge are indicated in parentheses.

Tissue recoveries of S. epidermidis

The recoveries of S. epidermidis from peritoneal samples of mice surviving one week after 10^8 cfu ip challenge are presented in Figure 2. Significantly greater numbers of bacteria remained associated with the peritoneum of uremic mice compared to sham-operated controls. All peritoneal washings of sham mice were culture negative whereas 3 of 19 specimens collected from uremic mice harboured viable bacteria.

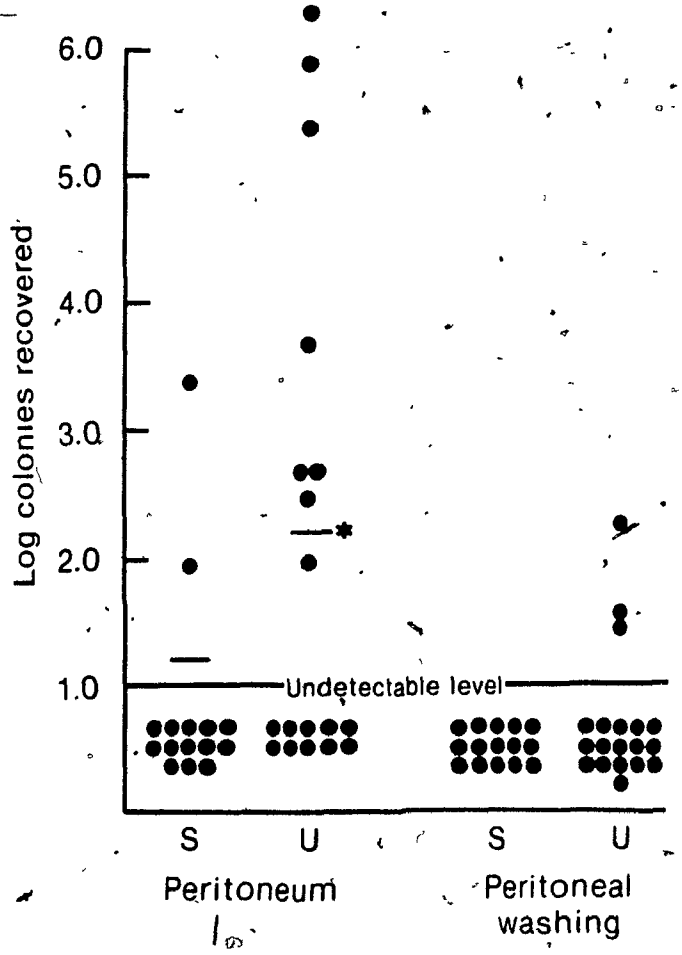


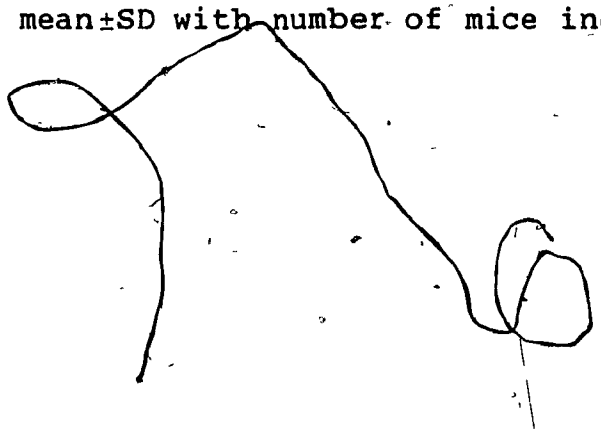
Figure 2. Recovery of Staphylococcus epidermidis from parietal peritoneum (per gram) and peritoneal washings (per ml) 1 week after 10^8 colony forming units challenge in sham-operated (S) and chronically uremic (U) mice. *Significantly greater ($p<0.05$) recovery in uremic mice compared to controls.

Total leucocyte and differential counts in peritoneal washings were similar for uremic and sham-operated mice (Table 2). Cellularity was twice that of a resident population (data not shown) harvested in the absence of bacterial challenge and a small elevation of polymorphonuclear cells was evident.

Table 2. Cellular constituents of peritoneal washings[a]

	Sham (15)	Uremia (19)
Leucocytes ($x10^6$/ml)	1.5±0.6	1.6±0.7
Macrophages (%)	43.7±12.4	41.6±10.8
Lymphocytes (%)	50.9±16.4	52.2±14.3
Polymorphs (%)	5.4±4.7	6.2±7.2
Mast cells (%)	0.1±0.3	0.1±0.2

[a]Results expressed as mean±SD with number of mice indicated in parentheses.



The time course of S. epidermidis clearance from the peritoneum of mice following a 10^6 cfu ip inoculation is summarized in Table 3. Peritoneal washings of normal and sham-operated mice were culture negative by 24 hours post challenge and 2 of 9 specimens from uremic mice yielded S. epidermidis. In contrast specimens of parietal peritoneum harbored large numbers of bacteria 24 hours post inoculation and recoveries were significantly greater from uremic mice than from controls. All animals effectively cleared the bacterial challenge but the process was delayed in uremic mice.

Table 3. Bacterial recoveries from normal, sham-operated and uremic mice[a]

	Normal	Sham	Uremia
Log colonies per g parietal peritoneum			
4 hours	3.2±1.2 (11)	3.8±0.9 (11)	4.0±1.1 (8)
24 hours	1.6±0.7 (11)	2.6±0.7 (12)	3.4±1.2 (9)[b]
48 hours	1.0±0 (8)	1.3±0.6 (8)	1.9±1.0 (8)
72 hours	1.1±0.3 (8)	1.1±0 (8)	1.3±0.7 (8)
Log colonies per ml peritoneal washing			
4 hours	2.4±0.4 (11)	1.8±0.7 (11)	1.6±0.6 (8)
24 hours	1.0±0 (12)	1.0±0 (12)	1.3±0.6 (9)
48 and 72 hours	No bacterial growth in all groups (8 each).		

[a]Results are expressed as mean ± SD with number of mice indicated in parentheses.

[b]Significantly greater ($p<0.05$) recoveries in uremic mice compared to sham-operated controls

Discussion

Utilizing a mouse model of chronic uremia, the influence of renal failure on the response to a range of inoculum sizes of S. epidermidis has been delineated. Lethality following a large ip inoculum of 10^9 cfu was accelerated in uremic mice and mortality in this group was significantly greater following the smaller 10^8 cfu ip challenge compared to sham-operated controls. In those mice surviving one week after 10^8 cfu inoculation and in mice followed to 72 hours after a 10^6 cfu challenge, peritoneal clearance was delayed in renal failure mice in contrast to controls.

Considerable controversy exists regarding the possible role of renal failure in the increased incidence of infections observed in patients with end-stage renal disease. It has been well documented that renal failure is accompanied by a number of immunological deficits affecting cell-mediated immunity particularly (1,2), however, a clear correlation between renal failure and increased susceptibility to infection remains elusive. The heterogenous nature of patient populations in terms of underlying disease, duration of renal failure and treatment modality may partially account for the existing controversy.

Studies in which animal models of uremia have been challenged with viable bacteria are few and unlike results reported herein, the outcome of these investigations have not concluded a fundamentally immunocompromised uremic host (4,5,8). To our knowledge no other animal study has addressed susceptibility to S. epidermidis ip challenge in renal failure

animals having duration and severity of uremia similar to that presented by this mouse model.

Regarding the mechanism whereby renal failure inhibits response to bacterial challenge, one component may be the impairment of neutrophil mobilization towards an infected site (8). We have previously reported (7) that following ip challenge and concurrent with significantly greater S. epidermidis recoveries from parietal peritoneum of uremic mice, peritoneal neutrophil response in renal failure animals was diminished compared to normal and sham controls. The increase in circulating neutrophils following bacterial challenge was similarly attenuated in uremic and sham-operated mice compared to normal controls. In this current report, peritoneal leucocyte and differential counts were similar in uremic and sham mice one week following 10^8 cfu challenge even though tissue recoveries of S. epidermidis were significantly greater in renal failure animals. These findings may signify an inappropriate inflammatory cell response to the persistent ip presence of S. epidermidis in chronically uremic mice.

Specimens of parietal peritoneum, although thoroughly rinsed, remained culture positive for S. epidermidis when peritoneal washings presented no bacterial growth. These findings suggest a secure association between the bacteria and some aspect of the peritoneum and may correspond to the clinical situation where culture negative dialysis effluents are recovered in CAPD patients otherwise presenting symptoms of peritonitis.

In the current studies, susceptibility to ip S. epidermidis inoculation was increased by renal failure, and,

therefore, the influence of uremia on host defenses to infection must be considered in the pathophysiology of CAPD peritonitis.

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Section 4.2

Impaired bacterial clearance and inflammatory response in renal failure mice subjected to intraperitoneal *Staphylococcus epidermidis* challenge

Presented in part at the 54th Annual Meeting of the Royal College of Physicians and Surgeons of Canada, Vancouver, Canada, September 8-12, 1985 and the 18th Annual Meeting of the American Society of Nephrology, Washington, D.C. December 15-18, 1985.

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ABSTRACT

The role of renal failure in the pathogenesis of the Staphylococcus epidermidis peritonitis presented by endstage renal disease patients treated with continuous ambulatory peritoneal dialysis was investigated in a mouse model of surgically-induced renal failure. Six weeks after the surgery an ip inoculum of 10^6 colony forming units S. epidermidis was administered to renal failure mice and their sham-operated and normal controls and assessment of bacterial clearance and inflammatory response was conducted over the next 72 hours. Peritoneal clearance of S. epidermidis was complete in most animals however the process was significantly delayed in renal failure mice compared to sham-operated controls. Viable bacteria invariably remained associated with the peritoneum after peritoneal washings had become culture negative. Peritoneal inflammatory response was markedly diminished in renal failure mice, the early polymorphonuclear cell response being particularly affected. Peripheral response consisted of a prompt and short lived polymorph increase which was similar in renal failure and sham-operated mice. The factors responsible for the observed impairment of local inflammatory response in association with delayed bacterial clearance in renal failure mice following ip challenge with S. epidermidis remain to be defined.

INTRODUCTION

Peritonitis is a frequent complication of patients with endstage renal disease treated with continuous ambulatory

peritoneal dialysis (CAPD) (1-3). Recent technical advances in design of ancillary equipment have been aimed primarily at decreasing the risk of touch contamination which is thought to occur at the time of dialysis exchange. Despite these efforts, the predominant offending organism remains the skin borne bacterium, Staphylococcus epidermidis. The mechanisms responsible for this potentially serious infection may be multifactorial (4,5). Renal failure results in an immunosuppressed state with severe dysfunction of cell-mediated immunity (6-8); its effect on resistance to infection however is less clear (9,10). As a foreign body, the indwelling peritoneal catheter may serve as a nidus for microorganisms (11) or impair local immune responses (12). In addition, peritoneal dialysis solutions cause inhibition of peripheral leucocyte function in vitro (13) and are cytotoxic to peritoneal cells in vitro (14). Another consequence of peritoneal dialysis is the intermittent dilution and drainage of immune reactants, soluble and cellular, from the peritoneal cavity (15-17).

We have addressed the relative role of renal failure in the CAPD-associated S. epidermidis peritonitis employing, as model, mice with surgically-induced renal failure in which defects of cell-mediated immunity have been previously demonstrated (18-20). Using ip inoculation with 10^6 cfu S. epidermidis we have investigated three aspects of the response to bacterial challenge. The first is whether renal failure influences peritoneal clearance of the inoculum, the second is the characterization of the local and systemic inflammatory

reaction to the bacterial challenge, while the third is whether in vivo inflammatory response of renal failure mice differs from that of sham-operated controls. This latter consideration is of particular importance, given the reported deficit in host defense mechanisms during renal failure.

MATERIALS AND METHODS

Animals

Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY, USA) at five weeks of age. The animals were allowed to acclimatize in holding facilities for one week prior to use. All animals had free access to water and mouse chow.

Renal failure

The mouse model of renal failure, which includes sham-operated controls, has been described previously (21). Briefly, six week old mice were anesthetized with ether and a small flank incision was made through which the right kidney was separated from the adrenal gland and freed of perirenal fat. Particular caution was taken to avoid trauma to the ureter. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, Calif., USA) and excluding a 2mm margin around the renal pelvis, the exposed surface of the kidney was electrocoagulated. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Two weeks after right kidney electrocoagulation, a left nephrectomy was done in those animals that would constitute the renal failure group and in sham-operated controls, surgical exploration of the left renal

area was conducted. Six to eight weeks after the second surgery the mice were used for experimentation. Normal control mice were also included in all experiments.

Blood analyses

Under ether anesthesia animals were exsanguinated by cardiac puncture and blood was collected in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for biochemistry and hematology analyses. Levels of urea nitrogen (BUN) and creatinine in plasma were determined with an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass, USA). Routine hematology was done utilizing a Coulter counter (Model 2B1, Coulter Electronics Inc., Hialeah, Fla, USA) and differential cell counts were performed on Wright-stained blood smear preparations.

Bacteria

Aliquots of S. epidermidis, reference number 29260, (a clinical isolate from peritoneal fluid kindly provided by Dr. S.I. Vas, Toronto Western Hospital, Ontario, Canada) were stored frozen in glycerol broth. The characteristic antibiotic sensitivity profile of this organism facilitated positive identification. As demonstrated by trypan blue staining of glass adherent material following incubation in trypticase soy broth, this organism is a "slime producing" S. epidermidis (22). The bacterial suspension for inoculation was prepared from overnight cultures grown on Columbia agar. Bacterial viable units were enumerated by serial dilution and pour plate techniques.

Experimental challenges

Intraperitoneal injection of 10^6 colony forming units (cfu) viable bacteria in 1 ml phosphate buffered saline (PBS)

was done under light ether anesthesia into the right lower quadrant of the abdomen. After selected time periods animals were sacrificed and the bacteriological status of peritoneal washings and specimens of the ventral parietal peritoneum was determined. Optimum inoculum size and sampling times post challenge had been previously established in preliminary studies in normal C57BL/6 mice.

Collection and processing of specimens

Following body weight measurement and blood collection, the abdomen was washed with alcohol and the skin was retracted aseptically to expose the intact abdominal wall. Ten ml of minimum essential medium (Eagle Modified) containing 10% heat inactivated fetal calf serum and 20 mM HEPES buffer was injected into the peritoneal cavity. The peritoneal washing was slowly withdrawn through a 21G needle and syringe. Cells of each effluent were counted in Neubauer counting chambers and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, Ill, USA). An aliquot of each sample was inoculated onto 5% horse blood Columbia agar and colonies recovered were expressed as cfu per ml peritoneal washing.

A specimen of the ventral parietal peritoneum (approximately 2 cm^2) was excised aseptically and rinsed in PBS. Specimens were blotted dry and placed into pretared vessels containing 2 ml PBS. Vessels were again weighed, tissue specimens were homogenized and inoculated into blood agar to obtain bacterial numbers. After correction for dilution in PBS bacterial recoveries were expressed as cfu per gram of peritoneum.

Colonies were enumerated after 24 hours incubation. Positive identification of bacteria recovered from collected specimens was ascertained by colony morphology, gram stain, catalase production and the characteristic antibiotic sensitivity profile.

Statistical analysis

Colonies recovered were converted to log units and results are expressed as mean±SD. Student's "t" test for unpaired data sets was used for single comparisons between groups of sham-operated and renal failure mice and between baseline assessments and those of various test times after inoculation. Normal mice were included in all experiments for interest but were not included in statistical analysis.

RESULTS

Renal failure model

Some characteristics of this mouse model of renal failure are presented in Table 1. As these parameters were not influenced by the inoculation procedure, data from animals sacrificed at all test times following inoculation were pooled. Blood urea nitrogen, which is the key assessment factor of renal function in this model, was appropriately elevated in renal failure animals compared to sham-operated controls. A tenfold increase of plasma creatinine was observed in renal failure animals compared to control values. It should be noted that normal plasma creatinine levels of mice are much lower than those of man. A strong inverse correlation ($p<0.001$) existed between levels of hemoglobin and BUN (data not shown) such that six to eight weeks after the induction of renal failure mean hemoglobin in renal failure animals was 60% of

Table 1

Selected characteristics of the mouse model of chronic renal failure[a,b].

Status of animals:	Normal	Sham	Renal failure
Blood urea nitrogen (mg/dl)	20.1±5.6 (88)	27.1±5.8 (78)	111.2±35.9[c] (75)
Plasma creatinine (mg/dl)	0.1±0.1 (14)	0.1±0.1 (12)	1.0±0.7[c] (36)
Hemoglobin (g/dl)	13.5±1.0 (80)	13.4±0.7 (80)	8.6±1.6[c] (70)
Body weight (g)	22.3±2.3 (84)	22.2±2.5 (71)	20.3±3.1[c] (71)

[a]Results expressed as mean± SD.

[b]Sample size indicated in parenthesis.

[c]Significant difference ($p<0.05$) between uremic mice and controls.

controls levels. The body weight of renal failure mice was significantly less than that of sham-operated controls.

Bacterial recoveries from the peritoneal cavity and peritoneum after ip bacterial challenge

The time course of S. epidermidis clearance from peritoneal washings and parietal peritoneum in the three groups of mice is depicted in Figures 1 and 2, respectively. With the exception of two mice in the renal failure group, peritoneal washings were culture negative 24 hours after ip inoculation whereas the majority of peritoneum samples still harbored viable bacteria. Culture positive peritoneum specimens were collected from sham-operated and renal failure animals at 48 hours and by 72 hours post inoculation only one animal in each of the groups presented positive peritoneum samples.

The inoculum was effectively cleared by all mice, however, peritoneal clearance was delayed in renal failure animals. This delay was most apparent ($p<0.05$) 24 hours after S. epidermidis challenge when 3.4±1.2 cfu (mean log±SD) per gram peritoneum was recovered from renal failure mice compared to 2.6±0.7 cfu per gram peritoneum from sham-operated controls. Subsequently, at 48 hours after inoculation peritoneum specimens from 4 of 8 renal failure mice were culture positive compared to 2 of 8 sham-operated controls.

Cellular recoveries from the peritoneal cavity after ip bacterial challenge

The nature of the cellular constituents of peritoneal washings harvested at various times after ip bacterial challenge are presented in Figure 3. Following inoculation there was an immediate and significant decrease in peritoneal

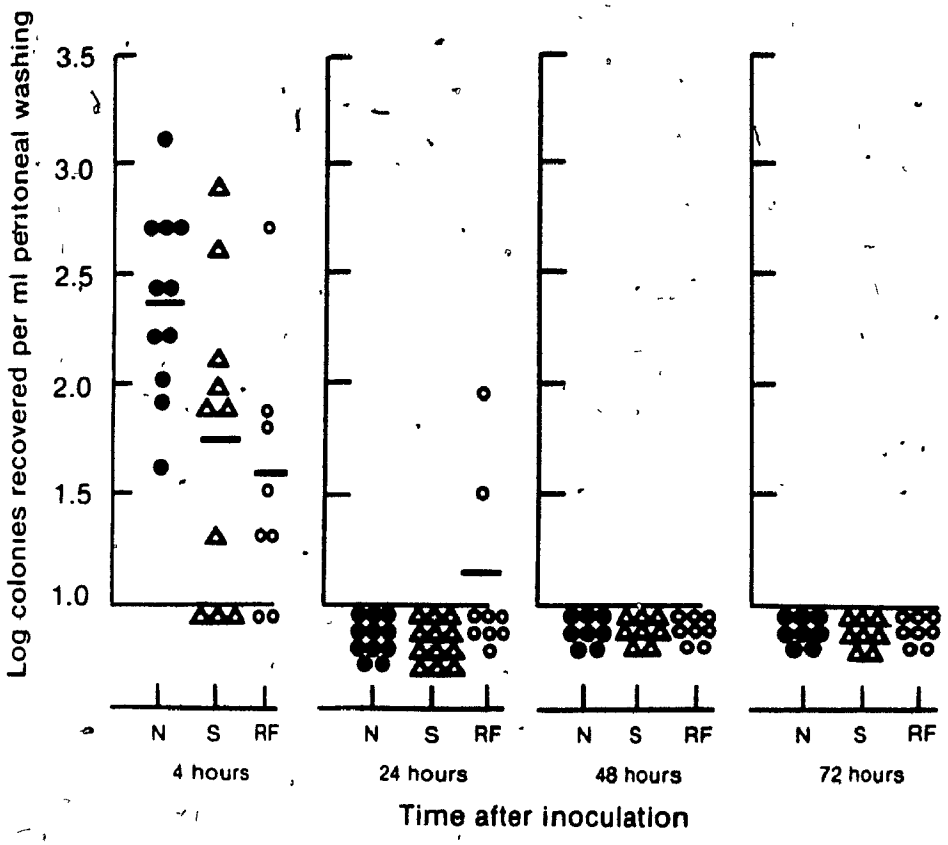

Figure 1: Recovery of Staphylococcus epidermidis from peritoneal washings after ip challenge (10^6 cfu) in renal failure (RF) mice and in normal (N) and sham-operated (S) control animals. Horizontal bars indicate mean values.

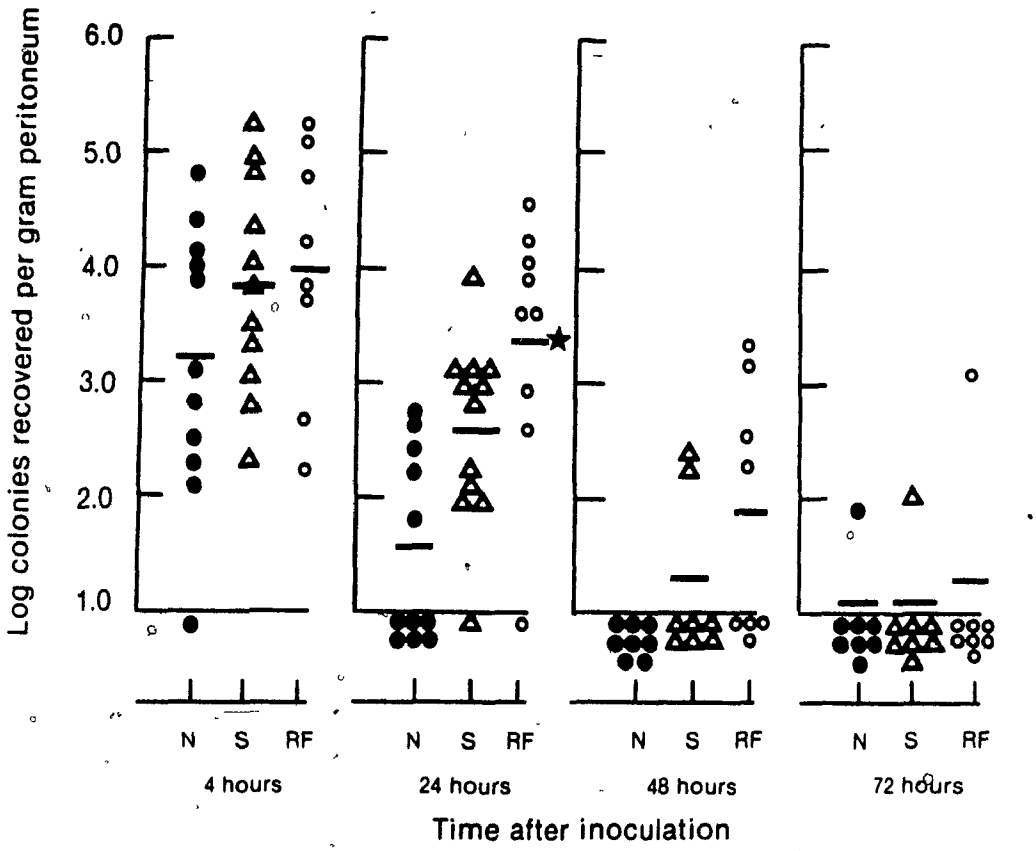

Figure 2. Recovery of Staphylococcus epidermidis from samples of parietal peritoneum after ip challenge (10^6 cfu) in renal failure (RF) mice and in normal (N) and sham-operated (S) control animals. A star indicates a significantly greater recovery ($p<0.05$) from renal failure mice compared to sham-operated control animals. Horizontal bars indicate mean values.

leucocytes which was most extreme in samples harvested from renal failure mice. In sham-operated controls leucocyte counts returned to preinoculation levels 24 hours after challenge, and thereafter exceeded baseline levels however, peritoneal leucocyte counts in renal failure mice remained depressed throughout the 72 hour follow up period. The fall in peritoneal leucocytes could be attributed to an immediate decrease in macrophages which was most extreme and prolonged in renal failure mice compared to sham-operated controls. Peak depression of peritoneal macrophages occurred between 2 and 4 hours post inoculation and counts gradually increased to 48 hours after challenge. Concomitant to the immediate fall in peritoneal leucocytes the absolute number of peritoneal polymorphs promptly increased in all animals after inoculation. Polymorph response was most apparent between 1 and 12 hours after challenge and thereafter rapidly diminished. Within 2 hours of inoculation a rise in peritoneal polymorphs was observed in all mice. Peak polymorph response, however, was markedly attenuated in renal failure mice compared to sham-operated controls.

Peripheral response to ip bacterial challenge

Hematology assessment of the three groups of mice at various test times is summarized in Table 2. In the C57BL/6 mouse strain, lymphocytes are the predominant circulating leucocytes and neutrophils are usually the only circulating polymorphs observed (23). In all mice there was a brisk and short lived peripheral polymorph response that peaked 2 hours after S. epidermidis challenge and small numbers of stab forms, which were never observed in the resting state, appeared

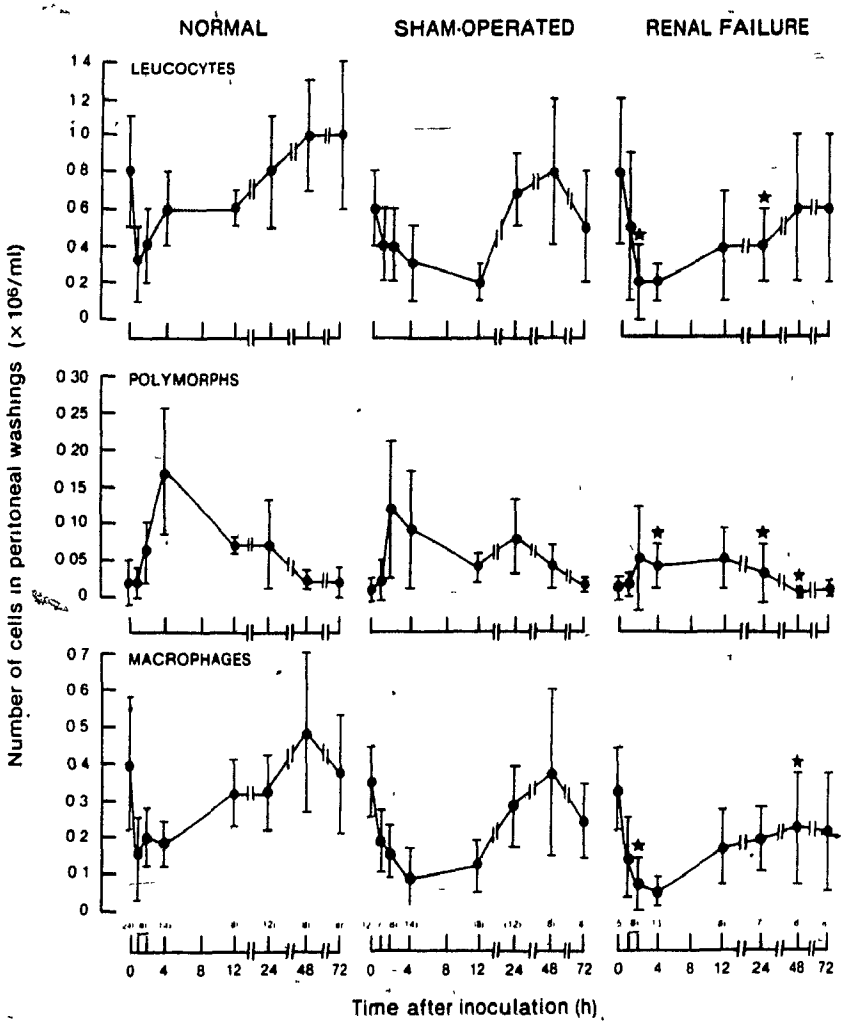

Figure 3. Peritoneal inflammatory response of renal failure, sham-operated and normal control animals following intraperitoneal Staphylococcus epidermidis challenge. Mean values±SD are presented at each test time. Stars indicate significant differences ($p<0.05$) between renal failure and sham-operated control mice. Number of animals assessed at each test time is indicated immediately above the horizontal axis.

Table 2

Changes in circulating leucocytes following ip challenge with *Staphylococcus epidermidis* in chronically uremic mice and in normal and sham-operated control animals[a,b]

	Time post inoculation (hours) 0	1	2	4	12	24	48	72
Normal								
Leucocytes/mm^3($x10^3$)	4.4±1.4	5.3±2.1	4.2±1.2	4.2±2.7	7.1±2.8	4.4±1.5	3.7±1.2	3.4±1.9
Polymorphs (%)	3.9±4.1	13.8±7.6	20.3±8.9	22.1±10.1[c]	15.5±2.6	5.2±2.9	11.9±7.7	6.9±4.2
Stab (%)	0	0	0	0.4±0.7	0.3±0.5	0	0.3±0.5	1.3±1.9
Lymphocytes (%)	96.1±4.1	85.4±7.2	79.3±8.6	77.3±10.2	82.6±2.8	94.7±3.0	87.6±7.4	91.9±4.9
Monocytes (%)	0	0.9±0.6	0.5±0.8	0.3±0.7	1.6±1.1	0.2±0.4	0.3±0.7	0
	(14)	(8)	(8)	(14)	(8)	(12)	(8)	(8)
Sham								
Leucocytes/mm^3($x10^3$)	5.7±2.3	3.9±2.2	5.0±1.8	3.8±1.8	6.5±3.1	5.1±1.9	4.9±2.0	4.6±1.9
Polymorphs (%)	5.9±4.8	9.6±3.9	15.6±9.8	13.8±7.4[c]	10.9±4.1	4.6±3.2	8.4±6.2	8.9±5.1
Stab (%)	0	0.1±0.4	1.5±1.6	1.1±2.4	0.1±0.4	0	0	0.4±0.7
Lymphocytes (%)	93.9±4.8	89.7±4.0	82.3±11.0	85.6±8.5	88.9±3.9	95.3±3.2	91.6±6.2	91.0±5.6
Monocytes (%)	0.2±0.6	0.6±0.8	0.9±0.6	0.4±0.9	0.1±0.4	0	0	0.1±0.4
	(13)	(7)	(8)	(16)	(8)	(12)	(8)	(8)
Renal failure								
Leucocytes/mm^3($x10^3$)	5.8±3.4	2.5±0.8	5.4±1.9	3.1 1.8	5.4±1.7	3.8±2.4	5.2±2.3	4.3±1.5
Polymorphs (%)	8.0±5.4	7.6±2.5	14.1±13.4	12.2 7.0	9.8±5.3	4.7±1.0	7.9±3.9	6.8±2.7
Stab (%)	0	0	0.4±0.7	0.1 0.3	0.1±0.4	0	0	0.1±0.4
Lymphocytes (%)	92.0±5.4	91.5±2.2	84.4±14.1	87.7 7.0	89.8±5.6	95.3±1.0	92.0±3.8	93.1±2.5
Monocytes (%)	0	0.9±1.0	1.1±0.8	0.1 0.3	0.4±0.7	0	0.1±0.4	0
	(10)	(8)	(8)	(13)	(8)	(6)	(8)	(8)

[a]Results expressed as mean±SD.
[b]Sample size indicated in parenthesis.
[c]Significant difference ($p<0.05$) between test time and respective zero time controls.

within 4 hours of inoculation. Polymorph counts returned to baseline levels 24 hours after bacterial challenge. Peripheral leucocyte response did not differ significantly between renal failure and sham-operated mice. However, in comparison to baseline levels, the rise in peripheral polymorphs following inoculation reached statistical signficance in sham-operated mice while that of renal failure mice did not, presumably due to their slightly higher resting state levels of circulating polymorphs.

Response of normal mice to ip S. epidermidis challenge

Mice that were not subjected to any surgical procedure were included in all experiments and represented the normal response to the bacterial challenge for mice of this strain, age and sex.

DISCUSSION

We have previously reported that renal failure renders mice more susceptible to ip challenge with a large S. epidermidis inoculum (10^8 cfu) with ensuing mortality in 30% of animals and increased bacterial recoveries in surviving mice (24). Since these observations may find explanation in a renal failure related impairment of host defense mechanisms, we examined the effect of renal failure on bacterial clearance and development of inflammatory response to a small S. epidermidis inoculum (10 6cfu). While this smaller S. epidermidis inoculum induced no mortality, its peritoneal clearance was delayed in renal failure. Local inflammatory response was strikingly diminished in renal failure animals compared to sham-operated controls. The deficit was primarily

characterized by a marked attenuation of the early polymorph rise in the peritoneal cavity following ip challenge. A sharp fall in the numbers of peritoneal leucocytes and more specifically macrophages was observed immediately following bacterial challenge in all animals, however, the magnitude and duration of this decline was greatest in renal failure mice. Circulating polymorph response which readily follows ip bacterial challenge with S. epidermidis was similar in renal failure and sham-operated control mice.

The use of an inbred mouse strain for this model of renal failure facilitated the investigation of the influence of severe chronic renal failure on the host's ability to respond to ip challenge with S. epidermidis. Partial characterization of this mouse model has been previously reported (18-20). In this current study renal failure animals had appropriately elevated levels of BUN and creatinine and presented significantly decreased hemoglobin levels and body weight compared to sham-operated control mice. These changes subsequent to the surgical induction of renal failure also characterize some of the severe metabolic disturbances presented by endstage renal disease patients.

The inoculum size of S. epidermidis used in these experiments was larger than that which would be introduced into the peritoneal cavity of CAPD patients following an incident of touch contamination. A much larger inoculum is in fact required to cause infection in the C57BL/6 mouse strain which is relatively resistant to extracellular pathogens (25,26). The selected inoculum size was small enough to avoid animal death yet large enough to cause a measurable response that

could differentiate between the animal groups. Indeed all mice survived and the bacterial challenge promptly stimulated inflammatory responses.

Within twenty four hours of ip S. epidermidis inoculation peritoneal washings had become culture negative while viable organisms remained associated with the peritoneum. Given the aggressive nature with which the peritoneal washings were performed these findings suggest the existence of a secure association between S. epidermidis and some aspect(s) of the peritoneum. Adherence of S. epidermidis to the peritoneum in the absence of culture positive peritoneal washings may correspond to the clinical situation where sterile dialysis effluents are recovered in CAPD patients otherwise presenting symptoms of peritonitis. The pathological assessment of samples of peritoneum from renal failure mice and their controls at various times after ip bacterial inoculation is currently under study and may better define the nature of the association of S. epidermidis with the serosal membrane.

The 10^6 cfu S. epidermidis ip inoculum was effectively cleared in all mice regardless of their degree of renal function, however, clearance was delayed in the renal failure group compared to sham-operated control mice. Although the in vitro assessment of immune function in renal failure has been frequently documented in the literature, relatively few studies of renal failure animal models challenged with viable bacteria have been reported. In contrast to our observations, recent investigations utilizing rat models of renal failure, have failed to demonstrate increased susceptibility to ip challenge

with Escherichia coli and Pseudomonas aeruginosa (9, 27). This apparent disparity of results may be attributed to the use of different animal models, test microorganisms, inocula sizes, various sampling times and tissue sites selected for microbiological assessment. Expansion of in vivo assessments to include a wider range of inoculum sizes and follow-up testing to complete bacterial clearance may be required to settle these differences.

Following ip S. epidermidis inoculation, the prompt development of a local inflammatory response characterized by a rise in peritoneal polymorphs was evident in all mice. Despite the peritoneal polymorph response to bacterial challenge a striking decline of peritoneal leucocytes was observed which was primarily accounted for by a marked reduction in macrophages. This observed fall in macrophages cannot be attributed to sampling error since the same methodology was applied to all animals throughout the follow-up period and systematic examination of smears of peritoneal washings never revealed cellular debris or clumping. We cannot exclude the possibility that following bacterial challenge macrophages become more adherent and are lost through the processing of peritoneal washings or, that macrophages accumulate on the peritoneum from which large numbers of bacteria are recovered. However concurring with our observations, a reduction of peritoneal leucocytes predominantly due to decreased macrophages, following ip staphylococcal challenge has been previously reported (28,29). The phenomenon was attributed to the antiinflammatory influence of staphylococcal products which specifically inhibited locomotion of leucocytes predominantly

of the monocytic cell line. In the current study, systemic inflammatory response to the bacterial challenge was similar in renal failure and sham-operated mice and was not as profound as that presented by peritoneal leucocytes.

The mechanism whereby renal failure influences bacterial clearance is unknown, however, integrating temporal relationships of bacterial clearance and inflammatory response patterns leads to the following considerations. In the early phase following bacterial challenge peripheral mobilization of polymorphs in renal failure and sham-operated mice was similar yet emigrating cells did not accumulate at the peritoneal site of bacterial challenge in renal failure mice. Similar observations have been reported elsewhere, in which the early phase of polymorph accumulation at an inflammatory site was diminished in renal failure rats (30) and in renal failure mice (14) in spite of normal peripheral polymorph response. In this latter case eighteen hours after ip thioglycolate injection peritoneal accumulation of both polymorphs and macrophages were significantly reduced in renal failure mice compared to sham-operated controls. In the critical phase immediately following ip inoculation, initial destruction of staphylococci is carried out by resident macrophages and thereafter the influx of new phagocytic cells is crucial to control extracellular bacterial multiplication (31). Therefore, failure to promptly mount an appropriate peritoneal phagocytic cell response following S. epidermidis challenge may characterize a prominent immunological impairment of the uremic host. The attenuated early local polymorph response and the immediate and prolonged reduction of peritoneal macrophages presented by renal failure

mice may form the basis for the delayed S. epidermidis clearance.

The detrimental influence of surgical trauma on immune responsiveness has been well documented (32-34) and necessitated the inclusion of sham-operated control mice in the current studies. Sham-operated mice underwent right kidney electrocoagulation and two weeks later surgical exploration of the left renal area was conducted. All experimental assessments were done at least 6 weeks after the second surgery. Bacterial clearance from the peritoneum as well as inflammatory response to the S. epidermidis challenge were attenuated in sham-operated mice compared to normal controls. The possibility that long lasting ip changes occur following surgery remains to be established, however, we have previously shown (14) that 6 weeks after surgical preparation, the number and nature of resident peritoneal cells harvested from sham-operated mice was similar to normal controls.

Although renal failure caused a delay in the clearance of an ip inoculum of S. epidermidis, the final outcome of the bacterial challenge was the same in all mice. Peritoneal contamination alone, then, with a single small bacterial inoculum may not invariably lead to infection in a relatively young and otherwise healthy subject with renal failure. In a clinical setting, however, when superimposed on advanced age, debility, associated illnesses and local conditions affecting immunocompetence, renal failure may well increase the propensity to infection in CAPD patients.

This animal preparation provides a useful system in which to study the effects of renal failure on the response to ip challenge with S. epidermidis. Results of this study suggest

that delayed bacterial clearance in renal failure mice is the consequence of their inability to mount an appropriate inflammatory response to ip S. epidermidis challenge. Although the exact nature of the immunological defect is currently unknown, this is the first evidence for an impaired inflammatory response in the pathogenesis of the increased susceptibility to S. epidermidis infection observed in renal failure. Further studies may help illuminate the basic pathophysiology of peritonitis associated with continuous ambulatory peritoneal dialysis.

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Chapter 5

The influence of a permanently dwelling peritoneal catheter on the response of renal failure mice to peritoneal *Staphylococcus epidermidis* challenge

Section 5.1

The role of an intraperitoneal catheter in the pathogenesis of experimental *Staphylococcus epidermidis* peritonitis in renal failure mice

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ABSTRACT

The influence of a permanently dwelling peritoneal catheter on the response of renal failure and control mice to peritoneal inoculation with 10^6 cfu Staphylococcus epidermidis was assessed 48 hours after bacterial challenge. Two weeks after the surgical induction of renal failure or sham surgery a segment of a peritoneal dialysis catheter was implanted entirely within the confines of the peritoneal cavity of mice. One month later peritoneal S. epidermidis inoculation was performed by transcutaneous injection through the abdominal wall either directly into the peritoneal cavity (ip) or via the catheter lumen (ic). Following ip inoculation, minimal bacterial growth was recovered from the peritoneal structures of all mice, including the peritoneal catheter. In contrast, following ic S. epidermidis challenge, while peritoneal washings and parietal peritoneum again presented minimal bacterial recoveries, the catheter site remained heavily colonized. S. epidermidis recovery from the catheter site of renal failure mice was significantly greater than that of sham-operated controls. Scanning electron microscopy of catheter segments recovered from mice following ic inoculation revealed single cocci or microcolonies associated with the catheter surface and differential leucocyte counts of fluid aspirated from the catheter lumen revealed evidence of acute inflammation. Signs of inflammatory processes in peritoneal washings and peripheral blood, however, were never observed.

These results are discussed in relation to S. epidermidis peritonitis and continuous ambulatory peritoneal dialysis.

INTRODUCTION

Since its inception, the practice of continuous ambulatory peritoneal dialysis (CAPD) has consistently been limited by the frequent complication of peritonitis. Despite continued improvements in the design of ancillary equipment to minimize the risk of touch contamination, skin borne Staphylococcus epidermidis remains the causative organism in a majority of CAPD peritonitis episodes (1-4). Electron microscopy studies have clearly demonstrated the association of a variety of bacteria with the smooth surface and cuffs of permanently dwelling peritoneal catheters recovered from CAPD patients (5-7). S. epidermidis has been notoriously associated with foreign body infections (8) and slime production, by certain strains, facilitates attachment to smooth surfaces and affords protection from eradication by host defense mechanisms and antimicrobial therapy (9-12).

Previous studies done in our laboratory have demonstrated that renal failure mice successfully clear, albeit slowly, a small (10^6 cfu) peritoneal S. epidermidis challenge (13). In the current investigation, the influence of a permanently dwelling peritoneal catheter segment on response to an identical S. epidermidis inoculum has been assessed in mice with surgically induced renal failure and sham-operated controls. The catheter segment was placed entirely within the confines of the peritoneal cavity in order to preserve

peritoneal integrity which would otherwise be threatened by a functional peritoneal catheter having an exit site to the skin. Experimental bacterial challenge consisted of transcutaneous peritoneal inoculation through the abdominal wall either directly into the peritoneal cavity or into the lumen of the peritoneal catheter segment. The results of our assessment suggest that the peritoneal catheter represents an immunologically preferred site from which early clearance of S. epidermidis is impaired and that bacterial clearance is further compromised by renal failure.

MATERIALS AND METHODS

Animal model:

Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY, USA) at five weeks of age. The animals were allowed to acclimatize in holding facilities for one week prior to use. All animals had free access to water and mouse chow.

As previously described, renal failure was induced in mice by two consecutive surgical procedures (14). Briefly, six week old mice were anesthetized with ether and a small flank incision was made through which the right kidney was separated from the adrenal gland and freed of the perirenal fat. Particular caution was taken to avoid trauma to the ureter. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, Calif., USA) and excluding a 2 mm margin around the pelvis, the surface of the kidney was electrocoagulated. The incision was closed with a running

suture through the deep layers and surgical clips were applied to the skin. Two weeks after the right kidney electrocoagulation, either contralateral nephrectomy (renal failure mice) or surgical exploration of the left renal area (sham-operated controls) was performed.

Two weeks elapsed between nephrectomy or sham surgery and the peritoneal implantation of a custom made segment of an Oreopoulos-Zellerman CAPD catheter (Accurate Surgical Instruments Co., Toronto, Canada). The catheter segment (12 mm long, 5 mm outer diameter, 0.07 ml residual volume) was manufactured with bevelled ends and six perforations were regularly spaced along its length. Through a left flank incision the catheter segment was placed entirely within the confines of the peritoneal cavity and was secured to the lateral abdominal wall by a single anchoring suture passing through one of the perforations. The free end of catheter was directed towards the left lower quadrant of the abdominal cavity. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Four to six weeks later animals were used for experimentation.

Bacterial challenge:

Dr. S.I. Vas (Toronto Western Hospital, Toronto, Canada) kindly supplied us with a strain of Staphylococcus epidermidis that had been recovered from the peritoneal fluid of a CAPD patient. Aliquots of this clinical isolate, reference number 29260, were frozen in glycerol broth. The characteristic antibiotic sensitivity profile of this organism facilitated positive identification. Compared to reference S. epidermidis

strains (kindly supplied by Dr. G.D. Christensen, University of Tennessee, Memphis, Tennessee), isolate 29260 produced glass adherent material when grown in trypticase soy broth (TSB) demonstrating slime producing ability (9). The bacterial suspension for inoculation was prepared from overnight cultures grown on Columbia agar. Bacterial viable units were enumerated by serial dilution and pour plate techniques.

Under light ether anesthesia mice received peritoneal inoculation with one ml HEPES buffered (20 mM) normal saline containing 10^6 colony forming units (cfu) viable bacteria. The transcutaneous injections through the abdominal wall were either intraperitoneal (ip), into the right lower quadrant of the abdomen, or intracatheter (ic), with the needle directed into the lumen of the free end of the catheter. Forty-eight hours after inoculation animals were sacrificed for assessment.

Blood analysis:

Mice were anesthetized with ether, weighed and exsanguinated by cardiac puncture. Blood was collected in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for biochemistry and hematology analysis. Blood urea nitrogen (BUN) levels were determined with an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass, USA). Routine hematology was done utilizing a Coulter counter (Model 2B1 Coulter Electronics Inc., Hialeah, Fla., USA) and differential cell counts were performed on Wright-stained smear preparations.

Collection and processing of specimens:

The abdomen was washed with alcohol and the skin and loose

connective tissue were retracted aseptically to expose an intact translucent abdominal wall consisting of parietal peritoneum and scant connective and muscle tissue. The entire tissue will hereafter be referred to as peritoneal membrane or parietal peritoneum. Ten ml of minimum essential medium (Eagle Modified) containing 10% heat inactivated fetal calf serum and 20 mM HEPES buffer was injected into the peritoneal cavity. The peritoneal washout was slowly withdrawn through a 21 gauge needle and syringe. Total cell counts of peritoneal washings were performed in Neubauer counting chambers and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, Ill., USA). An aliquot of each sample was spread onto 5% horse blood Columbia agar. Bacterial recoveries were expressed as cfu per ml of peritoneal washing.

A specimen of the ventral parietal peritoneum (approximately 2 x 2 cm) was excised aseptically, rinsed, homogenized in 2 ml HEPES buffered (20 mM) normal saline and inoculated onto blood agar. After correction for dilution, bacterial recoveries were expressed as cfu per gram of tissue.

The catheter segment was removed by fine dissection of any adhering tissue and by cutting the anchoring suture to the abdominal wall. Fluid within the catheter lumen was aspirated and determinations of total cell counts as well as cytocentrifuge preparations for differential cell counts were conducted as for peritoneal washings. Granulation tissue always formed a continuous sheath around the catheter segment. As for specimens of parietal peritoneum, this tissue was aseptically removed and homogenized in 1 ml HEPES buffered

normal saline. When necessary tenfold serial dilutions were prepared and specimens were cultured on blood agar. After the appropriate correction for dilution, the colony counts were expressed as cfu per gram of tissue.

Bacteria associated with the catheter surface were quantitated following a modification of a previously described method (15). Using light downward pressure, the catheter segment was streaked across a blood agar plate ten times. The catheter was rotated 90° and again streaked ten times across the agar plate. After repeating this process four times, the catheter was incubated in 5 ml TSB. Bacterial recoveries from blood agar assessment were expressed as cfu per catheter surface and results of incubation in TSB were qualitatively expressed as culture positive or negative.

All samples cultured on blood agar were incubated at 37°C for 24 hours before colonial enumeration. Confirmation of the constant identity of bacteria recovered from collected specimens was ascertained by colonial morphology, gram stain, catalase production and the characteristic antibiotic sensitivity profile.

Scanning electron microscopy (SEM)

A 2 mm transverse section from the anchoring end of each recovered catheter segment was reserved for SEM. Specimens were processed for SEM using standard techniques. Catheter specimens were fixed in cacodylate buffered glutaraldehyde solution (pH 7.3), dehydrated with increasing concentrations of acetone and dried to a critical point. Specimens were then fixed to metal planchets, coated with gold and examined with a scanning electron microscope (model JSM-35U; JEOL, USA, Inc.).

Histological assessment

A 2 mm transverse section of the catheter associated tissue was peeled away from the anchoring end of each catheter segment and was reserved for light microscopy. Following standard techniques, the tissue was fixed in 10% buffered formalin and stained with hematoxylin and eosin.

Expression of results and statistical analysis

Quantitative bacterial recoveries were converted to log units per gram. A log value of 1 per gram represents the lower limit of detection of S. epidermidis by this microbiological technique. Specimens presenting no bacterial growth were assigned a value of log 1. All results are expressed as mean± SD. Student's t test was used to compare the unpaired sets of data.

RESULTS

Animal model

Renal failure:

Selected characteristics of this mouse model six weeks after the surgical induction of renal failure are presented in Table 1. Blood urea nitrogen, which is the primary biochemical indicator of renal failure in this model, was appropriately elevated in the renal failure mice compared to sham-operated controls. Hemoglobin concentration as well as body weight of renal failure mice were significantly less than those of control animals.

Catheter implantation:

Peritoneal implantation of the catheter segment two weeks after the second surgery was well tolerated and mice presented no evidence of infection or inflammation as a result of

Table 1

Selected characteristics of the mouse model of chronic renal failure[a,b]

Status of animals:	Sham-operated (21)	Renal failure (22)
Blood urea nitrogen (mg/dl)	21.5±3.7	88.8±34.0[c]
Hemoglobin (g/dl)	13.5±0.6	9.3±1.6[c]
Body weight (g)	20.5±1.8	19.3±1.9[c]

[a]Results expressed as mean±SD.
[b]Sample size indicated in parenthesis.
[c]Significant difference ($p<0.05$) between the two groups of mice.

preparatory surgery. Immediate peritoneal response to the implant was demonstrated by the rapid formation of granulation tissue which encompassed the catheter segment. Previously, histological assessments of the catheter associated granulation tissue revealed a thick, poorly vascularized tissue which consisted predominantly of fibroblasts and mononuclear leucocytes (16). Although this tissue completely surrounded the catheter segment, immediate peritoneal distribution of the inoculation volume was not impeded as demonstrated radiologically by ic injecton of Renographin-60. One month after implantation, the catheter and its associated tissue almost always remained free of adhesion to peritoneal structures.

Recovery of S. epidermidis following intraperitoneal or intracatheter inoculation

The recovery of S. epidermidis forty eight hours after ip or ic inoculation of 10^6 cfu in mice harbouring peritoneal catheter implants is summarized in Table 2. Previous studies done in our laboratory have documented the peritoneal recovery of S. epidermidis 48 hours after 10^6 cfu ip challenge in mice without peritoneal catheter implants (13). In comparison to those observations, the presence of a peritoneal catheter segment did not influence the microbiological status of peritoneal washings or parietal peritoneum following ip or ic inoculation of 10^6 cfu S. epidermidis. Regardless of the route of inoculation or the level of renal function, S. epidermidis recovery from peritoneal washings and parietal peritoneum was minimal. However, after ic inoculation large numbers of S. epidermidis were recovered from the catheter associated granulation tissue and a majority of recovered

catheter segments were culture positive in TSB. In contrast catheter segments and associated granulation tissue recovered from mice after ip inoculation were invariably culture negative.

Forty eight hours after ic inoculation, recovery of S. epidermidis was significantly greater from catheter associated granulation tissue of renal failure mice compared to sham-operated controls (Table 2 and Figure 1). Quantitative assessment of S. epidermidis associated with catheter segments recovered after ic inoculation is presented in Figure 2. A greater proportion of catheters from sham-operated animals yielded no bacterial growth compared to those recovered from renal failure mice. Of those catheter segments presenting positive S. epidermidis recoveries, colonies enumerated were less for catheters recovered from sham-operated mice.

Scanning electron microscopy

SEM assessments of catheters recovered from renal failure mice and sham-operated control animals were similar. Invariably, the luminal surfaces of catheters were littered with host cells which were commonly macrophages that were often activated, while lymphocytes were seen less frequently (17) (Figures 3a-3c). Bacteria were not observed on the surface of catheters recovered from mice following ip inoculation (6 specimens examined). Single cocci or isolated microcolonies, frequently in association with host cells, were observed on the luminal surface of catheters recovered from mice subjected to ic S. epidermidis inoculation (14 specimens examined) (Figure 4). Coccoid forms embedded in a matrix which made clear distinction of bacteria impossible, were also seen on

Table 2

Recovery of *Staphylococcus epidermidis* from peritoneal structures of mice 48 hours after 10^6 cfu inoculation[a,b]

Mode of peritoneal inoculation:	Outside the catheter (ip)		Through the catheter lumen (ic)	
Status of animals:	Sham-operated (9)	Renal failure (10)	Sham-operated (12)	Renal failure (12)
Peritoneal washings (log colonies per ml)	NBG	NBG	NBG	NBG
Parietal peritoneum (log colonies per g)	NBG	1.2±0.4	1.2±0.4	1.4±0.6
Catheter associated tissue (log colonies per g)	NBG	NBG	2.8±1.6	5.8±2.1[c]
Catheter (growth in TSB)	NBG	NBG	6/12	10/12

[a]Results expressed as mean±SD with NBG indicating no bacterial growth.
[b]Sample size indicated in parenthesis.
[c]Significant difference ($p<0.05$) between the two groups of mice.

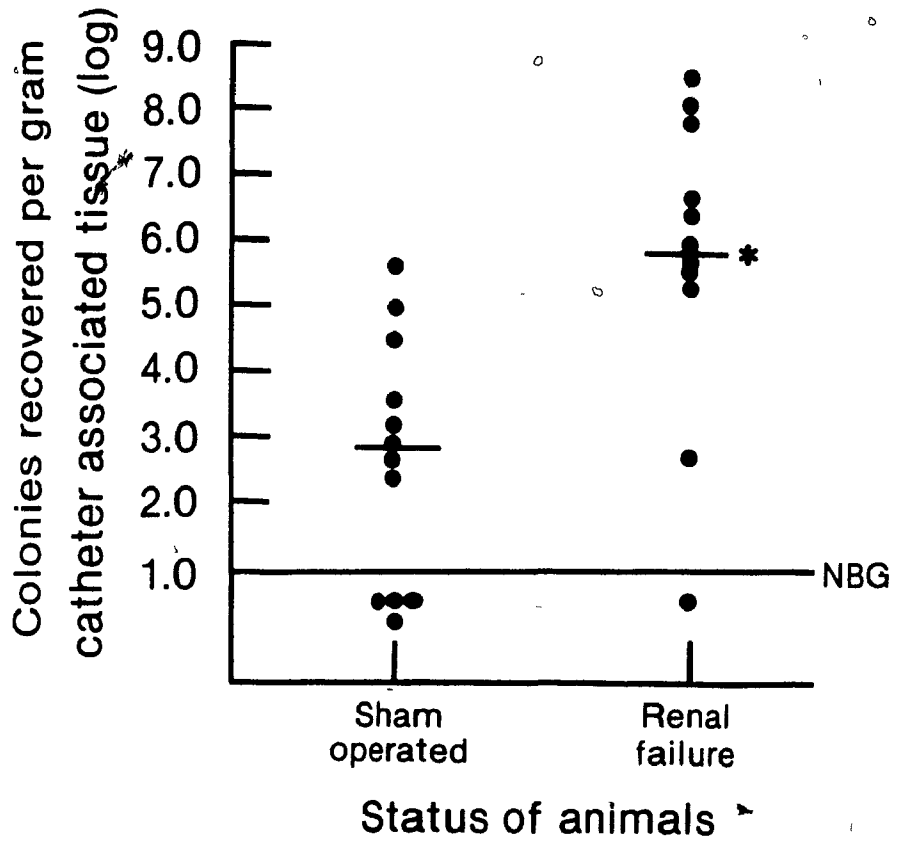

Figure 1. Recovery of Staphylococcus epidermidis from catheter associated granulation tissue from sham-operated and renal failure mice 48 hours after intracatheter inoculation (10^6 cfu). The horizontal bars represent mean values for each group and the asterisk indicates a significant difference ($p<0.05$) between renal failure and sham-operated controls. NBG indicates no bacterial growth and such data points are clustered in the separate section at the base of the figure.

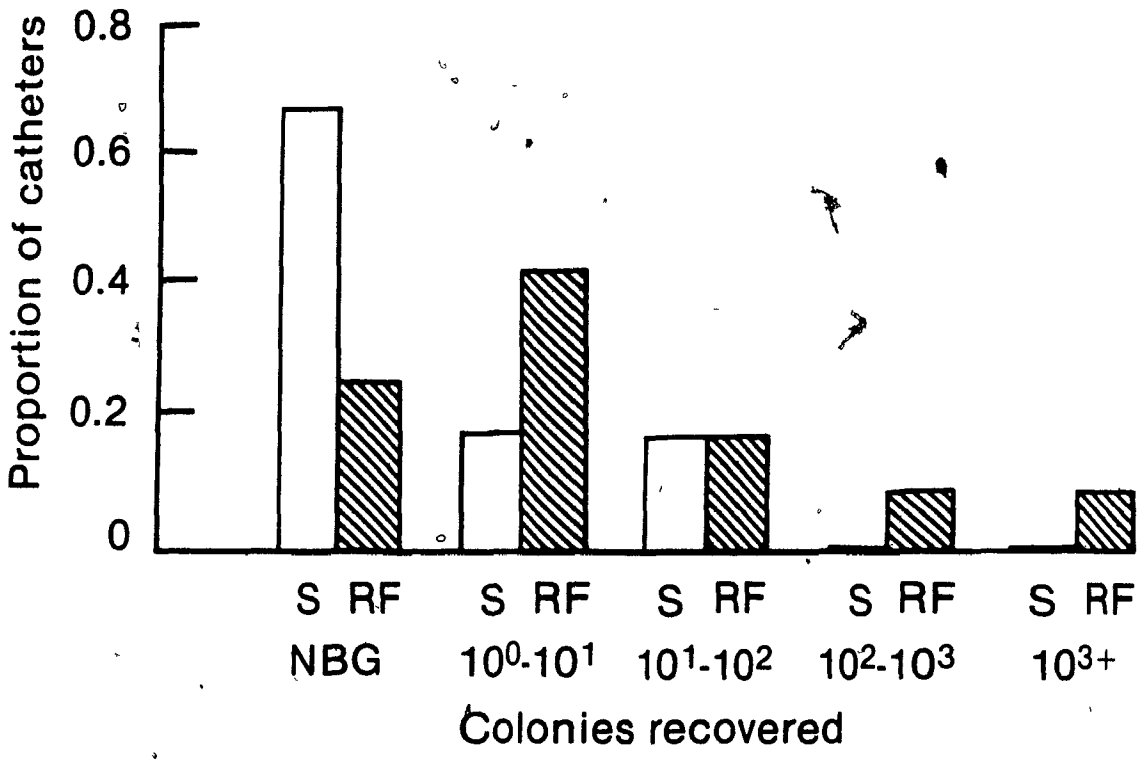

Figure 2. Recovery of Staphylococcus epidermidis from catheters of sham-operated (S) (open bars) and renal failure (RF) (hatched bars) mice 48 hours after intracatheter inoculation (10 cfu). These data are derived from the same animals represented in Figure 1. NBG indicates no bacterial growth.

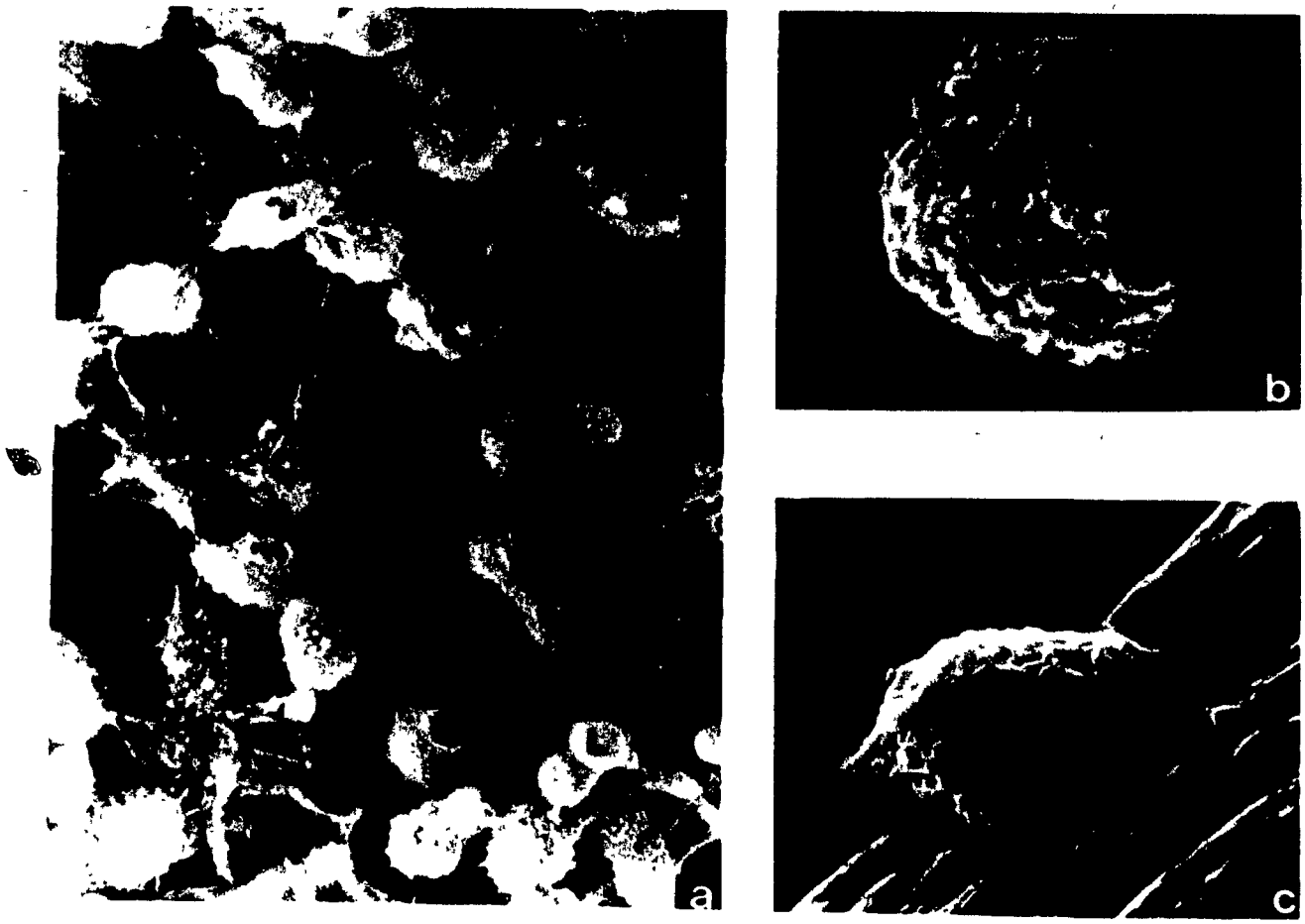

Figure 3. Scanning electron micrographs of host leucocytes on the luminal surface of peritoneal catheters recovered from mice 48 hours after Staphylococcus epidermidis inoculation (10^6 cfu). Leucocytes were invariably associated with the surface of catheters recovered from mice following either intracatheter or intraperitoneal inoculation.
(a) The catheter surface is littered with macrophages (M) and lymphocytes (L) (Magnification x 1,200).
(b) Unstimulated macrophage. The cell surface is covered by numerous irregular ridges (Magnification x 13,300).
(c) Activated macrophage. Cellular processes are extended over the catheter surface (Magnification x 6,000).

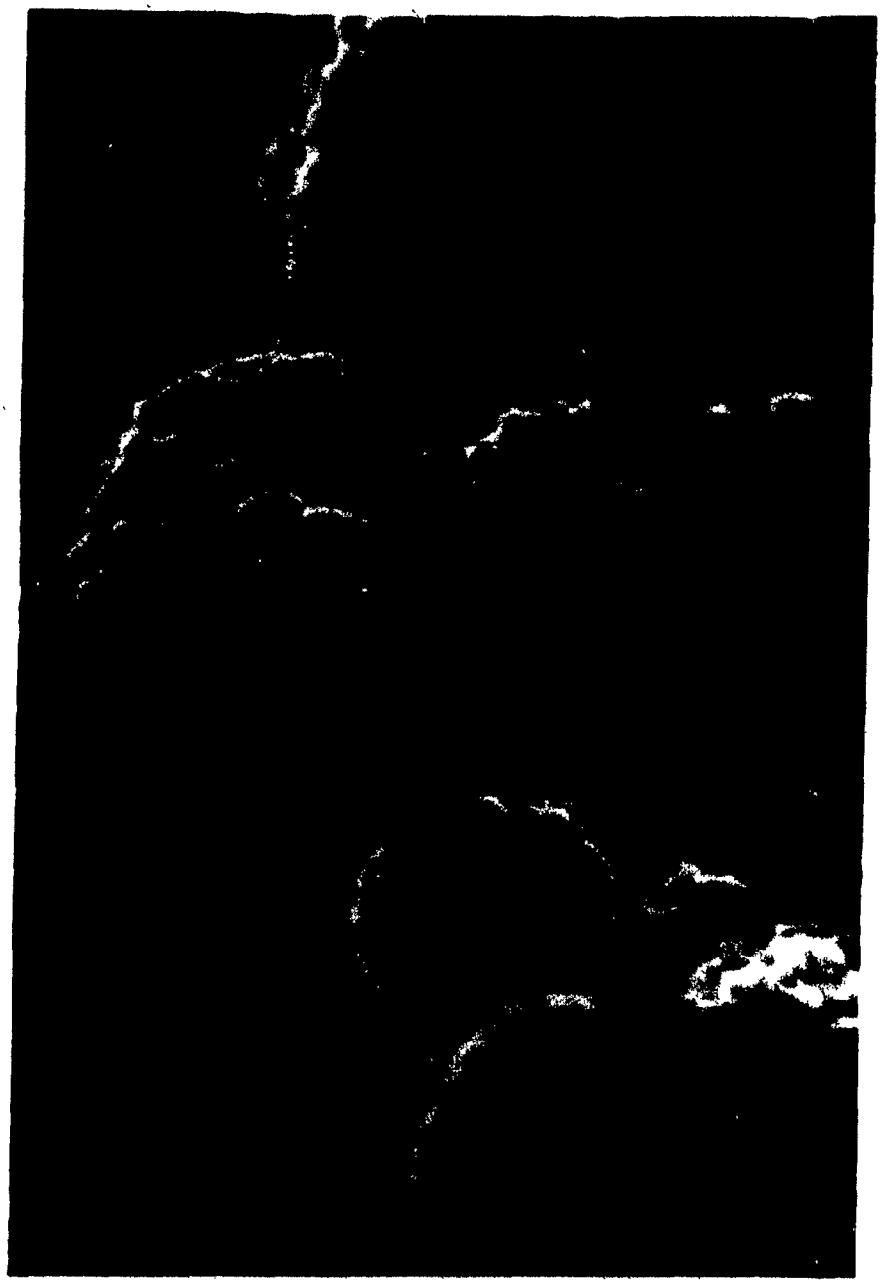

Figure 4. Scanning electron micrograph of the luminal surface of a catheter segment recovered 48 hours after intracatheter inoculation of 10^6 cfu Staphylococcus epidermidis. Single cocci or microcolonies are seen on the catheter surface and frequently in association with host leucocytes (Magnification x 11,000).

catheter surfaces following ic inoculation of mice. Microbiologic assessment of catheters recovered from mice following ic inoculation did not always concur with SEM assessment.

Inflammatory response

Forty eight hours after peritoneal inoculation (ip or ic) there was no evidence of inflammation measured by total and differential leucocytes in peritoneal washings and peripheral blood of mice (Table 3). Total peripheral and peritoneal leucocytes and polymorphs, which are predominantly neutrophils in C57BL/6 mice (18), were similar to levels in non inoculated control animals (data no shown). Fluid aspirates from the lumen of recovered catheter segments presented elevated leucocyte and polymorph counts compared to peritoneal washings. Following ic inoculation, both total cell and polymorph counts of catheter aspirates were elevated compared to those collected following ip inoculation. Significantly greater numbers of polymorphs were observed in aspirates of catheters recovered from renal failure animals compared to sham-operated mice following ic inoculation.

Following ip S. epidermidis challenge, histological assessment of catheter associated tissue (5 specimens examined) was similar to that of specimens collected under control conditions. In contrast, following ic inoculation (5 specimens examined) focal inflammation, marked by polymorph infiltration and interstitial edema, was evident (Figure 5).

Table 3.

Nature of cellular constituents of peritoneal washings, catheter exudate and peripheral blood of mice 48 hours after 10^6 cfu Staphylococcus epidermidis inoculation[a,b]

Mode of peritoneal inoculation:	Outside the catheter (ip)		Through the catheter lumen (ic)	
Status of animals:	Sham-operated	Renal failure	Sham-operated	Renal failure
Peritoneal washings	(9)	(10)	(12)	(12)
Leucocytes/ml (x10^6)	0.7±0.4	1.1±0.7	0.6±0.2	1.0±0.7
Macrophages (%)	49.4±9.5	35.2±7.7	49.9±5.7	50.0±9.4
Lymphocytes (%)	47.8±9.1	63.1±9.9	47.2±7.0	46.6±9.9
Polymorphs (%)	2.8±1.0	2.7±4.1	2.9±2.4	3.4±2.9
Mast cells (%)	0	0	0	0
Catheter exudate	(6)	(6)	(9)	(9)
Leucocytes/ml (x10^6)	1.9±2.3	2.6±2.8	7.1±6.3	8.3±10.5
Macrophages (%)	46.0±10.9	49.5±10.2	34.3±13.6	14.1±13.7[c,d]
Lymphocytes (%)	26.8±10.7	22.7±12.8	21.1±12.1	6.0±5.2[c,d]
Polymorphs (%)	27.2±11.1	27.8±11.9	44.6±20.1	79.9±18.4
Mast cells (%)	0	0	0	0
Circulating leucocytes	(9)	(10)	(12)	(11)
Total/ml (x10^3)	5.5±2.7	5.8±1.2	5.7±2.5	4.6±2.0
Neutrophils (%)	6.1±1.9	5.1±1.0	5.3±2.3	7.3±4.0
Stab (%)	0.1±0.3	0	0	0
Lymphocytes (%)	92.7±1.7	93.7±1.3	94.4±2.5	92.5±4.5
Monocytes (%)	1.0±1.1	1.0±1.1	0.3±0.5	0.3±0.9

[a]Results expressed as mean±SD.
[b]Sample size indicated in parenthesis.
[c]Significant difference ($p<0.05$) between renal failure mice and respective sham-operated controls.
[d]Significant difference ($p<0.05$) between ip and ic inoculation conditions.

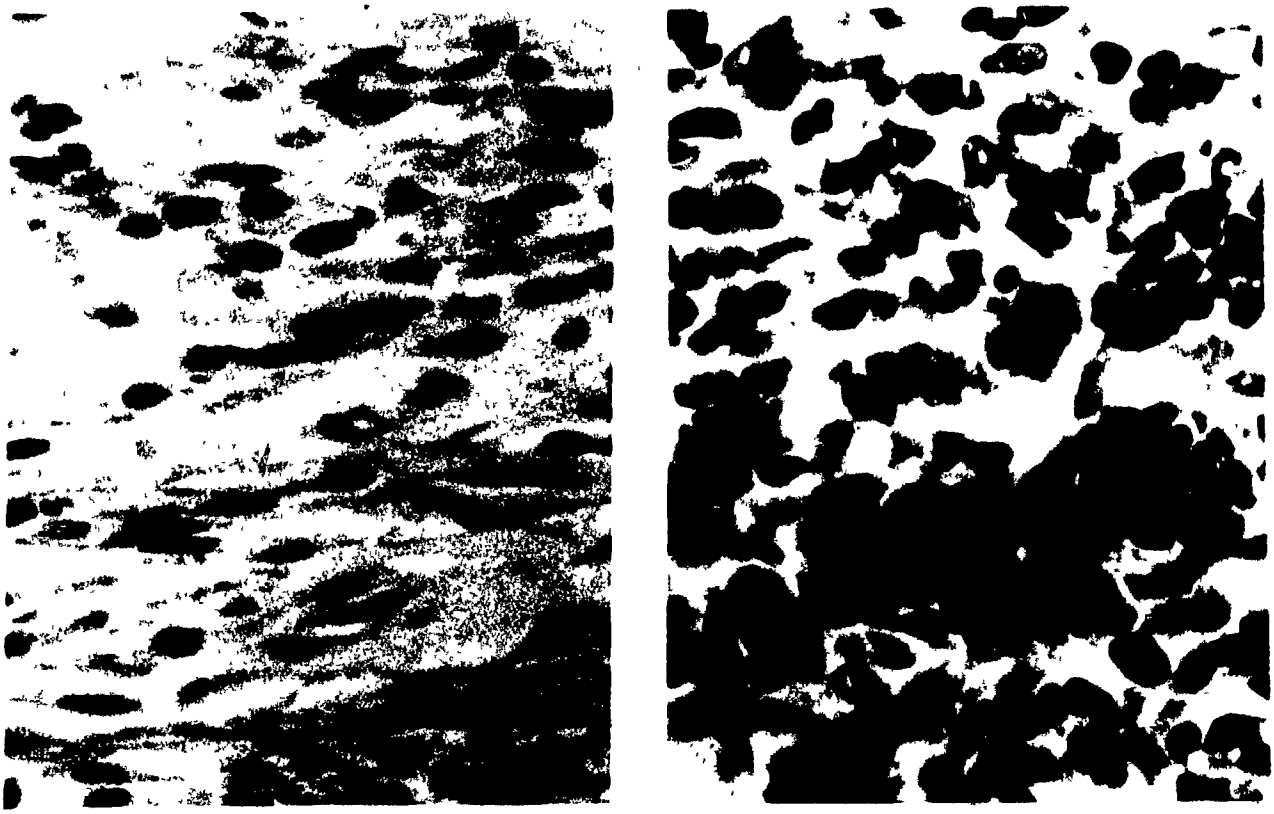

Figure 5. Left panel: Section from catheter associated granulation tissue recovered 48 hours after intraperitoneal inoculation of 10^6 cfu Staphylococcus epidermidis. Tissue is poorly vascularized and contains fibroblasts and mononuclear leucocytes. (Hematoxylin and eosin, Magnification x 400).
Right panel: Section from catheter associated granulation tissue recovered 48 hours after intracatheter inoculation of 10^6 cfu Staphylococcus epidermidis. Focal inflammation is demonstrated by mononuclear and polymorphonuclear leucocyte infiltration. (Hematoxylin and eosin, Magnification x 400).

DISCUSSION

We have assessed the influence of a permanently dwelling peritoneal catheter segment on the response of mice to peritoneal S. epidermidis challenge. Two weeks after the surgical preparation of the renal failure model, placement of a catheter segment entirely within the confines of the peritoneal cavity was well tolerated by the mice and the resulting animal preparation was free of complicating infections. Thus, experimental peritoneal S. epidermidis challenge was controlled and a comparison of intraperitoneal and intracatheter routes of administration could be conducted. Beyond the immediate catheter site, the implant did not compromise the local immune response of either sham-operated or renal failure mice to the peritoneal bacterial challenge, regardless of the mode of inoculation. The catheter site was invariably culture negative after ip inoculation whereas the catheter and its associated tissue presented large S. epidermidis recoveries after ic challenge. The defective early clearance of S. epidermidis from the catheter site following ic inoculation was further impaired in renal failure mice compared to sham-operated controls.

The surgical preparation of this animal model of renal failure has been previously described (14). Many of the manifestations presented by the renal failure mice are similar to those encountered in endstage renal disease patients and included severe anemia, florid osteodystrophy with striking bone marrow fibrosis and a state of malnutrition (19). In the current series of experiments which were conducted six weeks after the induction of renal failure, mice were azotemic and presented significant degrees of anemia and growth retardation.

Utilizing this mouse model, studies done in our laboratory have previously demonstrated an immunocompromised uremic host presenting reduced delayed-type hypersensitivity skin reaction (20-22) and increased susceptibility to bacterial challenge (13,23).

The development of long term peritoneal dialysis models, in animals having functional peritoneal catheters exiting through the skin, has been hindered by numerous complications. CAPD has been conducted in renal failure models in rabbits, dog and rats for periods ranging from two days to two months (24-26). Additions to the peritoneal dialysis solutions included high concentrations of heparin to prevent fibrin clot formation and prophylactic antibiotics. Despite these preventive measures, management of these animals was frequently complicated by peritoneal infection and obstruction of the peritoneal catheter. Finally, progressive bacterial colonization of the subcutaneous tunnel of the peritoneal catheter with resulting inflammation has been demonstrated in normal rabbits subjected to CAPD (27). In the current mouse model, the implantation of a permanently dwelling catheter segment into the peritoneal cavity of mice was never complicated by infection of the peritoneal cavity or surgical wounds. One month after implantation of the catheter, microbiological sterility of the peritoneal cavity was maintained presumably because the catheter did not exit through the abdominal wall. Although peritoneal dialysis was not conducted, access to the peritoneal cavity via the catheter was achieved successfully by transcutaneous injection into the catheter lumen. The resulting animal preparation could therefore accommodate controlled S. epidermidis challenge as

defined by our study objectives.

Peritoneal reaction to the catheter segment in mice was clearly evident one month after implantation, when a granulation tissue sheath completely surrounded the implant. Encapsulation of the peritoneal catheter in the chronic renal failure rat model of CAPD has been previously reported (26). Like any insoluble foreign material, the medical grade silicone rubber of the Oreopoulos-Zellerman CAPD catheter may cause chronic inflammation, even though it is chemically non reactive and generally induces minimal immunological and inflammatory response (28,29). In contrast to this animal preparation, similar evidence of foreign body reaction is not apparent when peritoneal catheters are surgically removed from CAPD patients (30,31). These observations may be explained by differences in the length of time that the peritoneal catheters reside in the peritoneal cavity or in the size and shape of the catheters (32,33).

The role of the peritoneal catheter in the development of S. epidermidis peritonitis in CAPD patients is currently a subject of great interest. Although the exact mechanism is unknown, foreign bodies have long been known to interfere with optimal immune responses in their vicinity. Zimmerli and colleagues have demonstrated impaired phagocytic and bactericidal functions of polymorphs within tissue cage implants (34). Involvement of S. epidermidis in foreign body infection has been well documented (8) and the production of slime by certain strains of S. epidermidis has been epidemiologically related to intravascular catheter sepsis (10) and recurrent S. epidermidis peritonitis in CAPD (35). SEM

assessment of peritoneal catheters recovered from CAPD patients has clearly demonstrated the presence of bacterial biofilms (frequently cocci) on catheter surfaces which may act as bacterial reservoirs causing persistent and recurrent peritonitis (5-7).

In the current investigation, the microbiological status of peritoneal washings and peritoneum following ip or ic S. epidermidis inoculation was not influenced by the presence of an indwelling peritoneal catheter segment. We are unaware of previous studies demonstrating the lack of influence of a peritoneal catheter implant on local response to bacterial challenge. Following ip inoculation the catheter site was invariably free of S. epidermidis, whereas following ic inoculation large numbers of bacteria were recovered from the catheter and its associated granulation tissue. Evidence presented here indicates that when the inoculum is administered into the catheter lumen the catheter site may act as a nidus for bacterial growth. Since the 10^6 cfu inoculum was administered in a one ml volume and the residual volume of the catheter was 0.07 ml, 7% of the original inoculum (log 4.85 cfu) at the most could remain within the catheter at the time of inoculation. Absolute recoveries of S. epidermidis from the catheter site exceeded log 4.85 in 40% of the renal failure mice while none of the sham-operated controls presented similar evidence of bacterial growth.

In spite of persisting S. epidermidis at the catheter site, there was no evidence of ongoing inflammatory processes measured by total cellularity and differential leucocyte counts of peritoneal washings and peripheral blood of mice. These

observations may be relevant to reported cases in which SEM assessments have demonstrated that peritoneal catheters recovered from CAPD patients were colonized with bacteria without apparent evidence of inflammation (5-7).

Although the immunosuppressive influence of renal failure on cell mediated immune response has been well documented (36-38), previous in vivo assessments, frequently conducted in rat models of renal failure, have not demonstrated a marked increase in susceptibility to bacterial challenge in the uremic host (26,39,40). Utilizing the current mouse model, increased mortality and accelerated lethality following large ip S. epidermidis inocula (10^9-10^8 cfu) was observed in renal failure mice (23). Peritoneal clearance of a smaller S. epidermidis challenge (10^6 cfu) was delayed in renal failure mice in contrast to sham-operated controls and early local inflammatory response during the first 24 hours after inoculation was strikingly attenuated in renal failure animals (13). Incorporating an indwelling peritoneal catheter into this model, the influence of renal failure on the response of mice to ic inoculation was again evident. Renal failure mice consistently presented greater bacterial recoveries from the catheter and its associated tissue than did sham-operated controls. At the time of our assessment, forty eight hours after inoculation, there was no evidence to support a defective inflammatory response and, in fact, concurrent with greater bacterial recoveries, renal failure mice presented elevated neutrophil counts in aspirates collected from the catheter lumen compared to sham-operated controls.

In agreement with microbiological assessments, SEM of

catheter segments recovered from mice following ip S. epidermidis challenge revealed no bacteria on the catheter surface. Single cocci or microcolonies of cocci were demonstrated on the surface of catheters following ic inoculation, however, microbiological and SEM assessments of specimens did not always concur. This disparity may be ascribed to sampling error. Only a small section of each recovered catheter was reserved for SEM and when S. epidermidis colonies were observed, bacterial growth was never evenly distributed across the catheter surface which was frequently obliterated by host cells.

The in vitro colonization of intravenous catheters by slime producing strains of coagulase negative staphylococci has been assessed by SEM (9). Colonization begins by adhesion of single cocci to the smooth catheter surface followed by the establishment of microcolonies which can induce surface erosion of the plastic material. In later stages the catheter surface is covered by a confluent single layer or thick multilayer of bacteria which is embedded in an amorphous cementing matrix, collectively referred to as biofilm. Forty eight hours after ic S. epidermidis inoculation of mice, early stages of staphylococcal colonization of the catheter segment were apparent. Whether or not long term colonization of the catheter, in the current animal preparation, would continue to the stage of established confluent biofilms remains to be determined. Unlike the in vitro dynamics of catheter colonization, the influence of host defense mechanisms opposing the development of the bacterial biofilm on peritoneal catheter segments must be considered.

The current animal preparation provides a system in which to investigate the relative roles of renal failure and peritoneal catheter implantation on susceptibility of mice to bacterial challenge. Through microbiologic studies and analysis of inflammatory responses, we have established that the catheter and its associated tissue remains the sole site of bacterial growth and inflammation 48 hours after S. epidermidis intracatheter inoculation. The microbiological fate of this "infected" site, remains an enigma. It is not yet known whether bacteria can be cleared from the catheter and if so, the factors that regulate clearance, including the effect of renal failure, have not been defined. Longer term experiments addressing these issues are currently being conducted in our laboratory.

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Section 5.2

Kinetics of *Staphylococcus epidermidis* clearance from the peritoneal catheter site of renal failure mice following intracatheter inoculation.

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Abstract

The response of mice with surgically induced renal failure and sham-operated controls to intracatheter peritoneal inoculation of 10^6 or 10^8 cfu S. epidermidis and the kinetics of bacterial clearance from peritoneal structures, including a peritoneal catheter implant was investigated. All animals survived experimental challenge with the smaller inoculum however significant mortality was observed following 10^8 cfu intracatheter inoculation and animal survival was always less in groups of renal failure mice than in controls. One week after 10^6 cfu inoculation most animals had cleared the bacterial challenge however a small number of renal failure mice continued to present culture positive specimens from the peritoneal catheter site up to one month after inoculation. At weekly intervals to one month post 10^8 cfu intracatheter inoculation, microbiological assessment of peritoneal structures of surviving mice demonstrated that the peritoneal catheter site was a reservoir for persisting S. epidermidis, a finding that was confirmed by scanning electron microscopy. Phenotypic variation was observed in isolates of S. epidermidis recovered from mice at least two weeks after inoculation. Unexpectedly, S. epidermidis recovery from the catheter site of renal failure mice was significantly less than that of sham-operated controls three and four weeks after 10^8 cfu inoculation. Inflammatory response was similar in sham-operated and renal failure mice in which peritoneal leucocytes and polymorphs were elevated during the first three

weeks after 10^8 cfu inoculation while systemic signs of inflammation consisting of a rise in circulating polymorphs persisted for the duration of the follow-up period. The results of the current investigation concur with previous studies of the early peritoneal distribution of S. epidermidis following intracatheter inoculation and reveal that the catheter site provides a preferred site for persistent bacterial colonization up to one month after inoculation particularly after a large bacterial challenge.

Introduction

The objective of the present study was to investigate the kinetics of Staphylococcus epidermidis clearance from the peritoneal catheter site of mice with surgically induced renal failure and sham-operated controls. This investigation is one of a series in which the pathogenesis of peritoneal S. epidermidis infection, a major complication of continuous ambulatory peritoneal dialysis, was studied in a recently developed mouse model of renal failure. We have previously demonstrated that chronic renal failure has an immunosuppressive influence on the response of mice to peritoneal S. epidermidis inoculation (1-3). The original mouse model was expanded to include a permanently dwelling peritoneal catheter which was implanted one month before experimental inoculation. Forty eight hours after intracatheter inoculation, S. epidermidis was effectively cleared from other peritoneal structures however bacteria remained associated with the catheter site. In addition, S. epidermidis recovery from the catheter site was significantly

greater in renal failure mice compared to sham-operated controls. On the basis of our previous studies we have addressed two questions: 1) Is S. epidermidis cleared from the catheter site during a long term (1 month) follow-up? 2) Does renal failure have a measureable influence on long term response of mice to intracatheter S. epidermidis inoculation? Groups of sham-operated and renal failure mice underwent intracatheter inoculation of either 10^6 or 10^8 cfu S. epidermidis. At specified intervals during the following month animals were sacrificed and microbiological, hematological and biochemical assessments were conducted. In addition, surfaces of recovered catheters were examined by scanning electron microscopy. The bacteriologic status of peritoneal structures as well as the local and systemic inflammatory response of sham-operated and renal failure mice to the experimental inoculum were compared.

Materials and Methods

Surgical induction of renal failure

Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY, USA) at five weeks of age. The animals were allowed to acclimatize in holding facilities for one week prior to use. All animals had free access to water and mouse chow.

The surgical preparation of renal failure and sham-operated mice has been described previously (4). Mice were anesthetized with ether and a small flank incision was made through which the kidney was separated from the adrenal

gland and freed of perirenal fat. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, CA, USA) and excluding a 2 mm margin around the renal pelvis, the exposed surface of the kidney was electrocoagulated. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Two weeks after right kidney electrocoagulation, those animals that would constitute the renal failure group underwent left nephrectomy while sham-operated controls underwent surgical exploration of the left renal area.

Catheter implantation

Two weeks elapsed between nephrectomy, or sham surgery, and peritoneal implantation of a custom made segment of an Oreopoulos-Zellerman CAPD catheter (Accurate Surgical Instruments Co., Toronto, Canada). Both ends of the segment were bevelled and the 1.2 cm length contained six regularly spaced perforations. According to the manufacturer's specifications, catheter segments were washed in mild detergent, rinsed several times in distilled water and gas sterilized prior to implantation. Through a left flank incision the catheter segment was placed entirely within the confines of the peritoneal cavity and was secured to the lateral abdominal wall by a single anchoring suture passing through one of the side perforations. The free end of the catheter was directed towards the left lower quadrant of the abdominal cavity. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Four to six weeks later animals were subjected to experimental inoculation.

Bacterial challenge:

The test S. epidermidis, reference number 29260, was recovered from the peritoneal fluid of a CAPD patient with peritonitis and was kindly supplied to us by Dr. S.I. Vas (Toronto Western Hospital, Toronto, Canada). Aliquots of this clinical isolate were maintained frozen in glycerol broth. The characteristic antibiotic sensitivity profile of this organism facilitated positive identification. Compared to reference slime-producing S. epidermidis strains (kindly supplied by Dr. G.D. Christensen, University of Tennessee, Memphis, Tennessee) isolate 29260 produced glass adherent material when grown in trypticase soy broth (TSB) demonstrating slime-producing ability (5). The bacterial suspension for inoculation was prepared from overnight cultures grown on Columbia agar. The density of viable bacteria (colony forming units) was enumerated by serial dilution and pour plate techniques.

Under light ether anesthesia mice received peritoneal inoculation with either 10^6 colony forming units (cfu) or 10^8 cfu S. epidermidis in 1 ml HEPES buffered (20mM) normal saline. The catheter segment was manipulated to palpate the catheter lumen and transcutaneous intracatheter injection was performed with a 25 gauge needle directed into the lumen of the free end of the catheter. In one experiment mice without peritoneal catheters received 10^8 cfu S. epidermidis inoculation intraperitoneally, by injection into the right lower quadrant of the abdomen. Assessments of mice were conducted at the specified weekly intervals after inoculation.

Blood analysis:

Mice were anesthetized with ether, weighed and

exsanguinated by cardiac puncture. Blood was collected in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for biochemistry and hematology analysis. Blood urea nitrogen (BUN) levels were determined with an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass, USA). Routine hematology was done utilizing a Coulter counter (Model 2B1 Coulter Electronics Inc., Hialeah, Fla., USA) and differential cell counts were performed on Wright-stained blood smear preparations.

Collection and processing of specimens:

The abdomen was washed with alcohol and the skin and loose connective tissue were retracted aseptically to expose an intact translucent abdominal wall consisting of parietal peritoneum and scant connective and muscle tissues. The entire tissue will hereafter be referred to as peritoneal membrane or parietal peritoneum. Ten ml of minimum essential medium (Eagle Modified) containing 10% heat inactivated fetal calf serum and 20 mM HEPES buffer was injected directly into the peritoneal cavity and the peritoneal washout was slowly withdrawn. Total cell counts of peritoneal washings were performed in Neubauer counting chambers and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, Ill., USA). An aliquot of each sample was quantitatively cultured onto 5% horse blood Columbia agar for bacterial content and identity. Bacterial recoveries were expressed as cfu per ml of peritoneal washing.

A specimen of the ventral parietal peritoneum (approximately 2 x 2 cm) was excised aseptically, rinsed,

homogenized in HEPES buffered normal saline and inoculated onto blood agar. After correction for dilution, bacterial recoveries were expressed as cfu per gram of tissue.

The catheter segment was removed by fine dissection of any adhering tissue and cutting the anchoring suture to the abdominal wall. Fluid within the catheter lumen was aspirated and determinations of total cell counts as well as cytocentrifuge preparations for differential cell counts were conducted as for peritoneal washings. Granulation tissue which surrounded the catheter segment was aseptically removed and homogenized in HEPES buffered normal saline. When necessary tenfold serial dilutions were prepared and specimens were inoculated onto blood agar. After the appropriate correction for dilution, colonies recovered were expressed as cfu per gram of tissue.

Bacteria associated with the catheter were quantitated following modifications of previously described methods (6,7). Using light downward pressure the catheter segment was streaked ten times across the agar plate. The catheter segment was rotated 90° and was again streaked across the agar plate. After repeating this process four times the catheter was incubated in TSB. Bacterial recoveries from blood agar assessment were expressed as cfu per catheter and results of incubation in TSB were expressed as culture positive or negative. Alternately, the recovered catheter segment was split lengthwise. One half of the catheter was processed as above. The remaining half was aseptically scraped with a sterile blade into HEPES buffered normal saline and the scraped material was broken up by Vortex mixing with glass beads for 30

seconds. When necessary tenfold serial dilutions of the saline wash were prepared and specimens were inoculated onto blood agar. After correction for dilution, colonies recovered were expressed as cfu per catheter.

Colony enumeration of all samples on agar was conducted after twenty-four hours incubation. Positive identification of recovered bacteria was ascertained by colony morphology, gram stain, catalase production and the characteristic antibiotic sensitivity profile. In selected cases, procedures for positive identification included automated identification by Vitek (Vitek Systems, McDonnel Douglas Health Systems Co., Hazelwood, MO, USA).

Scanning electron microscopy (SEM)

A 2 mm transverse section from the anchoring end of recovered catheters was reserved for SEM. Specimens were processed for SEM using standard techniques. Catheter segments were fixed in cacodylate buffered glutaraldehyde solution (pH 7.3), dehydrated with increasing concentrations of acetone and dried to a critical point. Specimens were then fixed to metal planchets, coated with gold and examined with a scanning electron microscope (model JSM-35U; JEOL, USA, Inc.).

Experimental protocol

Table 1 summarizes the experimental protocol of the preparation of the animal model and the schedule of inoculation and microbiological assessment of mice.

Expression of results and statistical analysis

Bacterial recoveries were converted to log units. Specimens presenting no bacterial growth on agar were assigned a value of log 1 (10 cfu per ml) which represents the lower

Table 1. Experimental protocol

Weeks	Procedure
0	Electrocoagulation of the right kidney (all mice)
2	Left nephrectomy (renal failure mice) or exploration of the left renal area (sham-operated mice)
4	Implantation of the peritoneal catheter
8	Intracatheter inoculation with *Staphylococcus epidermidis* (10^6 or 10^8 cfu)
9	Assessment 1 week post challenge (10^6 and, 10^8 cfu)
10	Assessment 2 weeks post challenge (10^8 cfu)
11	Assessment 3 weeks post challenge (10^8 cfu)
12	Assessment 4 weeks post challenge (10^6 and, 10^8 cfu)

limit of detection of the microbiological assessment. All results are expressed as mean±SD. Statistical analysis was conducted utilizing Student's t test for the comparison of unpaired data sets, one way analysis of variance and correlation analysis.

Results

Animal model

Table 2 summarizes selected characteristics of the mouse model seven to ten weeks after the surgical induction of renal failure. Blood urea nitrogen (BUN) was used to assess the degree of renal failure in this animal preparation and in comparison to sham-operated controls, renal failure mice presented appropriately elevated BUN levels. Mean hemoglobin concentration was always significantly reduced in renal failure mice compared to sham-operated controls and as previously reported hemoglobin levels of renal failure animals were significantly correlated to BUN. Hemoglobin levels of renal failure mice subjected to either 10^6 or 10^8 cfu *S. epidermidis* challenge were similar. In contrast, sham-operated mice that were sacrificed at weekly intervals after 10^8 cfu *S. epidermidis* inoculation presented significantly reduced mean hemoglobin concentration compared to respective animals that received 10^6 cfu inoculation or non inoculated sham-operated mice (data not shown). The reduction of hemoglobin concentration was not affected by the sampling time following 10^8 cfu inoculation or by the magnitude of bacterial recovery from the catheter site. Mean body weight of renal failure and sham-operated mice was similar.

Peritoneal implantation of the catheter, two weeks after

Table 2. Characteristics of the mouse model of renal failure[a]

Animal status:	Sham-operated	Renal failure
Blood urea nitrogen (mg/dl)[b]	23.9±5.0 (52)	98.4±29.5 (52)[e]
Hemoglobin (g/dl)		
10^6 cfu inoculation[c]	12.7±1.7 (17)	9.6±1.5 (17)[e]
10^8 cfu inoculation[d]	11.0±1.3 (30)[f]	8.7±1.7 (31)[e]
Body weight (g)[b]	22.5±2.3 (53)	21.7±1.8 (52)

[a]Results are obtained in 15-19 week old C57BL/6 inbred mice 7-10 weeks after the surgical induction of renal failure or sham surgery. Results are expressed as mean±SD and sample size is indicated in parentheses.

[b]No significant difference ($p<0.05$) was observed between animals subjected to 10^6 or 10^8 cfu Staphylococcus epidermidis challenge and therefore results were pooled.

[c]Results from mice sacrificed one week and one month after intracatheter inoculation with 10^6 cfu Staphylococcus epidermidis.

[d]Results from mice sacrificed at weekly intervals from one to four weeks after intracatheter inoculation with 10^8 cfu Staphylococcus epidermidis.

[e]Significant difference ($p<0.05$) between renal failure and sham-operated mice.

[f]Significant difference ($p<0.05$) between mice subjected to 10^6 and 10^8 cfu Staphylococcus epidermidis inoculation.

nephrectomy or sham surgery, was well tolerated by all mice. One month later, at the time of experimentation there was no evidence of peritoneal contamination as a result of preparatory surgical procedures or catheter implantation. Invariably, granulation tissue encompassed the catheter segment and has been characterized previously as a low grade inflammatory response of the peritoneum to the implant (8). Histological assessments of the catheter associated granulation tissue revealed a thick poorly vascularized tissue which consisted primarily of fibroblasts and mononuclear leucocytes. Immediate peritoneal distribution of the inoculation volume was not impeded by the catheter associated tissue. With the exception of rare occasions, the catheter and its associated tissue remained free of adhesion to peritoneal structures.

Response of mice to 10^6 cfu intracatheter S. epidermidis challenge

Recovery of S. epidermidis from peritoneal structures of mice one week and one month after a 10^6 cfu intracatheter peritoneal challenge is summarized in Table 3. All animals survived during the follow-up period and, with the exception of a small number of mice, S. epidermidis was effectively cleared from all peritoneal sampling sites, including the peritoneal catheter. One week after experimental challenge, S. epidermidis remained associated with the catheter site of one animal in each of the sham-operated and renal failure groups. One month after S. epidermidis challenge, two of seven renal failure mice presented positive recoveries from the catheter site while specimens from sham-operated animals invariably presented no bacterial growth.

Table 3. Recovery of *Staphylococcus epidermidis* from peritoneal structures of mice at various times after 10^6 cfu intracatheter peritoneal challenge[a,b]

Time after inoculation	1 week		1 month	
Animal status:	Sham-operated (9)	Renal failure (10)	Sham-operated (8)	Renal failure (7)
Number of mice with positive *S. epidermidis* recoveries	1	1	0	2
Site of bacterial recovery:				
peritoneal washings	0	0	0	0
parietal peritoneum	0	1	0	0
catheter site	1	1	0	2

[a]Results are obtained in 15-19 week old C57BL/6 inbred mice 7-10 weeks after the surgical induction of renal failure or sham surgery.
[b]Sample size is indicated in parentheses.

There was no evidence of ongoing inflammatory processes as measured by total and differential leucocyte counts in peripheral blood, peritoneal washings and fluid aspirated from the catheter lumen (data not shown). One week or one month following 10^6 cfu S. epidermidis intracatheter challenge, peritoneal and circulating leucocyte populations were not significantly different than the normal resident or resting state conditions. Leucocytes aspirated from the catheter lumens did not present evidence of acute inflammation and their differential nature was similar to that of leucocytes collected from lumens of non infected catheters.

Response of mice to 10^8 cfu S. epidermidis challenge

Survival:

Survival of mice to one month after 10^8 cfu intracatheter S. epidermidis challenge is summarized in Figure 1. Previously, during the development of this animal model, we investigated the response of sham-operated and renal failure mice without peritoneal catheter implants to intraperitoneal inoculation of 10^8 cfu S. epidermidis. For interest, results of this previous investigation are also included in Figure 1. Depending on the route of inoculation, there was a striking difference in the pattern of survival of mice such that mortality was least following intracatheter challenge with a majority of deaths occurring 3 to 4 weeks post inoculation. In contrast, in those animals that underwent intraperitoneal inoculation mortality was much greater and occurred within a shorter period of time after inoculation. With the exception of one sham-operated control, all mortality was accounted for by renal failure mice.

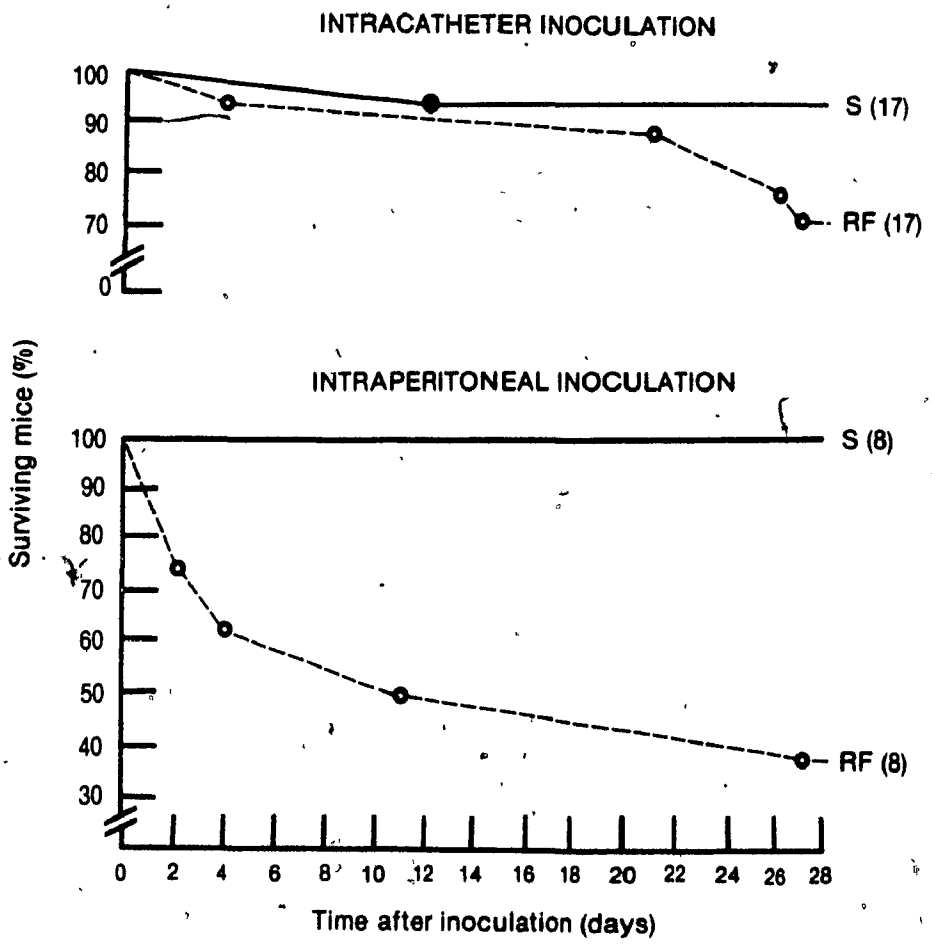

Figure 1. Survival curves of sham-operated (S) (solid line) and renal failure (RF) (dashed line) mice during a one month period after 10^8 cfu Staphylococcus epidermidis inoculation. The top panel represents results following intracatheter inoculation and the bottom panel summarizes experiments conducted in mice without peritoneal catheter implants subjected to intraperitoneal inoculation. Number of mice is indicated in parentheses.

Recovery of S. epidermidis from peritoneal structures:

The microbiologic status of peritoneal structures of surviving mice at weekly intervals to one month after 10^8 cfu intracatheter inoculation is summarized in Table 4 and Figures 2 and 3. In contrast to large recoveries of S. epidermidis from the catheter site, minimal or no bacterial growth was recovered from other tested peritoneal structures at all assessment times. S. epidermidis recovery from the catheter and its associated tissue was similar in sham-operated and renal failure mice for the first two weeks after inoculation. Thereafter, three and four weeks post inoculation, recovery of S. epidermidis from the catheter associated tissue of sham-operated controls was unexpectedly greater ($p<0.05$) than that from renal failure mice. One month after S. epidermidis challenge 5 of 11 renal failure mice presented culture positive specimens, while specimens from all of 15 sham-operated mice remained S. epidermidis culture positive. Recovery of S. epidermidis from catheters, assessed by streaking across blood agar, followed a similar pattern (Figure 3). Large numbers of bacteria were associated with the surface of catheters from renal failure and sham-operated mice, however, three and four weeks after inoculation, a large proportion of catheters recovered from renal failure mice presented smaller bacterial recoveries or no bacterial growth while those of sham-operated controls continued to present large S. epidermidis recoveries.

Inflammatory response:

Total leucocyte and polymorph counts of peritoneal washings and peripheral blood of surviving mice at weekly intervals to 1 month following 10^8 cfu intracatheter

Table 4. Recovery of Staphylococcus epidermidis from mice at various times after 10^8 cfu challenge[a,b]

Sampling site:	Peritoneal washings (log colonies per ml)		Parietal peritoneum (log colonies per gram)	
Animal status:	Sham-operated	Renal failure	Sham-operated	Renal failure
Time after inoculation (weeks)				
1	NBG (8)	1.03±0.5 (12)	2.2±1.8 (8)	1.6±1.2 (12)
2	NBG (5)	NBG (6)	1.5±1.1 (5)	NBG (6)
3	NBG (8)	NBG (7)	NBG (8)	NBG (7)
4	1.04±0.2 (15)	1.3±0.9 (11)	1.1±0.3 (15)	1.2±0.7 (11)

[a]Results are obtained in 15-19 week old C57BL/6 inbred mice 7-10 weeks after the surgical induction of renal failure or sham surgery.
[b]Results are expressed as mean±SD and sample size is indicated in parentheses.
[c]NBG indicates no bacterial growth.

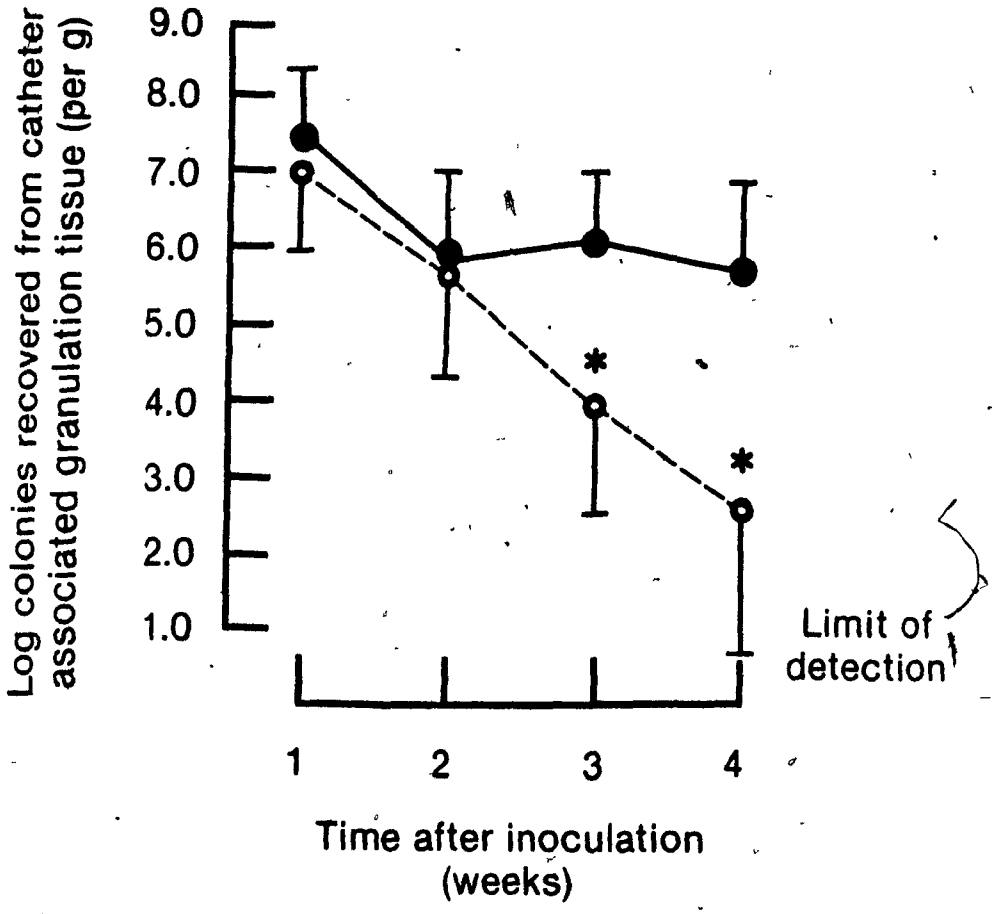

Figure 2. Recovery of Staphylococcus epidermidis from catheter associated granulation tissue from sham-operated (solid line) and renal failure (dashed line) mice during a one month period following 10^8 cfu intracatheter inoculation. Mean values and standard deviations are indicated at weekly intervals after inoculation. An asterisk indicates a significant difference ($p<0.05$) between renal failure mice and respective sham-operated controls.

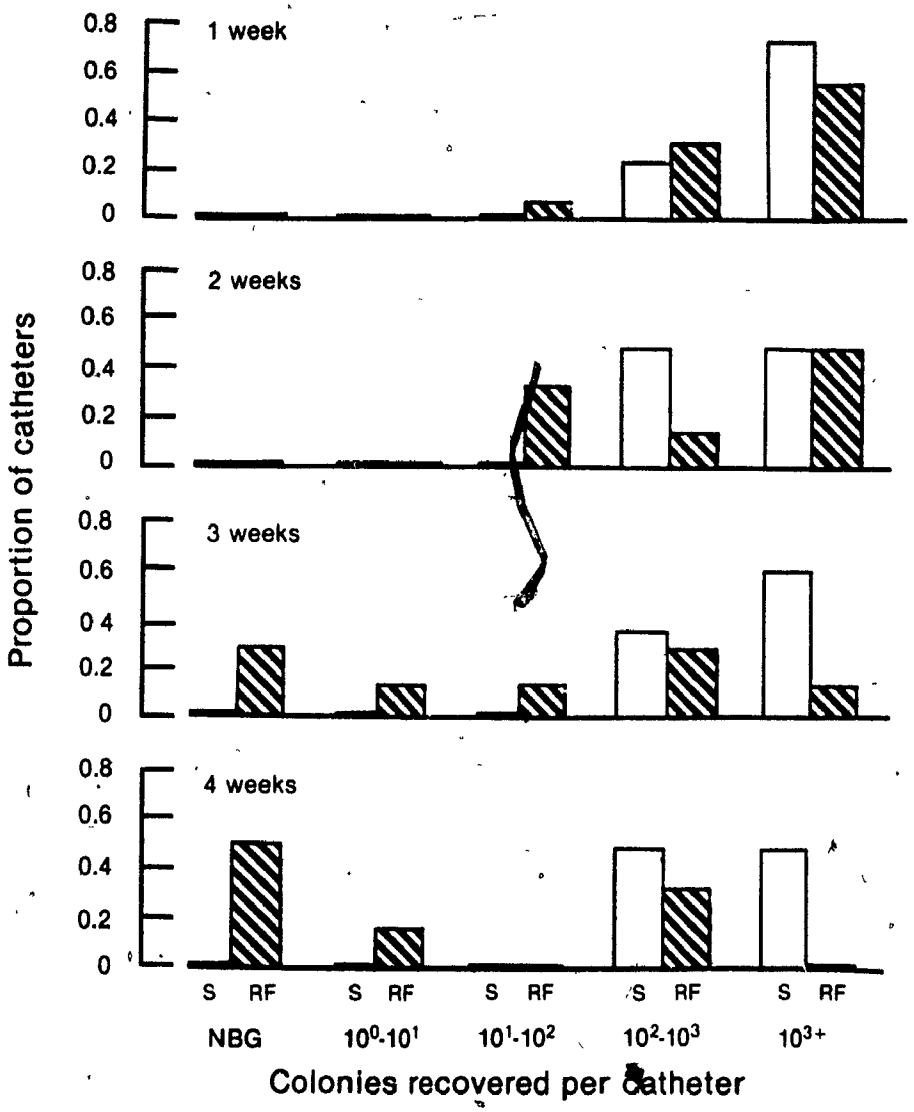

Figure 3. Recovery of Staphylococcus epidermidis from peritoneal catheters recovered from sham-operated (S) (open bars) and renal failure (RF) (hatched bars) mice at weekly intervals to one month after 10^8 cfu intracatheter inoculation. These data are derived from the same animals represented in Figure 2 and represent colonies recovered per catheter surface after streaking across an agar plate. NBG indicates no bacterial growth.

inoculation are presented in Figure 4. Compared to peritoneal and circulating leucocytes of non inoculated mice (data not shown) local and peripheral inflammatory response was evident in all mice following inoculation. Peritoneal polymorph counts were positively correlated ($p<0.05$) with the magnitude of bacterial recovery from the catheter site. Peritoneal polymorph response was greatest during the first two weeks after S. epidermidis challenge and thereafter declined such that three (sham-operated mice) and four (sham-operated and renal failure mice) weeks post inoculation peritoneal polymorphs were significantly less than levels of respective mice one week after challenge and compared favorably to a normal resident leucocyte population in spite of positive recovery of S. epidermidis from the catheter site. Total peritoneal leucocytes were also elevated for three weeks following inoculation compared to non inoculated mice. Circulating total leucocyte counts were not measurably affected by the experimental inoculation however there was a significant increase in circulating polymorphs over resting state conditions (no inoculation) which persisted for the duration of the follow-up. Inflammatory response, measured in terms of peritoneal and circulating leucocytes, was not significantly different between renal failure and sham-operated mice followed to one month after 10^8 cfu intracatheter S. epidermidis challenge.

Total leucocyte and differential counts of fluid aspirated from the lumen of catheters recovered from mice demonstrated acute inflammation. At one and four weeks after inoculation the leucocyte differential counts were similar, ranging from 75

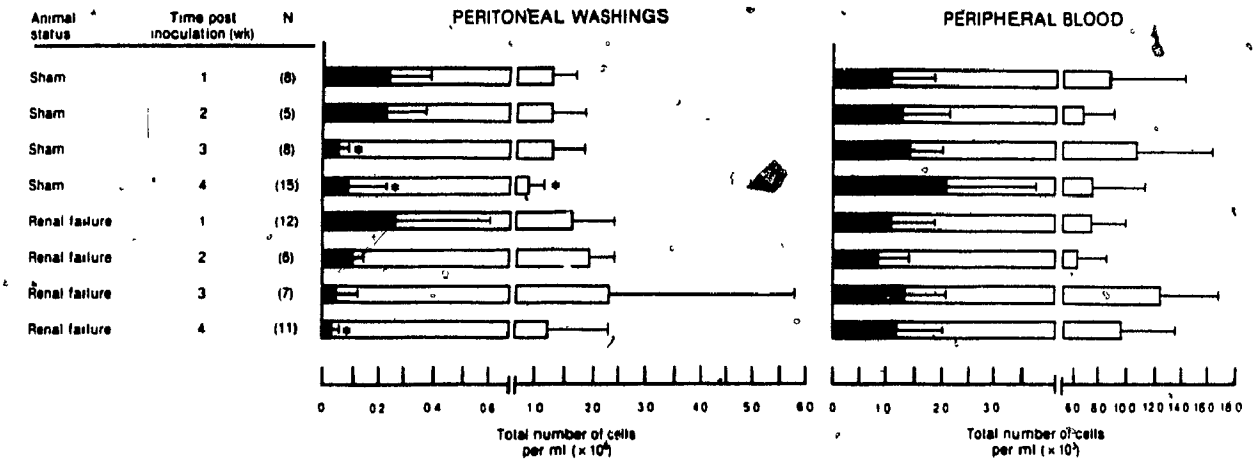

Figure 4. Mean peritoneal and circulating leucocytes (horizontal bars) and polymorphs (hatched areas) of sham-operated and renal failure mice at weekly intervals after 10^8 cfu intracatheter Staphylococcus epidermidis inoculation. These data are derived from the same animals represented in Figure 2. Horizontal lines represent standard deviations and numbers of mice are indicated in parentheses. Asterisks indicate significant differences ($p<0.05$) between values recorded at one week post inoculation and the indicated test time.

to 90% polymorphs, and were not apparently influenced by the renal function of the mice. Differential counting at 2 and 3 weeks after inoculation was not possible because of extensive cell clumping and debris.

Phenotypic colonial variation of S. epidermidis isolates

Variation of colonial morphology was observed in S. epidermidis isolates recovered from mice two to four weeks after 10^8 cfu inoculation (Figure 5). Three to five colonies of each type were successively subcultured three times and colonial morphology remained stable. Colonies varied in size and color, ranging from pale grey to opaque white (parent phenotype) and having a diameter of 0.5 to 3.0 mm (latter measure is that of parent phenotype). Assessments of the isolated colonial variants which included automated identification by Vitek revealed that the recovered phenotypes had identical antibiotic sensitivity profiles and biochemical reactions.

Tissue and catheter specimens recovered one week after 10^8 cfu inoculation presented only the parent S. epidermidis colonial phenotype. In contrast, two to four weeks after inoculation a majority (59.7%) of specimens of catheter associated granulation tissue and recovered catheter segments revealed phenotypic colonial variants of the inoculated S. epidermidis. All three of the culture positive specimens of parietal peritoneum recovered from mice during this period also produced colonial morphology variants.

The most commonly observed phenotypic colonial variant was similar in size to the white parent phenotype, but was grey in color. The frequency with which phenotypic colonial variation

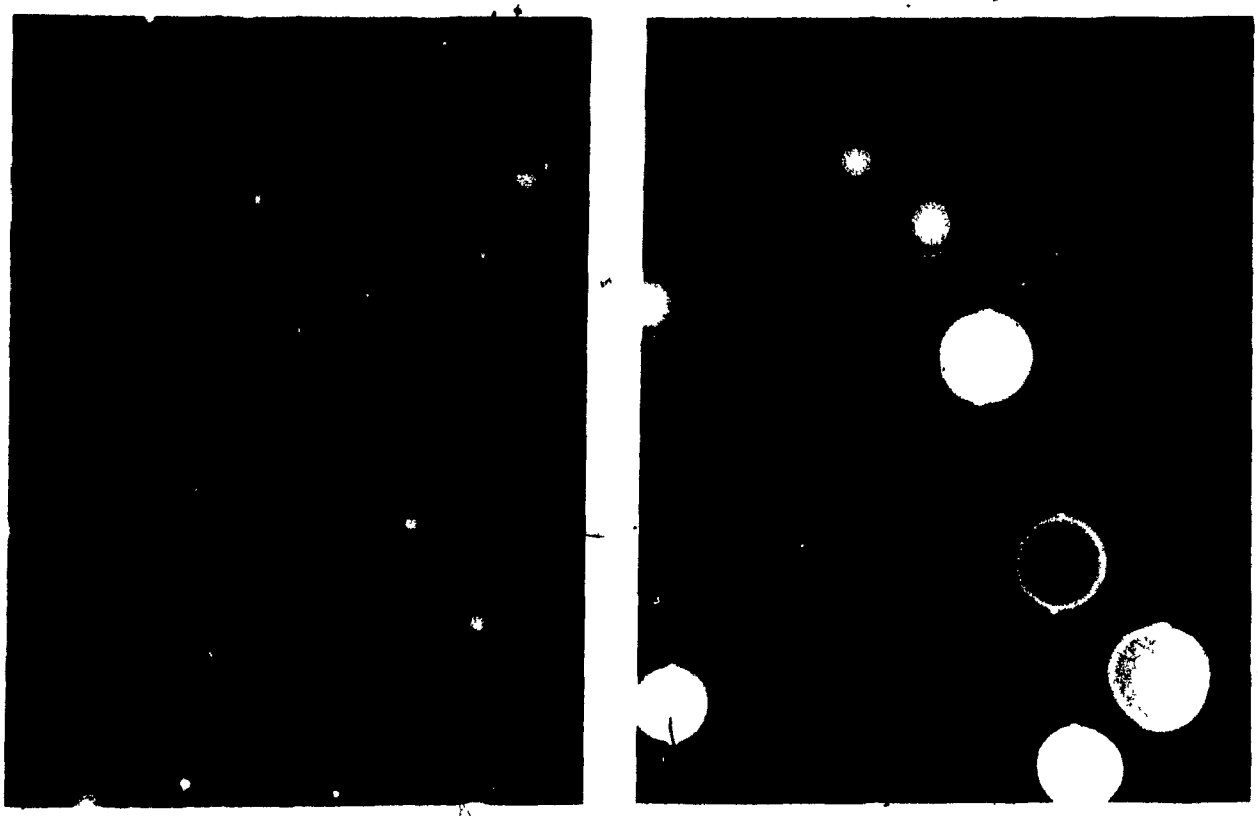

Figure 5. Primary culture on 5% horse blood Columbia agar of catheter associated granulation tissue recovered from a mouse three weeks after 10^8 cfu intracatheter Staphylococcus epidermidis inoculation showing large white (parent phenotype) large grey and small white colonial morphologies. The right panel is a photographic enlargement demonstrating the observed phenotypic variants. Photographs were taken after 24 hours incubation at 37°C.

was observed and the proportion of colonies presenting this phenotype in culture was not apparently affected by the sampling time (2, 3 or 4 weeks after inoculation), the sampling site (catheter or associated granulation tissue and peritoneum) or the level of renal function of the host. Pooling all isolates presenting phenotypic colonial variants, the percent of recovered S. epidermidis colonies presenting the grey phenotype was 38.7±32.3. One other colonial morphology variant which was much less frequently observed (9%) was markedly smaller than the parent phenotype but was similarly white.

Microbiologic assessments of catheter segments

Fifty four catheter segments were split lengthwise and one half was streaked across blood agar while the other half was scraped and the scraped material was broken up by Vortex mixing with glass beads. This latter method of assessment was conducted in order to detach any bacteria adhering to the catheter surface in a bacterial biofilm. The results of both microbiological assessments generally concurred. Catheters assessed according to these methods were recovered from renal failure and sham-operated mice 3 and 4 weeks after 10^8 cfu inoculation and 1 and 4 weeks after 10^6 cfu inoculation. Qualitative assessment of growth by the two methods was in agreement for 49 of 54 catheters (91%). In 29 cases, both methods of assessment yielded culture negative results and the remaining 20 cases were culture positive. In 12 of the 20 (60%) culture positive assessments, greater S. epidermidis recoveries were recorded following assessment by catheter scraping compared to catheter streaking. Of the 5 of 54 (9%)

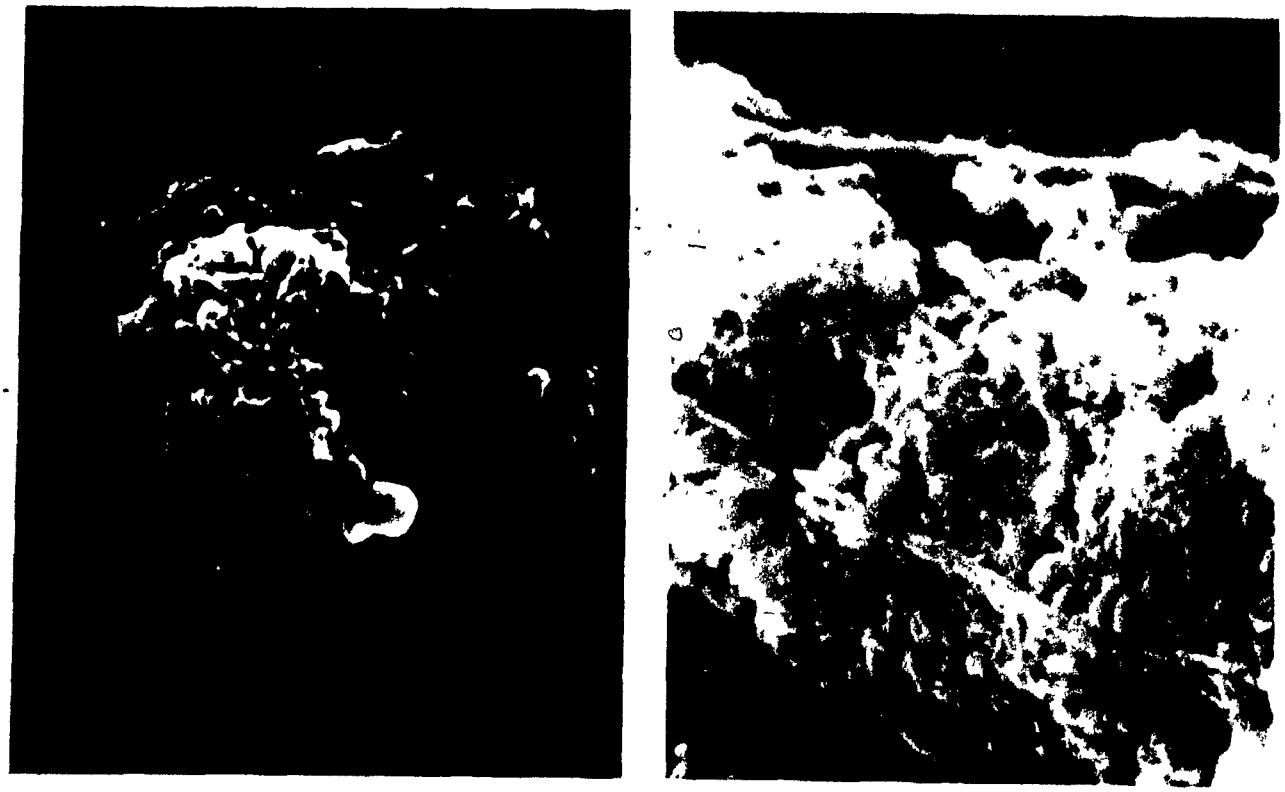

Figure 6. SEM of the luminal surface of catheters recovered from mice following intracatheter inoculation of 10^8 cfu Staphylococcus epidermidis.
Left panel: Macrocolony of cocci isolated on the surface of a catheter recovered one week after inoculation (Magnification x 4,000).
Right panel: Macrocolony of cocci isolated on the surface of a catheter recovered one month after inoculation. Coccoid forms are evident in an amorphous matrix (Magnification x 7,200).

discordant pairs, 4 were accounted for by culture negative scraping assessment and culture positive streaking results.

Scanning electron microscopy (SEM)

The luminal surface of catheter segments recovered from mice, one week (6 specimens) and four weeks (14 specimens) after 10^8 cfu intracatheter S. epidermidis challenge, were examined with SEM (Figure 6). One week after 10^8 cfu challenge, isolated macrocolonies of S. epidermidis were visualized on the catheter surface in association with macrophages and lymphocytes. After one month colonies of cocci were no longer clearly visualized however, coccoid shapes were frequently observed in isolated masses of amorphous material that littered the catheter surfaces. Leucocytes were always abundant on the surfaces examined. One week after 10^6 cfu intracatheter challenge, catheter segments (6 specimens) recovered from sham-operated and renal failure mice were also examined with SEM. Cocci were not observed on the catheter surfaces while lymphocytes and macrophages were evident. SEM assessments of catheters recovered from sham-operated and renal failure mice were similar.

Discussion

We have investigated the kinetics of S. epidermidis clearance from peritoneal structures of mice up to one month after either 10^6 or 10^8 cfu intracatheter peritoneal inoculation. All mice survived following inoculation of 10^6 cfu and one month after bacterial challenge, a small number of renal failure mice presented persistent S. epidermidis at the catheter site while all sham-operated controls had effectively cleared the inoculum. Survival of renal failure mice during

the one month following 10^8 cfu S. epidermidis intracatheter inoculation was markedly less than that of sham-operated controls and the peritoneal catheter site provided a preferred nidus for bacterial persistence in a majority of surviving animals. Unexpectedly, S. epidermidis clearance from the catheter site of renal failure mice was significantly accelerated 3 and 4 weeks after 10^8 cfu inoculation compared to sham-operated controls. Inflammatory response was similar in all animals and peritoneal inflammation was evident for the first 3 weeks after 10^8 cfu challenge while a systemic polymorph response persisted throughout the one month follow-up period.

The mouse model of chronic renal failure has been previously described (4,9) and was originally developed to study the influence of chronic uremia on host immune response. In order to address the influence of CAPD treatment variables, on the response of mice to peritoneal bacterial challenge, the animal preparation was expanded to include a permanently dwelling peritoneal catheter (8). Placement of the catheter segment entirely within the confines of the peritoneal cavity preserved the microbiological sterility of the peritoneum and the resulting animal preparation was not limited by bacterial contamination related to a catheter exit site to the skin. At the time of our assessments, seven to ten weeks after the surgical induction of renal failure, mice were azotemic and presented significant anemia. Presumably as a consequence of persisting S. epidermidis infection of the catheter site, sham-operated mice presented significant anemia during the one month follow-up after 10^8 cfu intracatheter inoculation

compared to those sacrificed one month after 10^6 cfu inoculation or non infected sham-operated controls. Furthermore, unlike previous observations where body weight of sham-operated mice was significantly greater than that of renal failure animals, body weight of both groups of mice in this series were similar.

In the literature there is a scarcity of in vivo investigations in which chronic renal failure animals have been directly challenged with viable bacteria and results of the few reported studies have failed to demonstrate a fundamentally immunocompromised uremic host (10-12). The results of the current studies, and those conducted previously in our laboratory utilizing the same animal preparation, may help to clarify some of the difficulties in the investigation of the influence of chronic uremia on susceptibility to infection. In previous experiments which were designed to differentiate between renal failure and sham-operated mice we have demonstrated the importance of the experimental inoculum size, the parameter chosen to measure response and the time of assessment relative to bacterial inoculation. Comparing our current observations with those of preliminary studies, the outcome of 10^8 cfu inoculation in terms of survival of mice varied between conditions of intracatheter or intraperitoneal inoculation. These apparently conflicting results may find explanation in a multifactorial relationship between renal failure, host immune response and virulence of the infecting microorganism.

Through a systematic approach with consistent microbiological assessment, we have investigated the roles of a

series of CAPD treatment variables in the pathogenesis of peritoneal S. epidermidis infection. Utilizing the mouse model, the immunosuppressive influence of chronic renal failure has been demonstrated in terms of accelerated lethality (with 10^9 cfu), increased mortality with (10^8 cfu) and impaired peritoneal clearance (with 10^6 cfu) following peritoneal S. epidermidis challenge (1,2). Peritoneal catheter implantation one month before peritoneal inoculation did not influence the response of sham-operated or renal failure mice to the S. epidermidis challenge (3). In mice bearing peritoneal catheter implants, intracatheter S. epidermidis inoculation revealed that bacteria remained preferentially associated with the catheter site and repeated peritoneal instillation of commercial peritoneal dialysis solution did not markedly influence the outcome of the experimental inoculation (3,13). The investigation of the long term clearance of S. epidermidis from the catheter site was undertaken as a result of previous studies in which the early clearance of a 10^6 cfu inoculum from the catheter implant was ineffective and was further impaired in renal failure mice (3). In the current investigation, most mice had cleared the 10^6 cfu S. epidermidis inoculum one month after challenge while a majority of those animals that underwent 10^8 cfu inoculation continued to present large S. epidermidis recoveries from the catheter site one month after challenge. The accelerated clearance from the catheter site of renal failure mice three and four weeks after 10^8 cfu S. epidermidis inoculation was an unexpected finding which may reflect an unfavorable environment for persisting S. epidermidis at the catheter site of chronic renal failure mice.

One week after 10^6 cfu S. epidermidis intracatheter inoculation there was no evidence of inflammatory response in sham-operated or renal failure mice. An impaired early peritoneal inflammatory response during the first twelve hours post inoculation in renal failure mice has been previously reported (2), yet in the current investigation inflammatory response of renal failure and sham-operated mice was similar at one week to one month after 10^8 cfu S. epidermidis challenge. During the follow-up period, peritoneal polymorph response was positively correlated to bacterial recovery from the catheter site. However, four weeks post challenge, in spite of persisting albeit diminishing numbers of S. epidermidis, there was no measureable peritoneal inflammatory response in mice. In contrast, peripheral polymorph response continued throughout the one month follow-up period reflecting a persistent inflammatory stimulus.

Slime production by certain strains of S. epidermidis enhances bacterial attachment to smooth surfaces and interferes with the phagocytosis and bactericidal functions of host leucocytes (14-17). Catheter colonization with slime-producing S. epidermidis resulting in confluent biofilm formation has been demonstrated on peritoneal catheters recovered from CAPD patients (7,18,19). Forty eight hours after intracatheter inoculation of mice with slime-producing S. epidermidis, SEM assessment of recovered catheters revealed early stages of biofilm formation (3). Our current SEM assessments of catheters recovered one week and one month after 10^8 cfu S. epidermidis intracatheter challenge did not reveal confluent S. epidermidis biofilms. However, isolated macrocolonies (1 week)

and coccoid forms embedded in an amorphous matrix (1 month) were observed on the luminal surfaces of catheters and represent later stages of biofilm formation (15).

Microbiologic assessment of catheters colonized with slime-producing S. epidermidis may be hampered by the firm attachment of bacteria to the smooth surface of the catheter (7,20). In the current investigation two methods of microbiological assessment (streaking the catheter across agar or scraping and Vortex mixing with glass beads) were compared and the results agreed qualitatively in most cases (91%). However quantitative results varied between the two methods of assessment, neither method yielding consistently larger or smaller recoveries. Further studies may be required to indentify a method of reliable quantitation of slime-producing strains of S. epidermidis which colonize the smooth surfaces of CAPD catheters.

The ability of microorganisms, including staphylococci, to adapt to altered or unfavorable growth conditions is demonstrated by the emergence of phenotypic and genotypic variants (21-25). A balance between host cellular and humoral bacteriostatic mechanisms and modified microbial metabolism may allow persistent bacterial growth to occur without symptoms of infection (22). Variation of colonial morphology was demonstrated in the current investigation by S. epidermidis isolates that were recovered from mice at least two weeks after 10^8 cfu inoculation. The proportion of colonial variants recovered was not apparently influenced by the site of recovery (catheter, catheter associated tissue or parietal peritoneum) or by the status of the host (renal failure or sham-operated

mice). Whether the variant isolates have comparable virulence as the parent S. epidermidis remains to be investigated.

Utilizing this animal preparation, we have demonstrated that the peritoneal catheter is a preferred site for long term persistence of S. epidermidis. The accelerated clearance of S. epidermidis from the catheter site of renal failure mice was an unexpected finding however, our results nevertheless reveal the potential importance of the peritoneal catheter in the pathogenesis of persistent CAPD peritonitis. The results of our current investigation provide a basic animal model in which to assess antimicrobial therapies for the eradication of persisting bacterial colonization of peritoneal catheters. Finally, the emergence of phenotypic colonial variants of S. epidermidis at least two weeks following intracatheter inoculation may be a significant observation and further study to characterize the nature of the recovered variants is warranted.

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Chapter 6

The influence of peritoneal instillation of dialysis solution on response of renal failure mice to intraperitoneal *Staphylococcus epidermidis* challenge

Section 6.1

Cytotoxicity of commercial peritoneal dialysis solutions towards peritoneal cells of chronically uremic mice

Presented in part at the III International Symposium on Peritoneal Dialysis, Washington, D.C., June 17-20, 1984, the 53rd Annual Meeting of the Royal College of Physicians and Surgeons of Canada, Montreal, Canada, September 10-13, 1984, and the 21st National Reticuloendothelial Society Meeting, Montreal, Canada, October 14-17, 1984.

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Abstract

A gradual loss of cell viability was observed during *in vitro* incubation of peritoneal cells from chronically uremic mice in commercial peritoneal dialysis solutions. The magnitude of this cytotoxicity toward peritoneal cells harvested from uremic mice and controls was comparable. Resident peritoneal cells were always found to be more susceptible than thioglycolate-elicited peritoneal populations of either macrophages or polymorphonuclear cells. In order to elucidate the factors contributing to this phenomenon, resident peritoneal cells recovered from normal mice were incubated *in vitro* for one hour in various solutions of known pH and osmolarity consisting of buffered and unbuffered commercial peritoneal dialysis solutions. The results clearly show that the major part of the cytotoxicity is attributable to the low pH of the solutions. Once pH was corrected, the hyperosmolarity of these solutions had no effect on cell viability; however a small but significant cytotoxicity remained. Factors other than those addressed in this study probably account for the observed residual cytotoxicity.

Introduction

Recent studies in which *in vitro* assays have been used to assess the impact of commercial peritoneal dialysis (PD) solutions on immune cell functions have increased our understanding of the deleterious effects of the extreme pH and osmolarity characteristics of these solutions.[1-3] Application of these assays to circulating blood leucocytes of normal individuals showed that the combination of low pH and

high osmolarity of PD solutions adversely affected the phagocytosis and bactericidal activity of polymorphonuclear cells. Both of these functions were markedly impaired when polymorphs were incubated in culture media having a pH adjusted to that of commercial PD solutions. Comparing 4 different PD solutions having dextrose concentrations ranging from 0.5 to 4.25%, these investigators observed inhibition of both polymorph phagocytosis and bactericidal activity by the 2.5 and 4.25% PD solutions. In addition, comparison of the effect on polymorphs of various substances, either drugs frequently added to PD solutions or endogenous metabolic products, showed that heparin, urea, lactate and acetate depressed chemiluminescence whereas insulin and creatinine did not. These investigators did not comment on the viability of cells maintained in PD solutions.

To further investigate this inhibitory effect of PD solutions and to determine its relationship to cells obtained from the peritoneal cavity of uremic subjects, we assessed the viability of peritoneal cells recovered from normal, sham-operated and chronically uremic mice during <u>in vitro</u> incubation in PD solutions. We found marked cytotoxicity of all tested PD solutions (0.5, 1.5 and 4.25% dextrose) compared with phosphate buffered saline (PBS). Resident peritoneal cells were much more susceptible than thioglycolate elicited ones, either macrophages or polymorphs. The same pattern of cytotoxicity was found across the three mice groups. Cell viability improved strikingly with correction of the pH of PD solutions, although a small but significant cytotoxicity was still present independent of osmolarity. These results

demonstrate that the low pH of PD solutions is the most significant factor affecting cell viability. Some factors influencing the viability of peritoneal cells maintained in PD solutions have been defined but those factors responsible for the observed residual cytotoxicity have yet to be identified.

Materials and Methods

Animals: Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, N.Y., USA) at five weeks of age. The animals were left to acclimatize in holding facilities for one week prior to use. All animals were allowed free access to water and mouse chow.

Induction of renal failure: Renal failure was induced surgically by a two step procedure as previously described.[4] Under ether anesthesia a small incision was made through which the right kidney could be freed of the adrenal gland and perirenal fat. Particular caution was taken to avoid trauma to the ureter. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, Calif., USA) the entire kidney surface was electrocoagulated. The abdominal incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. The second surgery was performed twelve to fifteen days later. In those animals which would constitute the uremic group a left nephrectomy was performed. In those mice which would constitute the sham-operated controls, a left flank incision was made through which the kidney was only visualized. Six weeks after the second surgery animals were used for experimentation.

Blood analyses: At the time of sacrifice mice were anesthetized with ether, weighed and blood was collected by cardiac puncture in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for hematological and biochemical analyses. Routine hematology was done utilizing a Coulter counter (Model 2B1, Coulter Electronics, Inc., Hialeah, Fla, USA) and differential cell counts were performed on Wright-stained blood smear preparations. Levels of urea nitrogen (BUN) and creatinine in plasma were assessed by an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass, USA).

Collection of peritoneal cells and incubation conditions: To obtain an elicited peritoneal cellular population, mice received an intraperitoneal injection of 1 ml Brewer's thioglycolate broth (Difco Laboratories, Detroit, Mich., USA) either 18 hours (polymorphonuclear cells) or 3 days (macrophages) prior to sacrifice.[5] Following exsanguination, the abdominal skin was retracted to expose the intact peritoneum. Ten ml of peritoneal wash solution was rapidly injected into the peritoneal cavity and the effluent was slowly withdrawn. Peritoneal cells were harvested in this manner using either commercial PD solutions containing lactate (Abbott Laboratories Ltd., Montreal, Quebec, Canada; Travenol Canada Inc., Mississauga, Ontario, Canada), PD solutions supplemented with 20 mM HEPES buffer or phosphate buffer saline (PBS) as the peritoneal wash solution. The collected volume was measured, transferred into polypropylene conical tubes and stored on ice for the remainder of the processing. Cells of each effluent were counted in Neubauer counting chambers and differential

cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, Ill., USA). Peritoneal effluents were centrifuged (1500 rpm, 10 min, 4°C) and the pellet was resuspended with the respective solution to a concentration of $2x10^6$ cells per ml. Aliquots of 0.5 ml of each effluent were added to three polypropylene incubation tubes; one a zero time control and the others were incubated for 20 or 60 minutes at 37°C and 5% CO_2. At zero time and following the specified incubation period, exposure was stopped by the addition of 3 ml cold minimal essential medium (MEM Eagle modified) containing Earle's salts, glutamine, 10% heat inactivated fetal calf serum (FCS) and 20 mM HEPES buffer. Samples were then returned to the incubator to complete a total 60 min incubation period. Following the addition of MEM the osmolarity and pH of the suspending solution approximated physiological conditions. Samples were again centrifuged (1500 rpm, 10 min, 4°C) and the pellet was resuspended to the original volume with cold MEM with 10% FCS and 20 mM HEPES buffer. Differential cell counts and viable cell counts, measured by trypan blue exclusion, were recorded for each sample. Osmolarity was measured on fresh samples with an Advanced Cryomatic Osmometer (Model 3C2, Advanced Instruments Inc., Needham Heights, Mass., USA).

Statisticial analysis: Results are presented as the mean and standard deviation. Significance was established at levels of $p<0.05$ using Student's t test to compare means of independent samples.

Results

Renal failure model

Data on the metabolic and hematological features of mice with renal failure and their control littermates are summarized in Table I. The BUN and plasma creatinine levels of renal failure mice were 114.9±34.5 and 0.7±0.3 mg/dl (mean±SD) compared to normal control values of 19.8±5.4 and 0.1±0.1 mg/dl, respectively. Values of BUN of uremic mice varied considerably ranging from 77 to 191 mg/dl. The retention of nitrogenous compounds was inversely correlated to hemoglobin concentrations, the mean of which had fallen to 7.7±1.1 g/dl by the time of sacrifice. None of the other usual hematological parameters, including circulating leucocyte counts and platelet numbers, were affected in the present series. No change was observed in leucocyte differential counts (data not shown) where lymphocytes account for over 90% of circulating leucocytes in this mouse strain.[6] The body weight of uremic mice was moderately but significantly reduced compared to sham operated and normal controls.

Table 1

Characteristics of the mouse model of renal failure[a,b]

	Normal	Sham	Uremic
Blood urea nitrogen (mg/dl)	19.8±5.4 (50)	26.3±6.8 (35)	114.9±24.5[c] (37)
Plasma creatinine (mg/dl)	0.1±0.1 (14)	0.2±0.1 (8)	0.7±0.3[c] (21)
Hemoglobin (g/dl)	13.1±0.8 (28)	13.0±0.7 (30)	7.7±1.1[c] (25)
Leucocytes/mm^3 ($x10^3$)	4.4±1.6 (28)	4.9±2.2 (30)	4.7±2.8 (25)
Platelets/mm^3	670±294 (18)	711±158 (19)	840±96 (19)
Body weight (g)	23.4±1.8 (34)	22.1±1.5 (31)	20.9±1.9[c] (34)

[a]Results are expressed as mean±SD.
[b]Number of animals examined in each group is indicated between parenthesis.
[c]Significant difference ($p<0.05$) between controls and uremic mice.

Peritoneal cell recoveries

Data on the peritoneal cells recovered from mice with renal failure and their control littermates are presented in Table II. Uremia per se had no influence on the differential nature of resident and elicited cell populations recovered from the peritoneal cavity. However, eighteen hours after the intraperitoneal injection of thioglycolate, cellularity of the peritoneal washout was significantly less in uremic mice than in controls. The predominant cell types recovered 18 hours and 3 days after thioglycolate injection were polymorphs and macrophages, respectively. No effect of surgery on peritoneal cell populations was seen in the sham-operated group.

Effect of commercial PD solutions on peritoneal cell viability

Data on the ability of commercial PD solutions to maintain mouse peritoneal cells in vitro are presented in Figures 1 and 2 and Table III. When mouse peritoneal cells were incubated in a PBS control solution, loss of viability was minimal after one hour (Figures 1 and 2). This was true for resident as well as both elicited peritoneal populations recovered from mice with renal failure or their control littermates. The overall cell viability after one hour incubation in PBS solution was 87.0± 7.0, 81.5±7.4, and 85.4±7.6% for normal, sham-operated and uremic mice, respectively. In contrast, a significant proportion of peritoneal cells incubated in 4.25% PD solution were nonviable after one hour. The incubation produced greatest losses amid the resident cell population with more than a half of the cells dead at the end of the incubation period (Figure 1), whereas over 60% of the elicited population consisting predominantly of macrophages (3 day exudate) and 75%

Table II

Characteristics of recovered peritoneal cell populations[a]

	Normal	Sham	Uremic
Resident cells			
Number of animals	24	12	16
Leucocytes x 10^6/ml	0.8±0.3	0.6±0.2	0.8±0.4
Differential count			
Macrophages(%)	49.9±8.2	61.1±11.4	47.5±13.6
Lymphocytes(%)	45.8±8.9	36.0±10.0	50.6±13.6
Polymorphs(%)	2.0±2.4	2.5±3.4	1.4±1.4
Mast cells(%)	2.2±1.6	0.3±0.9	0.5±0.9
18 hour inflammatory exudate			
Number of animals	13	7	14
Leucocytes x 10^6/ml	2.6±1.0	2.6±1.2	1.5 0.8[b]
Differential count			
Macrophages(%)	30.6±8.9	35.3±5.5	36.4±7.7
Lymphocytes(%)	12.8±9.6	11.0±7.7	8.8±5.7
Polymorphs(%)	56.5±12.6	53.6±12.5	54.8±10.8
Mast cells(%)	0±0	0±0	0±0
3 day inflammatory exudate			
Number of animals	26	23	21
Leucocytes x 10^6/ml	2.3±0.7	2.4±1.1	2.4±1.0
Differential count			
Macrophages(%)	74.0±8.1	73.7±8.2	70.4±7.1
Lymphocytes(%)	16.8±6.7	16.1±6.7	19.6±6.4
Polymorphs(%)	8.8±4.3	10.7±3.7	9.6±4.3
Mast cells(%)	0.4±0.9	0.0±0.2	0.1±0.2

[a]Results are expressed as mean±SD.
[b]Significant difference ($p<0.05$) between controls and uremic mice.

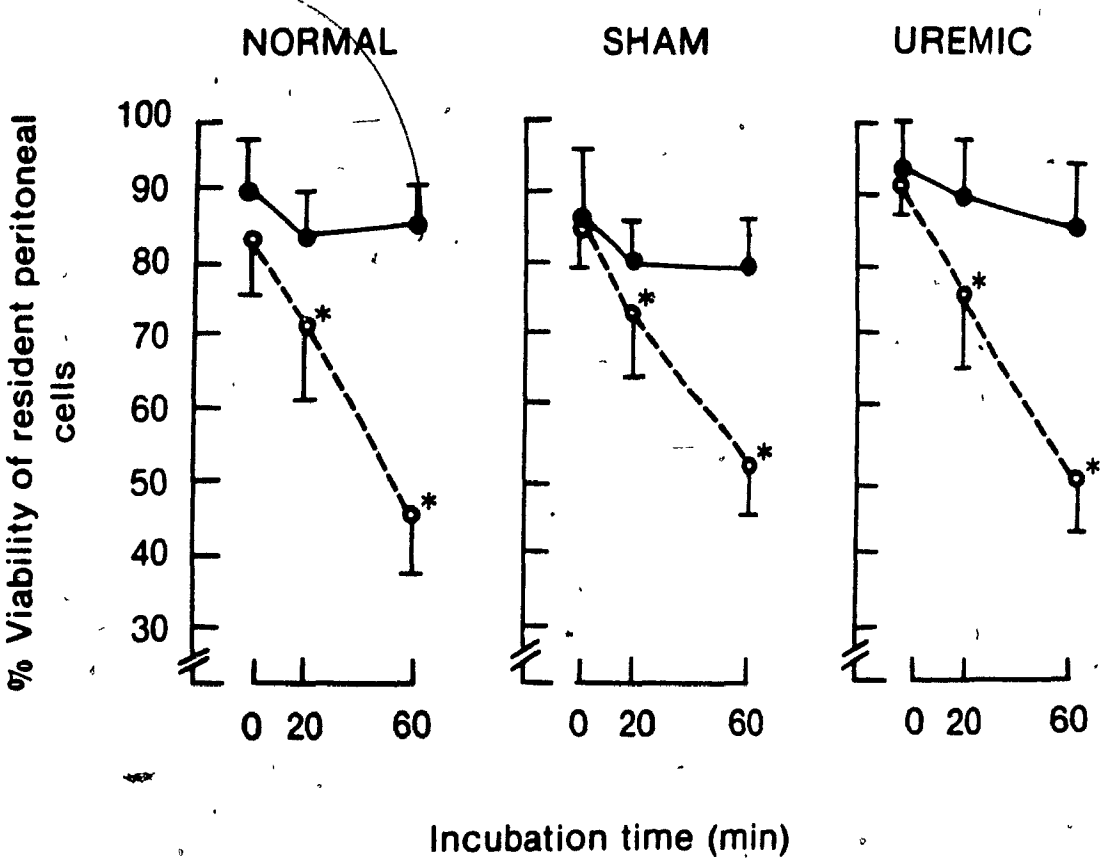

Figure 1: Time course of cytotoxicity of commercial 4.25% peritoneal dialysis solution (dashed line) exhibited in vitro towards mouse resident peritoneal cells compared to phosphate buffered saline control solution (solid line). Mean±SD percent viability of cells obtained from normal, sham-operated and chronically uremic mice are shown. Significant differences ($p<0.05$) between each incubation solution for the three test times are indicated by asterisks.

of the one having predominantly polymorphonuclear cells (18 hour exudate) remained viable (Figure 2).

It is noteworthy that the viability of the zero time controls of the elicited populations harvested in 4.25% dextrose PD solution was consistently less than that of the PBS controls. The cytotoxic effect observed during the incubation period was comparable for cells harvested from normal, sham-operated and uremic mice. Therefore results were pooled across the three groups of mice (Table III). A similar magnitude of cytotoxicity is observed when resident cells are incubated in 4.25% or 1.5% dextrose even though the osmolarity and pH characteristics of the latter solution are not as extreme as those of the former. In contrast, incubation of the elicited population consisting primarily of macrophages in 1.5% PD solution resulted in a significant reduction in viability but to a lesser degree than that observed during incubation in 4.25% dextrose.

To determine if one given cell type was more sensitive to the PD solutions, differential counts were performed at the end of each incubation period. No apparent difference in differential counts of the remaining cells was seen. In some instances, however, such as after the 60 min incubation of resident peritoneal cells in 4.25% PD solution, differential counting could not be carried out because cell morphology was changed beyond recognition.

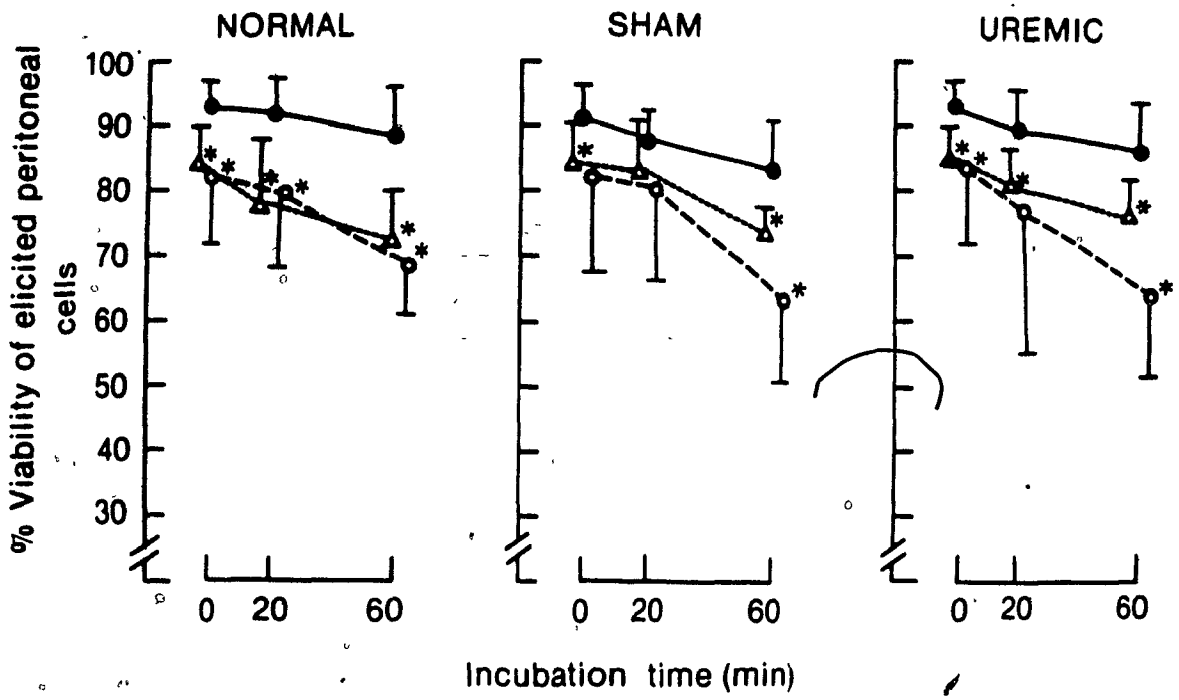

Figure 2: Time course of cytotoxicity of commercial 4.25% peritoneal dialysis solution (dashed line) exhibited in vitro towards mouse elicited peritoneal cells compared to phosphate buffered saline control solution (solid line). Peritoneal cells were recovered 18 hours (short dashes) and 3 days (long dashes) after a thioglycolate intraperitoneal injection. Mean±SD percent viability of cells obtained from normal, sham-operated and chronically uremic mice are shown. Significant differences ($p<0.05$) between the peritoneal dialysis solution and a PBS control solution for the three test times are indicated by asterisks.

Table III

Effect of PD solution on the viability of mouse peritoneal cells[a,b]

Cells	Nature of solution	Percent viability 0 time	20 min	60 min
Resident				
N=19	PBS	89.6±7.9	84.2±7.8	83.2±8.0
N=13	1.5%PD	81.2±4.2	ND[d]	52.9±9.4[c]
N=20	4.25%PD	85.5±6.6	72.0±9.5[c]	49.1±8.0[c]
Inflammatory 18 hour exudate				
N=9	PBS	93.4±3.8	94.9±2.6	88.3±2.2
N=25	4.25%PD	84.4±5.1[c]	80.7±7.0[c]	75.0±5.4[c]
Inflammatory 3 day exudate				
N=24	PBS	91.7±3.6	88.2±4.7	85.2±8.1
N=24	1.5%PD	89.5±8.2	ND[d]	76.8±10.9[c]
N=22	4.25%PD	82.3±11.6[c]	78.8±14.5[c]	65.2±9.8[c]

[a]Pooled viability of peritoneal cells harvested from normal, sham-operated and uremic mice incubated in either PD solution or PBS.
[b]Results are expressed as mean±SD.
[c]Significant difference ($p<0.05$) between control PBS and test PD solutions.
[d]Not determined.

Effect of buffered commercial PD solutions on peritoneal cell viability

Data on the characteristics of buffered commercial PD solutions and their ability to maintain mouse peritoneal cells *in vitro* are presented in Tables IV and V, respectively. In order to investigate the mechanism of the cytotoxicity of PD solutions towards mouse peritoneal cells, we set out to delineate the respective contribution of the high osmolarity and low pH of commercial PD solutions to the observed cytotoxicity. Accordingly viability of resident peritoneal cells from normal mice was determined before and after 60 min incubation in unmodified and buffered commercial PD solutions. In this series of experiments, the entire range of commercially available PD solutions was tested from 0.5 to 4.25% dextrose concentration. The addition of 20 mM HEPES buffer adjusted the pH of the PD solutions to that of the control PBS solution and also augmented their osmolarity by approximately 20 units. Thus, all buffered PD solutions had similar pH values (7.1-7.2) but differed markedly in osmolarity which ranged from 304 to 507 mOsm/kg.

In such a system, the incubation of resident peritoneal cells from normal mice in unbuffered PD solutions induced a marked loss in cell viability as shown earlier (Table V). The addition of HEPES buffer to the 1.5 and 4.25% PD solutions significantly improved the resulting cell viability, which was then comparable for both buffered PD solutions although they varied greatly in osmolarity. This data suggests that the observed cytotoxicity of PD solutions was largely accounted for by their low pH and that loss of viability was independent of

Table IV

Measured pH and osmolarity values of buffered and unbuffered PD solutions

Nature of solution	pH	Osmolarity (mOsm/kg_2O)
PBS	7.2	301
0.5% PD	5.8	281
Buffered 0.5% PD	7.2	304
1.5% PD	5.6	333
Buffered 1.5% PD	7.2	351
4.25% PD I	5.3	485
Buffered 4.25% PD I	7.1	501
4.25% PD II	5.0	491
Buffered 4.5% PD II	7.1	507

hyperosmolarity once the pH had been adjusted to physiological levels. However, a similar loss of viability was exhibited by cells incubated in buffered and unbuffered 0.5% PD solution having pH levels of 7.2 and 5.8 respectively. With the addition of buffer, pH and osmolarity values of the 0.5% PD solution were similar to those of PBS yet following 60 min incubation there was a small (16%) but significant reduction in cell viability compared to that in the PBS control solution. A similar degree of residual cytotoxicity was observed following incubation in all buffered PD solutions thereby suggesting that factor(s) other than pH and osmolarity are responsible for part of the cytotoxicity exhibited by commercial PD solutions.

Viability of peritoneal cells harvested from normal mice and incubated in 4.25% PD solutions supplied by two different manufacturers is also presented in Table V. After a one hour incubation, both PD solutions induced a marked cytotoxicity which was more pronounced in PD solution II. The addition of HEPES buffer to both PD solutions resulted in a significant reduction of this cytotoxicity, which was then comparable for the two brands. The greater cytotoxicity of unmodified PD solution II may be due to its comparatively lower pH (Table IV).

Table V

Effect of buffered and unbuffered PD solutions on the viability of mouse peritoneal cells[a,b]

Nature of solution (N)	Percent viability 0 time	60 min	Percent reduction in viability at 60 min
PBS (16)	90.3±5.7	84.4±7.1	6.5±5.4
0.5% PD (8)	85.1±10.3	68.6±9.5[c]	18.9±10.7[c]
Buffered 0.5% PD (7)	84.7±4.0	71.4±7.8[c]	15.8±7.2[c]
1.5 PD (12)	78.4±5.2[c]	50.1±7.3[c]	36.1±8.1[c]
Buffered 1.5% PD (8)	84.4±8.3	69.6±9.4[c]	17.7±9.9[c]
4.25% PD I (16)	83.8±7.7[c]	48.7±11.1[c]	41.8±13.2[c]
Buffered 4.25% PD I (16)	89.2±4.7	70.1±9.0[c]	21.9±8.8[c]
4.25% PD II (12)	78.2±7.0[c]	21.6±7.8[c]	72.3±10.6[c]
Buffered 4.25% PD II (8)	92.8±5.2	77.4±6.6[c]	16.2±10.3[c]

[a]Resident peritoneal cells recovered from normal mice.
[b]Results are expressed as mean±SD.
[c]Significant difference ($p<0.05$) between control PBS and test PD solutions.

Discussion

This study has extended previous observations of early reduction in cellular function after incubation with commercial PD solutions.[1-3] We have found in this study that a significant loss of cell viability in both resident and elicited peritoneal cell populations occurred within one hour of incubation with PD solutions. In addition, the PD solutions affected indiscriminately all cell types recovered from the peritoneal space regardless of their origin from animals with varying degress of renal function. In an effort to dissociate between the low pH and high osmolarity characteristics of PD solutions it was possible to ascribe the greater part of the observed cytotoxicity to the low pH and a lesser part to an as yet undefined factor distinct from osmolarity.

The experimental preparation used in this study facilitated assessment of the influence of chronic renal failure on viability of peritoneal cells maintained in PD solutions. With this model one is dealing with an entire animal preparation of a chronic and severe metabolic disorder and experiments can be carried out with mice whose peritoneal cell attributes and functions have been well characterized in normal physiological states.[7-9] Six weeks after the onset of renal failure, mean values of blood urea nitrogen and plasma creatinine were appropriately elevated when compared to normal and sham-operated animals. It is important to note that levels of plasma creatinine in mice of the strain used in these experiments are about one tenth of the normal values in man. The renal failure animals also manifested prominent hematological changes having markedly decreased hemoglobin

concentrations characteristic of the level observed in end-stage renal disease patients. In addition, uremic mice showed significant growth retardation in comparison to sham-operated controls. These observations, taken altogether, suggest that the surgical damage incurred by the kidneys is sufficient to induce the severe disturbances associated clinically with chronic uremia.

Uremia per se did not influence the differential nature of the peritoneal cell populations harvested nor their susceptibility to the cytotoxic effect of the dialysis solutions. However, eighteen hours after treatment with an inflammatory stimulus peritoneal cellularity was significantly less in uremic mice as compared to controls. The elicited populations were consistently more resistant to the deleterious effect of the PD solutions than was the resident population yet viability of all populations was significantly reduced. This greater resistance may reflect the normal function of elicited cell populations in the relatively acidic microenvironment of inflammatory sites.[10,11] Differential cell counts did not change during incubation in PD solutions, thus the cytotoxic effect appears to affect all cellular constituents equally.

From the experiments designed to identify the separate contributions of high osmolarity and low pH of the PD solutions to the observed toxicity the following conclusions can be drawn. Osmolarity did not appear to be a contributing factor once pH had been corrected since similar residual cytotoxicity was observed across the wide range of osmolarities examined. With the more extreme conditions of low pH presented by the 1.5 and 4.25% PD solutions the correction of pH with HEPES buffer

considerably increased cell viability during a one hour incubation. However, in the less extreme condition presented by 0.5% PD solution (pH 5.8) correction of pH did not alter the observed cytotoxicity. Since a residual cytotoxic effect was observed following one hour incubation in all buffered PD solutions including the buffered iso-osmolar 0.5% PD solution, factors other than those addressed here must account for the remaining loss of viability. Other possible contributing factors include dextrose degradation products and/or plasticizers leached from the dialysis solution bags both of which would be enhanced during heat sterilization processes. Potential factors that we did not study include the effect of the buffer (lactate, acetate or bicarbonate) in the commercial PD solutions.

It would be erroneous to extrapolate the observed cytotoxic effect during in vitro incubation of peritoneal cells in PD solutions to the clinical practice of peritoneal dialysis. The harvesting and processing of the cellular constituents of the peritoneal cavity may delete substances that afford cellular protection against the detrimental effects of the PD solution. Further, the physicochemical characteristics of the dialysis solutions which remain constant for the duration of the in vitro incubation period do not parallel the changing characteristics of dialysis solutions following their instillation into the peritoneal cavity. Iso-osmolarity is achieved after four and six hours of peritoneal dwell by 1.5 and 4.25% PD solutions, respectively, and pH equilibrates at 7.2 after one hour.[1,12] Although loss of cell viability increased with in vitro incubation time to

one hour, there was an immediate (0 time) and significant cytotoxic effect of 4.25% PD solution towards elicited peritoneal cells compared to PBS controls. In addition resident peritoneal cells incubated in 4.25% PD solution for 20 min incurred a significant loss of viability.

The cellular constituents that have been examined in this study are those that play an integral role in host defense of the peritoneum against bacterial infection. Since the inception of peritoneal dialysis, many advances have been made with regards to ancillary equipment to reduce the occurrence of peritonitis resulting from touch contamination and yet few patients are spared an event of peritonitis. Concurrent with an event of touch contamination at dialysis exchange, the inflowing PD solution presents the most extreme non-physiological characteristics of pH and osmolarity. It remains plausible that frequent exposure to large volumes of PD solutions may challenge local immunocompetence of the peritoneum. The influence of dialysis solutions dwelling in the peritoneal cavity of chronically uremic mice on their susceptibility to intraperitoneal bacterial challenge is currently under investigation.

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Section 6.2

Response of chronic renal failure mice to peritoneal *Staphylococcus epidermidis* challenge: Impact of repeated peritoneal instillation of dialysis solution.

Presented in part at the 55th Annual Meeting of the Royal College of Physicians and Surgeons of Canada, Toronto, Canada, September 21-25, 1986.

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Abstract

The effect of repeated peritoneal instillation of 4.25% dextrose peritoneal dialysis (PD) solution on the peritoneal clearance of a S. epidermidis challenge was investigated in a mouse model of surgically induced renal failure. Mice bearing peritoneal catheter implants underwent daily (3 ml) or twice daily (1.5 ml) peritoneal instillation of PD solution without peritoneal drainage, for periods of up to two weeks. Peritoneal infusion by transcutaneous injection into the catheter lumen was conducted repeatedly without bacterial contamination of the peritoneal cavity or catheter. Peritoneal instillation of PD solution did not have a marked influence on the microbiological status of peritoneal structures of mice following experimental intracatheter S. epidermidis inoculation with 10^6 cfu (assessment 48 hours post inoculation) or 10^8 cfu (assessment 1 week post inoculation). Microbiological and scanning electron microscopy assessment of recovered peritoneal catheters demonstrated that S. epidermidis remained associated with the catheter site after other peritoneal structures had become culture negative. A renal failure related impairment of bacterial clearance from the catheter site was apparent in mice following experimental challenge with the smaller 10^6 cfu inoculum only. During control experiments conducted in the absence of S. epidermidis inoculation repeated peritoneal instillation of PD solution caused acute local inflammation without evidence of a marked systemic inflammatory response. Furthermore, peritoneal inflammatory response to S. epidermidis challenge was augmented by the infusion procedure. Scanning

electron microscopy of the parietal peritoneum revealed striking morphological alterations of the mesothelial surface as a consequence of daily peritoneal instillation of PD solution. Although the animal preparation was limited to peritoneal infusion without drainage, the influence of repeated peritoneal instillation of hyperosmolar acidic PD solution on response of mice to peritoneal S. epidermidis challenge was successfully addressed.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is a widely accepted form of renal replacement therapy however, its development has been consistently complicated by peritonitis which is frequently caused by Staphylococcus epidermidis (1-5). Superimposed on the renal failure patient harbouring a permanently dwelling CAPD catheter is the practice of repeated peritoneal instillation of large volumes of peritoneal dialysis (PD) solutions which regularly, albeit temporarily, alters the peritoneal environment to conditions of non physiologic osmolarity and pH (6). In vitro investigations have demonstrated that PD solutions inhibited phagocytic and bactericidal functions of human peripheral blood leucocytes (7) and were cytotoxic to peritoneal leucocytes of renal failure mice (8). Peritoneal instillation of large fluid volumes of PD solutions may further impair local host defense mechanisms as has been demonstrated in studies conducted in rats where susceptibility to experimental bacterial peritonitis increased with increasing inoculation volume (9, 10). Furthermore during CAPD, the repeated instillation and drainage of PD solutions

imposes a continual dilution and loss of cellular and soluble immune reactants from the peritoneal cavity (11-13).

Utilizing a mouse model of chronic renal failure we have previously demonstrated the relative roles of renal failure and the permanently dwelling peritoneal catheter in the developemnt and persistence of peritoneal S. epidermidis infection. Susceptibility to peritoneal S. epidermidis challenge was increased in renal failure mice (14,15) and following intracatheter inoculation, bacterial clearance from the catheter site was ineffective (16,17). In the current investigation, we have assessed the influence of repeated peritoneal instillation of PD solution, without peritoneal drainage, on the response of chronic renal failure mice to peritoneal S. epidermidis challenge. Previous in vitro studies of the effect of PD solutions on host leucocytes have thereby been extended to an in vivo assessment in which response parameters included microbiological status of inoculated mice and host inflammatory responses. Although CAPD was not conducted in this animal preparation, the experimental procedure nevertheless permitted the evaluation of the effects of multiple variables (degree of renal function and peritoneal infusion procedure) on mechanisms of bacterial clearance.

Materials and Methods

Animals

Female C57BL/6 mice were obtained from Charles River Breeding Laboratories (Kingston, NY, USA) at five weeks of age. The animals were allowed to acclimatize in holding facilities for one week prior to use. All animals had free access to water and mouse chow.

The preparation of renal failure and sham-operated mice

was accomplished by two consecutive surgical procedures as described previously (18). Under ether anesthesia the right kidney was exposed and separated from the adrenal gland and perirenal fat. Using a single point cauterizer (Hyfrecator, Model X-712, Birtcher Corp, Los Angeles, Calif., USA) the exposed surface of the kidney was electrocoagulated. The incision was closed with a running suture through the deep layers and surgical clips were applied to the skin. Two weeks after right kidney electrocoagulation, a left nephrectomy was done in those animals that would constitute the renal failure group and surgical exploration of the left renal area was conducted in sham-operated controls.

Two weeks elapsed between nephrectomy or sham surgery and the peritoneal implantation of a custom made segment of an Oreopoulos-Zellerman CAPD catheter (Accurate Surgical Instruments Co., Toronto, Canada). Both ends of the segment were bevelled and the 1.2 cm length contained six regularly spaced perforations. Through a left flank incision the catheter segment was placed intraperitoneally and was secured to the lateral abdominal wall by a single anchoring suture passing through one of the side perforations. The free end of the catheter was directed towards the left lower quadrant of the abdominal cavity. The surgical wound was closed as above and animals were used for experimentation four to six weeks after catheter implantation.

Peritoneal dialysis solutions

Commercially available peritoneal dialysis (PD) solution (Abbott Laboratories Ltd., Montreal, Canada) containing 4.25% dextrose (pH 5.3, 485 mOsm/kg H_2O) was warmed to 37°C prior to peritoneal instillation. Peritoneal infusion was performed

under light ether anesthesia by transcutaneous injection directly into the catheter lumen.

Bacteria

Dr. S.I. Vas (Toronto Western Hospital, Toronto, Canada) kindly supplied us with a strain of S. epidermidis that had been recovered from the peritoneal fluid of a CAPD patient with peritonitis. Aliquots of this clinical isolate, reference number 29260, were frozen in glycerol broth. The characteristic antibiotic sensitivity profile of this organism facilitated positive identification. Compared to reference slime producing S. epidermidis strains (supplied by Dr. G.D. Christensen, University of Tennessee, Memphis, Tennessee, USA), isolate 29260 produced glass adherent material when grown in trypticase soy broth (TSB) demonstrating its slime-producing ability (19). The bacterial suspension for inoculation was prepared from overnight cultures grown on Columbia agar. The density of viable bacteria (colony forming units) was enumerated by serial dilution and pour plate techniques.

Experimental protocol

Three different experimental situations were studied in mice with surgically induced renal failure and sham-operated controls according to the experimental protocol outlined in Table 1. Experiments in which mice underwent peritoneal infusion and bacterial challenge were compared to control experiments in which mice received inoculation without infusion.

I. Peritoneal instillation of PD solution without bacterial challenge.

Over a two week period sham-operated and renal failure

mice underwent twice daily peritoneal infusion of 1.5 ml PD solution. After 14 days animals were sacrificed for assessment. Body weight was recorded daily throughout the infusion period.

II. Peritoneal instillation of PD solutions and 10^6 cfu *S. epidermidis* challenge: assessment 48 hours after inoculation.

For one week prior to bacterial challenge, sham-operated and renal failure mice underwent daily infusion of 3 ml PD solution. On day eight, animals received an intracatheter inoculation of 10^6 cfu *S. epidermidis* in 1 ml phosphate buffered saline (PBS). Daily infusion of PD solution continued for the next 48 hours after which animals were sacrificed for assessment. During the period of peritoneal infusion, body weight of mice was recorded daily. In control experiments in which animals were not subjected to daily infusion, sham-operated and renal failure mice received an intracatheter inoculation of 10^6 cfu *S. epidermidis* in 1 ml PBS and were sacrificed for assessment 48 hours after bacterial challenge.

III. Peritoneal instillation of PD solution and 10^8 cfu *S. epidermidis* challenge: assessment 1 week after inoculation.

As above, sham-operated and renal failure mice underwent peritoneal instillation of PD solution for one week prior to bacterial challenge, but in this series of experiments they received twice daily infusion of 1.5 ml PD solution. On day 8, 10^8 cfu *S. epidermidis* in 1 ml PBS was injected into the catheter lumen. The twice daily infusion procedure continued for one more week after which animals were sacrificed for assessment. Daily determination of body weight was conducted throughout the infusion procedure. Control experiments were

Table 1. Experimental protocol

Time (weeks)	Procedure
0	Right kidney electrocoagulation (all mice).
2	Left nephrectomy (renal failure mice) or surgical exploration of left renal area (sham-operated mice).
4	Implantation of peritoneal catheter segment (all mice).
8	Start of daily peritoneal instillation of peritoneal dialysis solution (4.25% dextrose).
	I. No bacterial challenge (1.5 ml infusion twice daily) / II. 10^6 cfu *S. epidermidis* (3 ml infusion once daily) / III. 10^8 cfu *S. epidermidis* (1.5 ml infusion twice daily)
9	Intracatheter bacterial challenge (II, III)
	Assessment (48 hours after challenge) (II)
10	Assessment (I) / Assessment (1 week after challenge) (III)

conducted in sham-operated and renal failure mice in which animals were subjected to intracatheter inoculation with 10^8 cfu S. epidermidis without daily infusion and were sacrificed one week after inoculation.

Blood analysis

At the time of sacrifice mice were anesthetized with ether, weighed and blood was collected by cardiac puncture in heparin-coated plastic syringes (Hepalean, Harris Laboratories, Toronto, Canada) for biochemistry and hematology analysis. Blood urea nitrogen (BUN) levels were determined with an IL-9 autoanalyzer (Instrumentation Laboratory Inc., Lexington, Mass., USA). Routine hematology was done utilizing a Coulter counter (Model 2B1, Coulter Electronics Inc., Hialeah, Fla., USA) and differential cell counts were performed on Wright-stained blood smear preparations.

Collection and processing of specimens

The abdomen of each animal was washed with alcohol and the skin and loose connective tissue were retracted aseptically to expose an intact abdominal wall. Ten ml of minimum essential medium (Eagle Modified) containing 10% heat inactivated fetal calf serum and 20 mM HEPES buffer was injected through the abdominal wall directly into the peritoneal cavity and the peritoneal washing was slowly aspirated. Total cell counts of peritoneal washings were enumerated in Neubauer counting chambers and differential cell counts were determined from cytocentrifuge preparations stained with Diff-Quick (American Scientific Products, McGaw Park, ILL., USA). An aliquot of each sample was quantitatively cultured onto blood agar for bacterial content and identity. Bacterial recoveries were

expressed as cfu per ml of peritoneal washing.

A specimen of the exposed abdominal wall (approximately 2 x 2 cm) was excised aseptically. The translucent abdominal wall, which consisted of the peritoneal membrane and scant connective and muscle tissue, will be hereafter referred to as parietal peritoneum or peritoneal membrane. Tissue samples were homogenized in PBS and inoculated onto 5% horse blood Columbia agar. After correction for dilution bacterial recoveries were expressed as cfu per gram of tissue.

The peritoneal catheter implant was removed after fine dissection of any adhering tissue and cutting the anchoring suture to the lateral abdominal wall. The granulation tissue surrounding the catheter was aseptically removed and homogenized in PBS. When necessary tenfold serial dilutions were prepared and specimens were inoculated onto blood agar. After correction for dilution, bacterial recoveries were expressed as cfu per gram of tissue.

Bacteria associated with the catheter were quantitated following a modification of a previously described method (20). The catheter segment was lightly streaked across a blood agar plate ten times, rotated 90° and again streaked ten times across the agar plate. This process was repeated through 360° and the catheter was then incubated in TSB. Bacterial recoveries from blood agar assessment were expressed as colonies per catheter surface and results of incubation in TSB were expressed qualitatively as culture positive or negative.

Colony enumeration of all samples on blood agar was conducted after twenty-four hours incubation at 37°C. Positive identification of bacteria recovered from collected specimens

was ascertained by colony morphology, gram stain, catalase production and the characteristic antibiotic sensitivity profile.

Scanning electron microscopy (SEM)

A 2 mm transverse section from the anchoring end of each recovered catheter was reserved for SEM. A 5 x 5 mm specimen of the ventral parietal peritoneum was excised from the upper right quadrant at a site far removed from that of the multiple intracatheter injections or that of the single intraperitoneal injection required for peritoneal washing. The excised specimen was spread flat on a piece of filter paper previously dampened with 10% buffered formalin. All specimens were processed for SEM using standard techniques. Samples were fixed in cacodylate buffered glutaraldehyde solution (pH 7.3), dehydrated with increasing concentrations of acetone and dried to a critical point. Specimens were then fixed to metal planchets, coated with gold and examined with a scanning electron microscope (model JSM-35U; JEOL, USA, Inc.).

Expression of results and statistical analysis

Bacterial recoveries were converted to log units. Samples presenting no bacterial growth on blood agar were assigned a log value of 1 (10 cfu per ml or less) which represents the lower limit of detection of the microbiological assessment. All results are expressed as mean± SD. Unpaired data for sham-operated and renal failure mice were compared by Student's t test and techniques for analysis of variance were utilized for multiple comparisons between experimental conditions of infusion or no infusion.

Results

Characteristics of the animal model

The surgical procedures for the preparation of the mouse model of renal failure were well tolerated. Within four days of nephrectomy approximately 20% mortality was observed in the renal failure animals due to excessive destruction of the renal parenchyma while the remaining 80% survived until their designated date of sacrifice. No post-operative mortality was observed in sham-operated control mice.

Selected characteristics of the mouse model, six to eight weeks after the onset of renal failure, are presented in Table 2. Severe impairment of renal function was present in renal failure mice as shown by the fourfold rise in blood urea nitrogen levels. Both hemoglobin levels and body weight of renal failure animals were significantly reduced compared to sham-operated mice.

The peritoneal implantation of the CAPD catheter segment two weeks after nephrectomy or sham surgery was well tolerated by all mice. As previously described, four to six weeks after catheter implantation, granulation tissue which consisted predominantly of fibroblasts and mononuclear leucocytes invariably surrounded the catheter segment (21). This catheter associated tissue, however, did not interfere with the immediate peritoneal distribution of the infusion volume as previously demonstrated radiologically by intracatheter injection of a radioopaque medium (21).

The peritoneal instillation of PD solution was well tolerated by all mice and their mobility and level of activity were not apparently influenced by the infusion procedure. Body weight of mice remained constant throughout the infusion period

Table 2. Characteristics of the mouse model of chronic renal failure[a,b]

Animal status:	Sham-operated	Renal failure
Blood urea nitrogen (mg/dl)	21.8±4.9 (41)	88.1±23.5[c] (41)
Hemoglobin (g/dl)	12.8±1.1 (36)	9.2±1.5[c] (39)
Body weight (g)	21.2±1.9 (41)	20.2±2.0[c] (41)

[a]Results obtained in 14 week old C57BL/6 inbred mice 6 to 8 weeks after the surgical induction of renal failure or sham surgery.

[b]Results are expressed as mean±SD and sample size is indicated in parenthesis.

[c]Significant difference ($p<0.05$) between renal failure and control mice.

and did not differ from their preinfusion weight.

Peritoneal instillation of PD solution without bacterial challenge:

After 14 days of twice daily peritoneal instillation of PD solution, sham-operated and renal failure mice were sacrificed for assessment. No bacterial growth was recovered from peritoneal structures including the catheter and its associated granulation tissue in any of the mice. However, in contrast to a normal resident peritoneal leucocyte population, there was a marked peritoneal inflammatory response as a consequence of the infusion procedure which was most severe in renal failure mice (Table 3). In renal failure mice both total peritoneal leucocyte and polymorph counts increased markedly as a consequence of the infusion procedure while sham-operated controls presented an unexplained fall in total peritoneal cellularity and a moderate increase in percent polymorphs. Sham-operated mice presented evidence of a mild peripheral inflammatory response while peripheral leucocytes of infused renal failure mice were similar to non infused controls. As previously shown in this mouse strain, lymphocytes are the predominant circulating leucocyte (22).

Effect of peritoneal instillation of PD solution on response of mice to 10^6 cfu S. epidermidis challenge

Recovery of S. epidermidis 48 hours after 10^6 cfu intracatheter inoculation from mice that had received once daily peritoneal instillation of 3 ml PD solution for one week prior to bacterial challenge is presented in Figures 1 and 2. Compared to control experiments in which mice received the identical inoculum without infusion the microbiological status

Table 3. Local and peripheral inflammatory response to peritoneal infusion of PD solution[a,b]

	No infusion		Infusion[c]	
	Sham-operated	Renal failure	Sham-operated	Renal failure
Peritoneal leucocytes	(12)	(16)	(5)	(5)
Total leucocytes per ml ($x10^6$)	0.6±0.2	0.8±0.4	0.1±0.1[e]	1.5±0.7[d,e]
Macrophages (%)	61.1±11.4	47.5±13.6	40.2±10.1[e]	33.4±7.8[e]
Lymphocytes (%)	36.0±10.0	50.6±13.6	44.4±19.0	25.6±17.6[e]
Polymorphs (%)	2.5±3.4	1.4±1.4	15.6±16.1[e]	41.0±15.9[e]
Mast cells (%)	0.3±0.9	0.5±1.0	0	0
Circulating leucocytes	(13)	(10)	(5)	(5)
Total leucocytes per ml ($x10^3$)	5.7±2.3	5.8±3.4	5.5±2.9	6.1±2.7
Neutrophils (%)	5.9±4.8	8.0±5.4	14.0±12.3	6.6±4.7
Stabs (%)	0	0	0	0
Lymphocytes (%)	93.9±4.8	92.0±5.4	85.0±13.7	93.0±4.9

[a]Results obtained in 14 week old C57BL/6 inbred mice 6 to 8 weeks after the surgical induction of renal failure or sham surgery.

[b]Results are expressed as mean±SD and sample size is indicated in parenthesis.

[c]Twice daily peritoneal infusion of 1.5 ml 4.25% dextrose PD solution.

[d]Significant difference ($p<0.05$) between sham-operated and renal failure infused mice.

[e]Significant difference ($p<0.05$) between infused and respective non infused mice.

of all tissues tested was unaffected by the peritoneal instillation of PD solution. Minimal bacterial growth was present in specimens of peritoneum and peritoneal washings from all mice irrespective of their level of renal function. In contrast, large numbers of bacteria were recovered from the catheter associated granulation tissue of all animals and recoveries from renal failure mice were significantly greater ($p < 0.05$) than those of sham-operated controls. Furthermore, S. epidermidis recovery from peritoneal catheters was not greatly influenced by the infusion procedure. In view of the small frequencies within each of the categories of bacteria recovered per catheter, statistical analysis was limited however a larger proportion of catheters presenting no bacterial growth were recovered from non infused compared to infused renal failure mice (Figure 2). Regardless of peritoneal infusion, a greater proportion of catheters recovered from sham-operated controls presented no bacterial growth compared to those or renal failure mice.

In mice that did not undergo peritoneal instillation of PD solution, there was no evidence of inflammation, in the peritoneal cavity or in the peripheral blood, 48 hours after 10^6 cfu inoculation (Figure 3). An identical inoculation in mice subjected to daily infusion generated a local polymorph response which achieved statistical significance compared to non infused mice in sham-operated controls only. Although there was a rise in the mean peritoneal polymorph count of infused renal failure mice compared to non infused controls, the response was variable and statistical significance was not established. Levels of peritoneal and circulating total

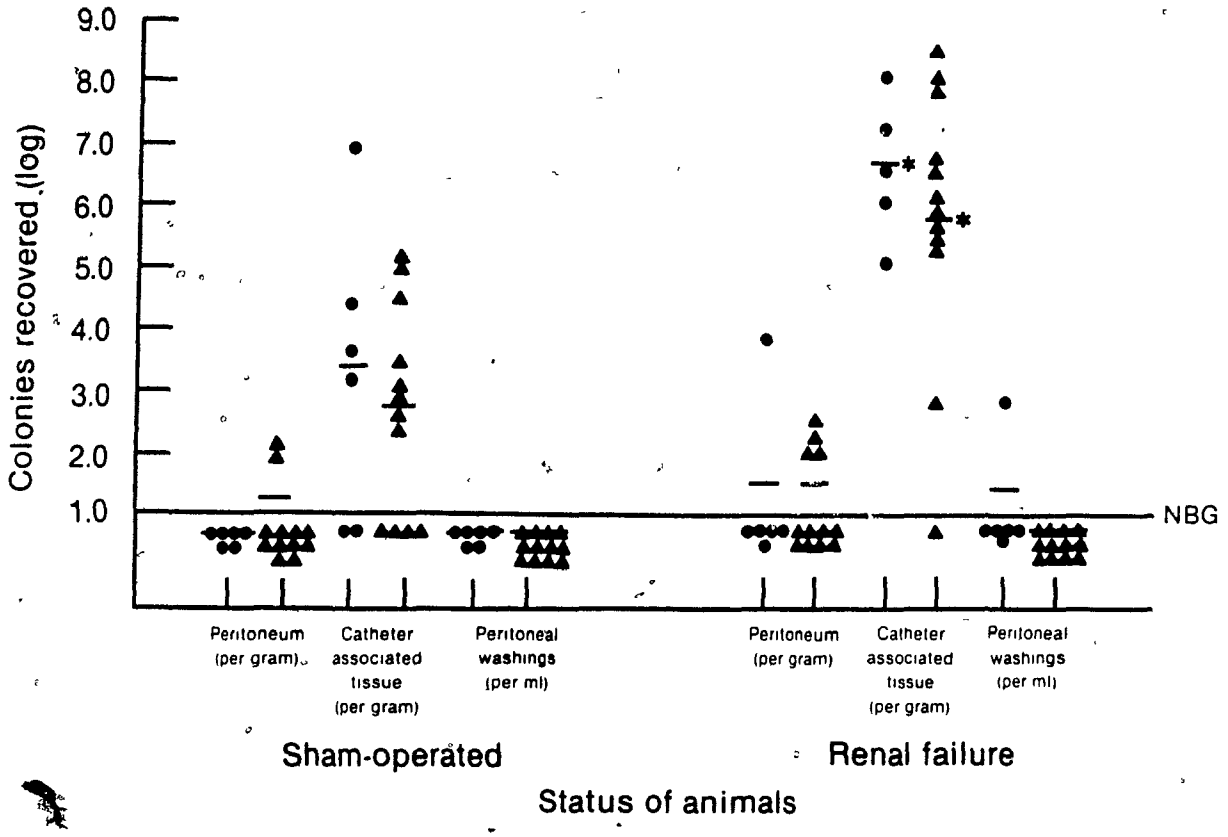

Figure 1. Recovery of Staphylococcus epidermidis from parietal peritoneum, catheter associated tissue and peritoneal washings of sham-operated and renal failure mice 48 hours after 10^6 cfu intracatheter challenge. Specimens from mice subjected to once daily peritoneal instillation of 3 ml 4.25% dextrose peritoneal dialysis solution (circles) are compared to respective non infused animals (triangles). Mean values are represented by horizontal bars and an asterisk indicates a significant difference ($p<0.05$) between renal failure and respective sham-operated mice. NBG indicates no bacterial growth.

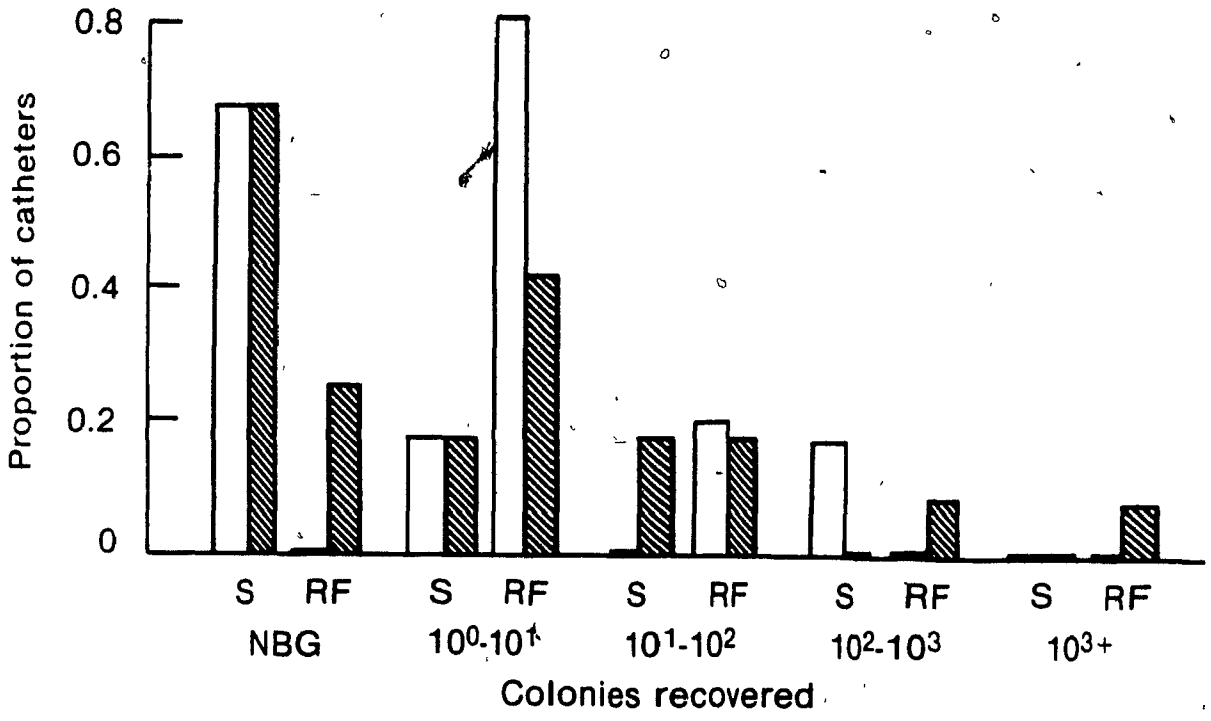

Figure 2. Recovery of Staphylococcus epidermidis from peritoneal catheters of sham-operated (S) and renal failure (RF) mice 48 hours after 10^6 cfu intracatheter challenge. Recovery from catheters of mice subjected to once daily peritoneal infusion of 3 ml 4.25% dextrose peritoneal dialysis solution (open bars) is compared to respective non infused (hatched bars) animals. NBG indicates no bacterial growth.

leucocytes as well as peripheral polymorphs were not significantly affected by the infusion procedure. Total counts of peritoneal and circulating leucocytes and polymorphs of sham-operated and renal failure mice were not significantly different.

Effect of peritoneal instillation of PD solution on the response of mice to 10^8 cfu S. epidermidis challenge

Recovery of S. epidermidis one week after 10^8 cfu inoculation from mice subjected to twice daily peritoneal infusion of 1.5 ml PD solution for a two week period is summarized in Figures 4 and 5. In contrast to assessments 48 hours after 10^6 cfu inoculation, sizable S. epidermidis recoveries were obtained from specimens of peritoneal membrane and peritoneal washings one week after 10^8 cfu inoculation. With the exception of peritoneal washings from which recovery of S. epidermidis was moderatly elevated in infused mice, the infusion procedure did not affect the microbiological status of any other peritoneal structure. A majority of mice in both experimental conditions presented heavily colonized peritoneal catheters (10^{3+} colonies per catheter). Recovery of S. epidermidis was similar in sham-operated and renal failure mice which again is in contrast to assessments following 10^6 cfu inoculation in which a renal failure related impairment of bacterial clearance from the catheter site was evident.

Local and peripheral inflammatory response of mice 1 week after 10^8 cfu intracatheter S. epidermidis challenge is summarized in Figure 6. Under conditions of twice daily peritoneal infusion with PD solution, peritoneal inflammatory response to S. epidermidis inoculation was increased as shown

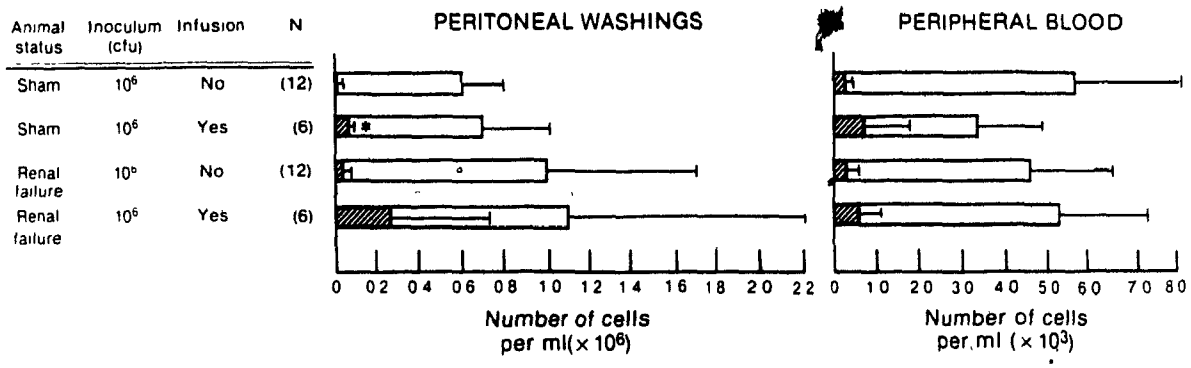

Figure 3. Mean peritoneal and circulating leucocytes (horizontal bars) and polymorphs (hatched bars) of sham-operated and renal failure mice 48 hours after 10^6 cfu intracatheter Staphylococcus epidermidis challenge. Inflammatory response of mice subjected to once daily peritoneal infusion of 3 ml 4.25% dextrose peritoneal dialysis solution is compared to respective non infused animals. Horizontal lines represent standard deviations and the number of mice is indicated in parentheses. An asterisk indicates a significant difference ($p<0.05$) between infused and respective non infused mice.

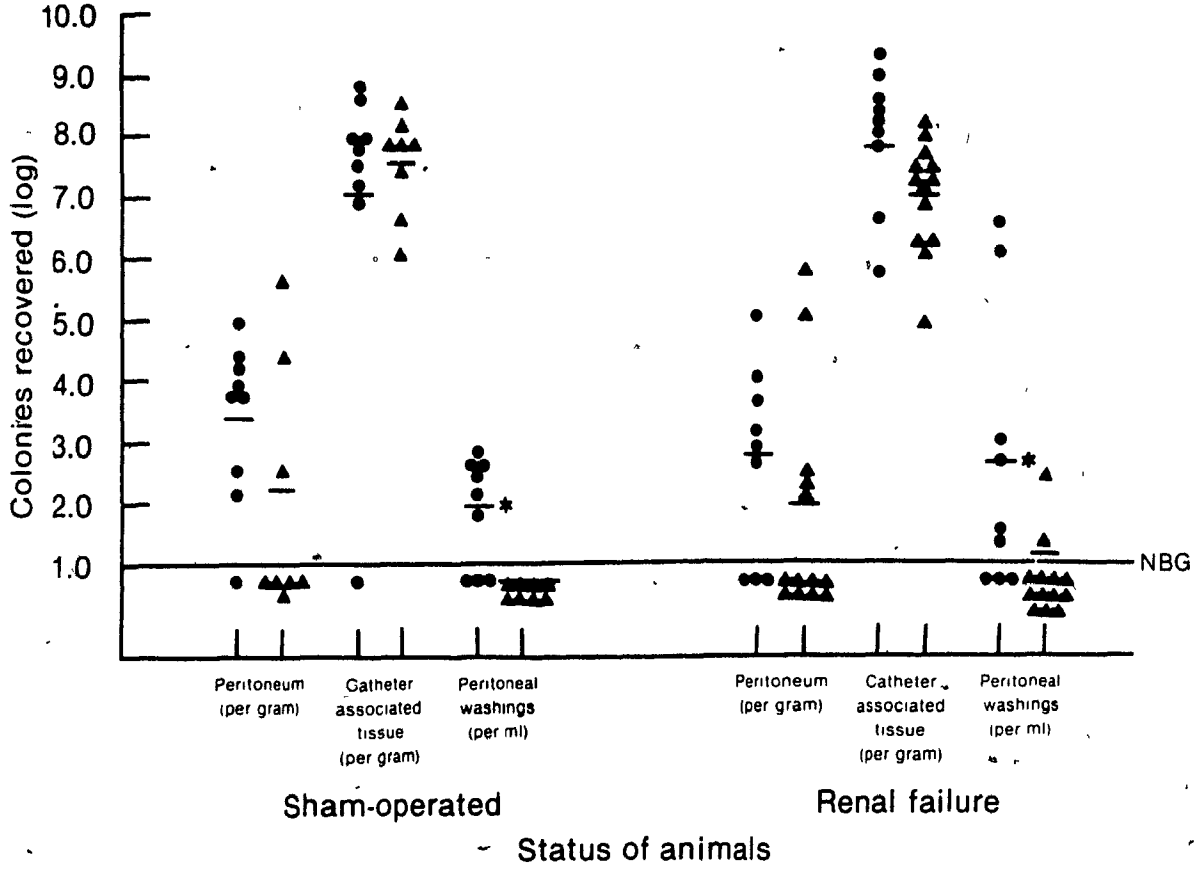

Figure 4. Recovery of Staphylococcus epidermidis from parietal peritoneum, catheter associated tissue and peritoneal washings of sham-operated and renal failure mice one week after 10^8 cfu intracatheter challenge. Specimens of mice subjected to twice daily peritoneal infusion of 1.5 ml 4.25% dextrose peritoneal dialysis solution (circles) are compared to respective non infused animals (triangles). Mean values are represented by horizontal bars and asterisks indicate significant differences ($p<0.05$) between infused and respective non infused mice. NBG indicates no bacterial growth.

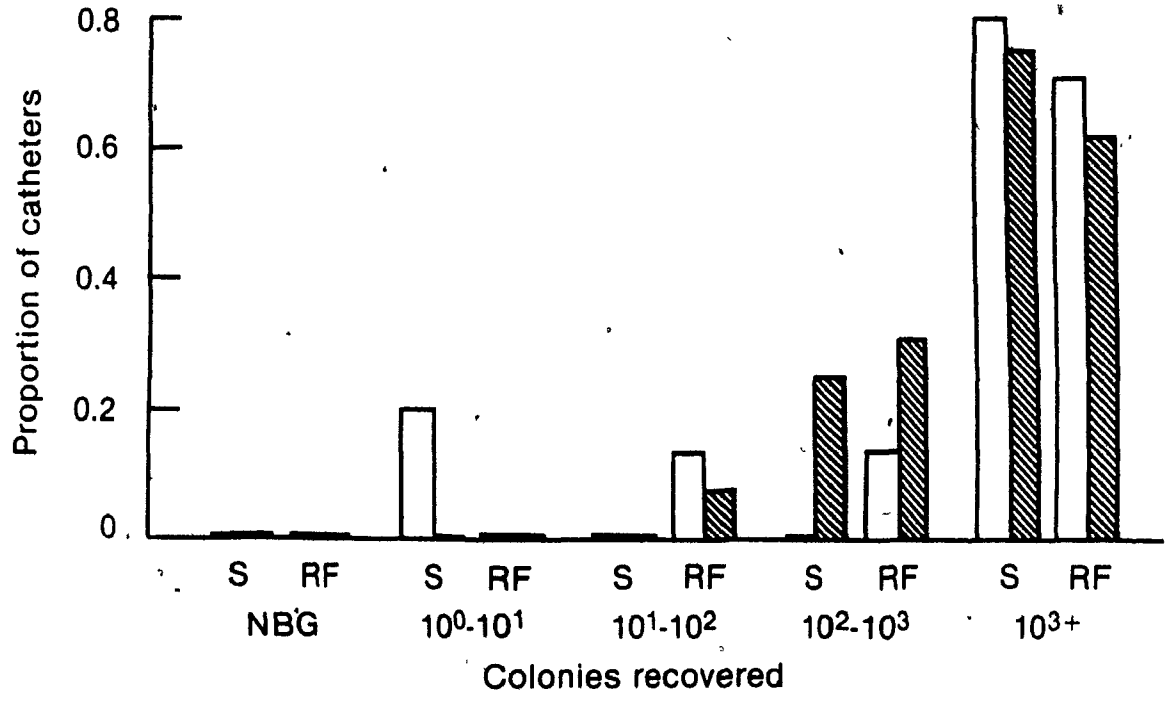

Figure 5. Recovery of Staphylococcus epidermidis from peritoneal catheters of sham-operated (S) and renal failure (RF) mice one week after 10^8 cfu intracatheter challenge. Recovery from catheters of mice subjected to twice daily infusion with 1.5 ml 4.25% dextrose peritoneal dialysis solution (open bars) are compared to non infused animals (hatched bars). NBG indicates no bacterial growth.

by elevations in the numbers of peritoneal leucocytes and polymorphs. The number and type (differentials) of peritoneal and peripheral leucocytes were similar in renal failure and sham-operated mice. Local inflammatory response 1 week after 10^8 cfu inoculation markedly exceeded that observed 48 hours after 10^6 cfu challenge with peritoneal and peripheral leucocyte and polymorph counts two to fourfold greater than those following 10^6 cfu inoculation.

Scanning electron microscopy (SEM)

Catheter segments recovered from mice receiving daily peritoneal infusion of PD solution and their non infused controls were assessed with SEM. Neither the infusion procedures nor the renal failure had an apparent influence on the bacterial association with the catheter surface. Forty eight hours after 10^6 cfu S. epidermidis intracatheter inoculation, single cocci or microcolonies were observed on the surface of catheters recovered from infused and non infused mice (data not shown). One week after 10^8 cfu intracatheter challenge, large colonies of cocci or isolated areas of confluent growth on the catheter surface were observed (Figure 7).

SEM assessments of the mesothelial lining of the parietal peritoneum of sham-operated and renal failure mice one week after 10^8 cfu intracatheter S. epidermidis inoculation were compared to those of mice that received the identical inoculum and underwent twice daily peritoneal instillation of 1.5 ml PD solution. Mesothelial morphology underwent striking alteration as a consequence of the infusion procedure and specimens recovered from renal failure or sham-operated mice were

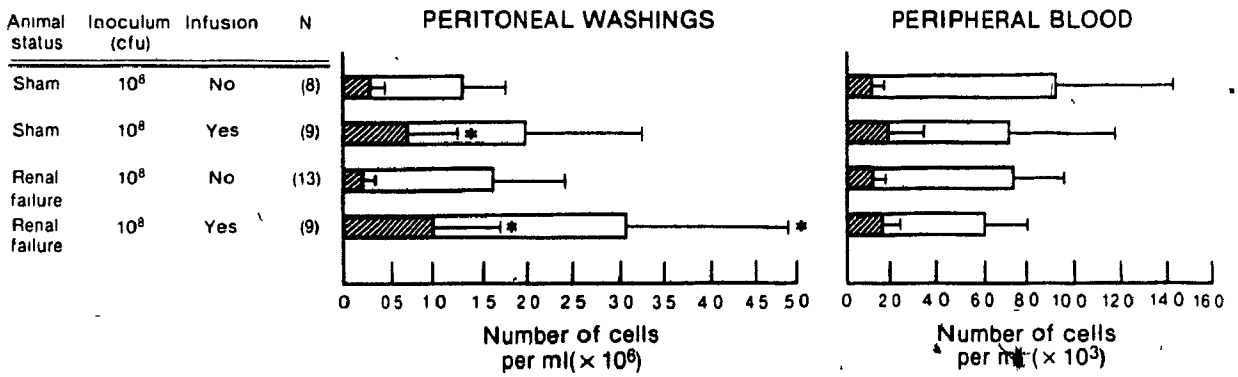

Figure 6. Mean peritoneal and circulating leucocytes (horizontal bars) and polymorphs (hatched areas) of sham-operated and renal failure mice 1 week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge. Inflammatory response of mice subjected to twice daily peritoneal instillation of 1.5 ml 4.25% dextrose peritoneal dialysis solution is compared to respective non infused animals. Horizontal lines represent standard deviations and the number of mice is indicated in parentheses. Asterisks indicate significant differences ($p<0.05$) between infused and respective non infused mice.

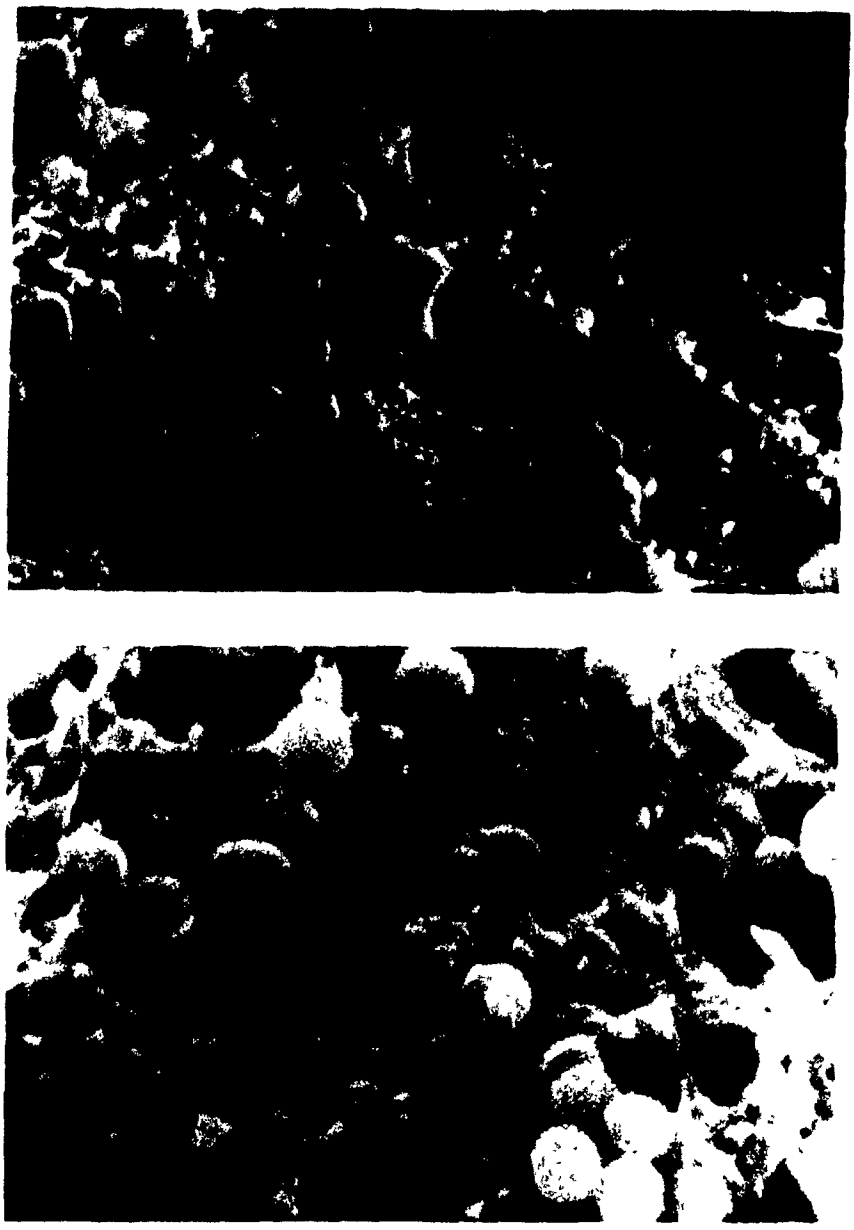

Figure 7. SEM of luminal surface of a peritoneal catheter segment recovered from a renal failure mouse subjected to twice daily peritoneal instillation of 1.5 ml 4.25% dextrose peritoneal dialysis solution and one week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge.
Top, low power SEM: Cocci are distributed across the catheter surface and leucocytes are also evident (Magnification x 1,600).
Bottom, high power SEM: Cocci are revealed in a fibrin web on the catheter surface (Magnification x 10,000).

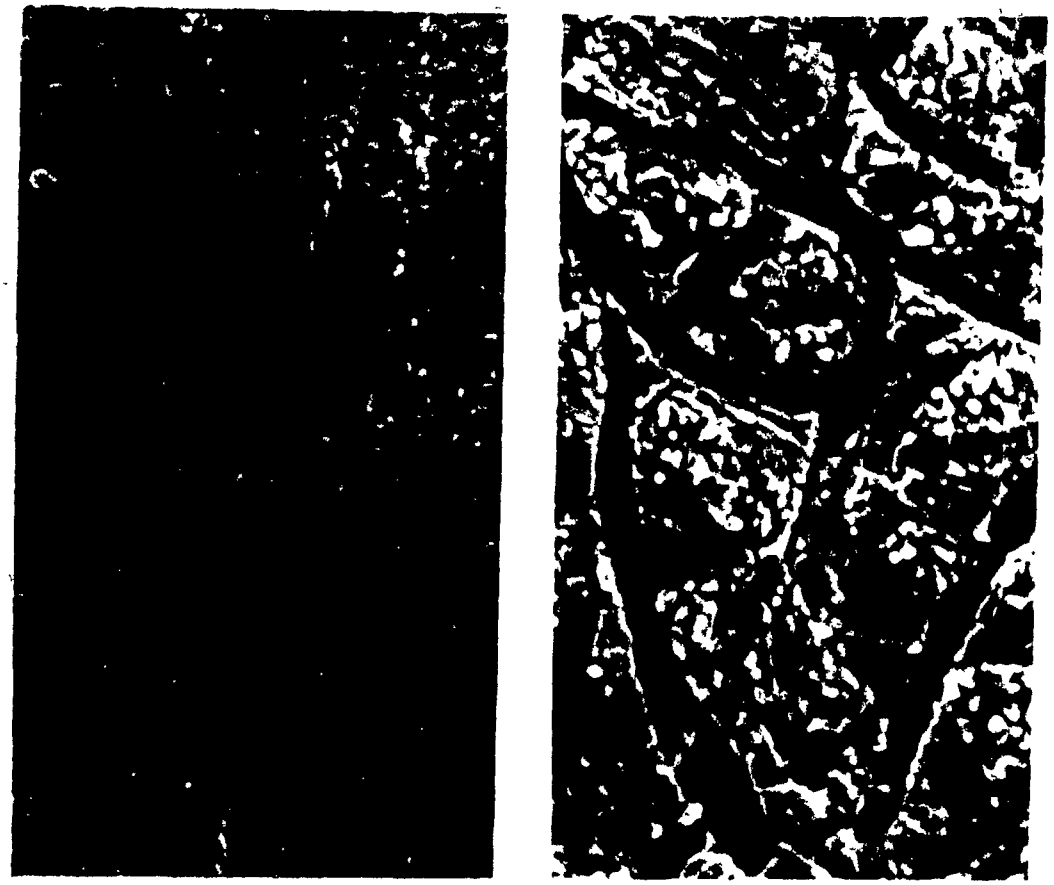

Figure 8. Left panel, low power SEM: Mesothelium of parietal peritoneum of a sham-operated mouse one week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge (Magnification x 200).
Right panel, low power SEM: Mesothelium of parietal peritoneum of a renal failure mouse subjected to twice daily peritoneal instillation of 1.5 ml 4.25% dextrose peritoneal dialysis solution and one week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge. Granulation tissue reaction with engorgement of the capillary lattice is evident and leucocytes litter the mesothelial surface (Magnification x 200).

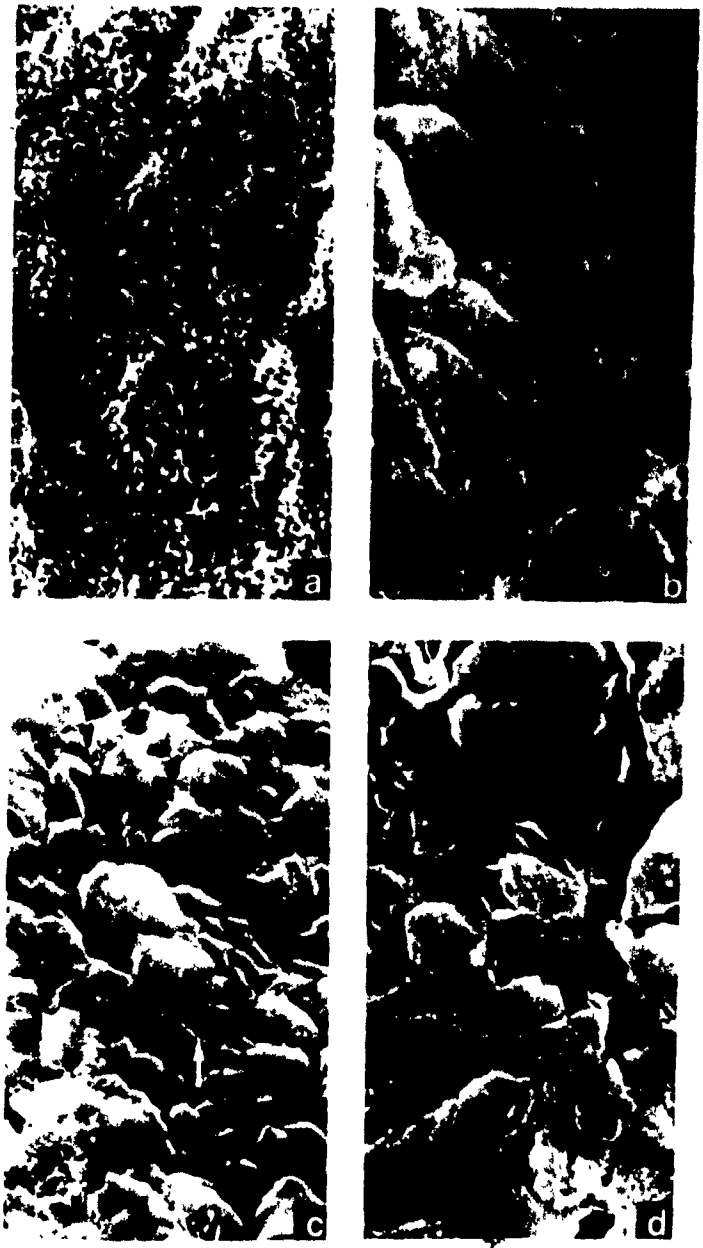

Figure 9. (a) High power SEM: Mesothelium of parietal peritoneum of a renal failure mouse one week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge. Microvillus projections obscure individual mesothelial cells (Magnification x 2,000).
(b-d) High power SEM: Mesothelium of parietal peritoneum of mice subjected to twice daily peritoneal instillation of 1.5 ml 4.25% dextrose peritoneal dialysis solution and one week after 10^8 cfu intracatheter Staphylococcus epidermidis challenge (Magnification x 2,000).
(b) Mesothelial cells are separated exposing intercellular junctions. Microvilli are markedly attenuated (specimen from a sham-operated mouse).
(c) There is focal disruption of mesothelial cells (arrows). Microvilli are absent (specimen from a sham-operated mouse).
(d) Mesothelial cells are severely damaged demonstrating disruption and degeneration (specimen from a renal failure mouse).

undistinguishable. Compared to non infused controls (Figure 8, left panel), the mesothelium of infused mice (Figure 8, right panel), appeared to undergo a granulation tissue reaction and numerous leucocytes littered the mesothelial surface. At higher magnification, SEM of parietal peritoneum of inoculated, non infused mice (Figure 9a) demonstrated abundant microvilli which obscured the contours of the mesothelial cells to which they were attached. The parietal peritoneum of mice subjected to inoculation and infusion presented varying degrees of mesothelial damage (Figure 9b-9c) which included attenuation of microvilli exposing intercellular junctions as well as focal disruption and degeneration of mesothelium.

Discussion

The results of the current investigation have demonstrated that repeated peritoneal instillation of hyperosmolar, acidic PD solution did not have a marked influence on the microbiological status of peritoneal structures of mice following peritoneal S. epidermidis challenge with either a small (10^6 cfu) or large (10^8 cfu) inoculum. Clearance of S. epidermidis from the peritoneal catheter was ineffective, regardless of PD solution infusion and SEM assessment of recovered catheters demonstrated colonies of cocci associated with the catheter surface. The impact of renal failure on the ability of mice to respond to bacterial challenge was only evident following the smaller S. epidermidis challenge where renal failure mice presented greater bacterial recoveries from the catheter site. The peritoneal infusion procedure itself caused acute local inflammation and when combined with S. epidermidis inoculation peritoneal polymorph response was

augmented. The systemic inflammatory response however was not significantly influenced by the infusion procedure. Scanning electron microscopy of the mesothelium of the parietal peritoneum revealed striking morphological alterations as a consequence of peritoneal instillation of PD solution.

The surgical preparation and the resulting characteristics of chronic uremia presented by this mouse model of renal failure have been previously described (18, 21, 22). In this series of experiments, six to eight weeks after the induction of renal failure, mice were azotemic and presented significant anemia and growth retardation. Previously described models of peritoneal dialysis in animals having functional peritoneal catheters exiting to the skin have frequently been complicated by peritoneal contamination and catheter obstruction (23-27). In the current mouse model, peritoneal access via the catheter was accomplished by transcutaneous injection into the catheter lumen. The sterility of the peritoneal cavity was thereby maintained and repeated intracatheter instillation of PD solution was not complicated by peritoneal contamination. The volume of the peritoneal instillate was selected since it produced only a moderate abdominal distention and it did not adversely affect the activity or mobility of the mice. Infusion volumes based on ratios of body weight or surface area of the mouse and man proved impractical. Unlike the practice of CAPD, the resulting model was limited to peritoneal infusion without drainage, a procedure that was well tolerated by all mice, including renal failure animals presumably due to their ability to produce large volumes of dilute urine (22).

Concurring with our results, investigations conducted in

sham-operated and uremic rats have demonstrated that twice daily peritoneal infusion of 10 ml 4.25% dextrose PD solution via a permanent peritoneal catheter for 8 days did not impair the clearance of a peritoneal inoculation of $2x10^7$ cfu Escherichia coli (26). Twenty-four hours after experimental inoculation, bacterial recovery from the spleen of infused rats was moderately elevated compared to non infused controls while all other peritoneal structures presented comparable E. coli recoveries during conditions of infusion and no infusion. In the current investigation, the modest increase in the recovery of S. epidermidis from peritoneal washings of infused mice following 10^8 cfu intracatheter inoculation may have resulted from the infusion of PD solution through a heavily colonized catheter site causing peritoneal dissemination of bacteria. In contrast to the catheter site, peritoneal washings and parietal peritoneum consistently presented no bacterial growth or much smaller bacterial recoveries 48 hours or 1 week after 10^6 or 10^8 cfu S. epidermidis inoculation, respectively.

The influence of renal failure on the response of mice to the bacterial challenge was not consistently demonstrated in the present studies insofar as a renal failure related impairment of bacterial clearance from the catheter site was demonstrated only following 10^6 cfu S. epidermidis inoculation. This impairment was observed in experiments with or without the daily infusion procedure. In previous investigations in which mice without peritoneal catheter implants underwent peritoneal S. epidermidis inoculation renal failure mice presented increased susceptibility in terms of survival time and mortality (with 10^8-10^9 cfu) and delayed

peritoneal clearance of the inoculum (with 10^6 cfu) compared to sham-operated controls (14,15). In the current investigation one week after 10^8 cfu intracatheter inoculation sham-operated and renal failure mice could not be distinguished on the basis of bacterial recovery from peritoneal structures. The observed inconsistencies regarding the effect of renal failure on host defense to bacterial challenge may reflect differing experimental variables of sampling time (48 hours vs 1 week) or the inoculation size (10^6 vs 10^8 cfu). For example, a maximal stimulus (10^8 cfu) may be required to evoke an appropriate host defense response in renal failure mice which was not demonstrated following inoculation with a smaller inoculum.

Peritoneal infusion of PD solutions in mice by itself caused a local polymorph response and inflammatory response to bacterial inoculation was augmented in mice subjected to the peritoneal infusion procedure. Our current observations which reflect later stages of the inflammatory response do not reveal differences between sham-operated and renal failure mice and are in contrast to our previous findings in which earlier phases of peritoneal inflammatory response were impaired in renal failure mice (15).

In vitro assessments of PD solutions have demonstrated their inhibitory influence on phagocytosis and bactericidal function of human leucocytes (7), their cytotoxic effect toward peritoneal leucocytes of mice (8) and their deleterious influence on opsonizing and bacteriolytic functions of human complement (28). These cellular and soluble components of the immune system play an integral role in host defense of the

peritoneal cavity against bacterial infection (12) yet our *in vivo* findings do not reflect the results of *in vitro* studies. Unlike *in vitro* conditions, where hyperosmolar and acidic characteristics of PD solution remain constant, pH and osmolarity of PD solutions approach physiological levels after one and four hours of peritoneal dwell, respectively (6). Furthermore, constituents within the peritoneal cavity may protect leucocytes from the deleterious effects of PD solution such that local immune responses are not apparently compromised. Also noteworthy in considering the impact of PD solutions on peritoneal defense to bacterial challenge is the fact that PD solutions do not provide an optimum growth medium for *in vitro* bacterial culture however, conditions for growth improve quickly with peritoneal dwell time (29,30). Finally if, in CAPD patients, host defenses are impaired as a consequence of exposure to PD solution they may not be of fundamental importance if the permanently dwelling peritoneal catheter provides an environment in which the same host defense mechanisms against bacterial growth are normally ineffective.

In previous studies and in the current investigation, microbiological and SEM assessments of peritoneal catheters recovered from mice following intracatheter *S. epidermidis* challenge demonstrated that bacteria remain associated with the catheter site after other peritoneal structures have become culture negative (16,17). SEM revealed macrocolonies of cocci and areas of confluent bacterial growth which, according to *in vitro* dynamics of colonization of catheter surfaces by slime-producing coagulase negative staphylococci, represent later stages of biofilm formation (31). A role of bacterial

biofilms in refractory bacterial infections such as the recurrent episodes of CAPD peritonitis has been proposed (32). The biofilm is envisioned as a "defensive" mode of growth in which the bacterial population embedded in the glycocalyx material has protection from antimicrobial therapy and host defense mechanisms. SEM assessments of peritoneal catheters recovered from CAPD patients with or without symptoms of peritonitis have also demonstrated the existence of bacterial biofilms on the catheter surface (33-35). Whether or not all CAPD patients harbour bacterial biofilms on their peritoneal catheters and if the biofilm microorganism is in fact responsible for observed peritonitis episodes is presently unknown.

In contrast to experimental conditions of inoculation alone, the mesothelial cell lining of the peritoneal cavity of mice underwent striking morphological alterations as a result of inoculation and repeated peritoneal instillation of 4.25% dextrose PD solution. It is unlikely that these changes could be attributed to mechanical distention of the peritoneum of infused mice since the instillation volumes were small (1.5-3 ml), and particularly in the case of the 1.5 ml volume, there was no evidence of abdominal distention following peritoneal infusion. In agreement with previous studies in CAPD patients and experimental animals subjected to peritoneal infusion with PD solution (36,37), repeated exposure of the peritoneal cavity to hyperosmolar, acidic PD solutions caused attenuation of mesothelial microvilli, shrinkage of mesothelial cells abolishing tight junctions between adjacent cells and in some areas caused cellular denudation. In addition, the peritoneum

of infused mice presented evidence of granulation tissue reaction and the mesothelial surface was frequently littered with inflammatory cells. These findings concur with the augmented local inflammatory response to peritoneal S. epidermidis challenge which was observed in mice subjected to repeated peritoneal instillation of PD solution. Whether or not the observed morphological changes of the mesothelium could interfere with dialysis processes across the peritoneal membrane remains to be defined.

Utilizing this animal preparation, we have demonstrated that repeated peritoneal instillation of PD solution, without peritoneal drainage, for periods of up to two weeks, did not have a marked influence on the outcome of peritoneal S. epidermidis challenge. Bacteria remained preferentially associated with the catheter site and in this milieu, growth and persistence of S. epidermidis was not measurably influenced by exposure to PD solutions. Since the bacterial biofilm affords protection from eradication by host defense mechanisms, the deleterious effects of PD solutions on leucocyte populations that have been observed in vitro may be of little consequence to host defense under these in vivo conditions.

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Chapter 7

General Discussion and Future Directions

Since the introduction of continuous ambulatory peritoneal dialysis, peritonitis caused by Staphylococcus epidermidis has consistently been a major complication of this dialysis modality (24, 27, 28). In order to gain a better understanding of the pathogenesis of CAPD associated S. epidermidis peritonitis, a mouse model was utilized to investigate the relative roles of (a) chronic uremia, (b) a peritoneal catheter implant and (c) repeated exposure of the peritoneum to peritoneal dialysis solutions, in the development and progression of this infectious process. Although actual peritoneal dialysis was not conducted in this animal preparation, this did not preclude the investigation of the role of those defined variables in the etiology of peritonitis associated with CAPD. The results of these investigations have demonstrated the immunosuppressive influence of chronic uremia and the role of the peritoneal catheter implant as a nidus for protracted S. epidermidis colonization. Repeated peritoneal exposure to hyperosmolar, acidic peritoneal dialysis solutions did not influence the response of mice to peritoneal S. epidermidis challenge.

i. The mouse models

The development of appropriate animal models was imperative to the conduct of this research project. Thus, three related mouse models were utilized: (a) the mouse model of chronic renal failure, which had been previously developed (203), was further characterized (b) the renal failure mouse

bearing a permanent peritoneal catheter implant was developed and was extended to include (c) repeated peritoneal instillation of dialysis solution. The use of well defined, inbred mouse models provided homogeneous, and therefore simplified, in vivo systems in which to investigate the progression of the peritoneal S. epidermidis infectious process under controlled experimental conditions. The investigation of the roles of the defined variables in the pathogenesis of peritoneal S. epidermidis infection was conducted while keeping constant, the inbred mouse strain, the test bacteria and the characteristics of the catheter implant. However, inherent to the theoretical advantages offered by inbred animal models and controlled experimental conditions in the absence of confounding clinical variables are the obvious and significant differences between the defined experimental conditions and those that prevail clinically. Furthermore, with the objective of mimicing all possible clinical conditions, the experimental microorganism used in the current investigation was a human S. epidermidis strain isolated from a CAPD patient. The inoculation of mice with a human S. epidermidis strain clearly does not reproduce natural events.

The preparation of the mouse models was labour intensive, requiring two or three surgical procedures, however large numbers of experimental animals could be prepared at one time without unrealistic technical requirements for their maintenance. The severity of the surgically induced renal failure was graded by the extent of kidney electrocoagulation. Animals were prepared with the objective of moderate uremia that did not preclude indefinite survival without dietary restriction and dialysis support and which correlated to BUN

levels approximating 100 mg/dl. Blood urea nitrogen levels of a similar magnitude may be observed clinically, particularly in dialysis patients maintained with intermittent therapy and in underdialyzed CAPD patients.

The C57BL/6 inbred mouse strain was selected for the mouse model of chronic renal failure. This strain was readily available from animal breeders which were located at a reasonably short distance from the laboratory. Furthermore, this strain was suitable for the mouse model of chronic renal failure since congenital renal abnormalities and spontaneous hypertension are rare (204, 205). The C57BL/6 mouse is immunologically well defined, is without major immunological difficiencies and is resistant to many extracellular pathogens (205-206). It was, therefore, a good candidate mouse strain for studies of host response to bacterial challenge.

The normal values of several biological parameters of mice differ from those of man, however these differences did not preclude the development of the current mouse model of renal failure. Plasma creatinine which, in mice, is approximately one tenth the normal level in man was, nevertheless, significantly elevated by the experimental induction of renal failure. In comparison to man, a larger proportion of lymphocytes (90%) and a respective smaller proportion of neutrophils (less than 10%) compromise circulating leucocytes of mice. However, systemic neutrophil response was promptly mounted in mice following experimental inoculation. Furthermore, renal failure mice presented several manifestations of chronic uremia including growth retardation, severe anemia, hyperphosphatemia, hyperkalemia, acidosis and

changes in bone histology which were consistent with secondary hyperparathyroidism.

The mouse model also included sham-operated animals to control for the immunosuppression and increased susceptibility to infection that is associated with surgical manipulation. Several defects of host immune response following surgical trauma have been reported and include depressed cell-mediated immune response measured by delayed type hypersensitivity response, decreased circulating lymphocytes, impaired blastogenic response and mixed lymphocyte reaction which may be attributed to increased suppressor cell activity (207-209). Furthermore, decreased leucocyte chemotaxis, impaired polymorph delivery to an inflammatory focus and reduced neutrophil bactericidal function have also been demonstrated in surgical patients (210-212). In the current mouse model, sham-surgery was conducted at least six weeks before experimental inoculation, yet peritoneal clearance of S. epidermidis, as well as local inflammatory response of sham-operated mice, was significantly impaired compared to normal control animals. These results underline the importance of including sham-operated controls in experimental animal models and reveal the potentially enduring influence of surgery on host immune response.

The mouse model of chronic renal failure was extended to include a peritoneal catheter implant. Unlike functional CAPD catheters, the mouse catheter segment resided entirely within the confines of the peritoneal cavity (no skin exit site) and did not, therefore, compromise the sterility of the peritoneum. Previously described animal models, having peritoneal catheters

exiting through the skin, have been limited by uncontrolled, spontaneous peritonitis which has required continuous antibiotic therapy. The use of such animal models would clearly limit the current investigations which required controlled experimental bacterial challenges.

The mouse preparation was subjected to repeated peritoneal instillation of commercially available peritoneal dialysis solution. However, drainage of the dialysis solution from the peritoneal cavity was not accomplished and therefore, this model was not utilized to address the roles of peritoneal dialysis and/or the continual loss to drainage of soluble and cellular peritoneal constituents (many of which may be immunoreactive) in the pathogenesis of peritoneal infection. Rather, this mouse model facilitated the investigation of the influence of repeated exposure of the peritoneum to non physiologic peritoneal dialysis solutions on the development and progression of a peritoneal S. epidermidis infection.

Repeated peritoneal instillation of dialysis solution was not complicated by bacterial contamination of peritoneal structures, however, there was evidence of peritoneal inflammation. In response to the infusion procedure, renal failure mice presented increased numbers of total peritoneal leucocytes and polymorphs, while peritoneal leucocyte counts of sham-operated controls were markedly decreased in spite of moderately elevated polymorph counts. The underlying mechanism (s) responsible for these observations is unknown, however, possible explanations include varying degrees of dilution of peritoneal leucocytes by the instillation volume as well as differing inflammatory response patterns in renal failure and

sham-operated mice subjected to infusion. Investigation of the response of normal mice to peritoneal instillation of dialysis solution may be useful in clarifying these issues.

The final extension of the mouse model to one of CAPD was not realized due to unresolved technical difficulties. Drainage of the instilled peritoneal dialysis solution was attempted by intracatheter aspiration and alternatively by peritoneal aspiration using a needle and syringe. In all cases drainage was blocked by omentum or granulation tissue associated with the catheter segment. This limitation precluded further investigations of the influence of CAPD on host defense of the peritoneum.

The three successfully defined mouse models facilitated the following investigations. The influence of chronic uremia, in the absence of dialysis treatment, on host susceptibility to S. epidermidis challenge was investigated. In a noninfected preparation of renal failure mice bearing an intraperitoneal catheter segment, the role of the catheter implant in the pathogenesis of peritoneal S. epidermidis infection was determined in a controlled experimental setting. Lastly, the impact of repeated exposure of the peritoneum (as well as the experimental inoculum) to peritoneal dialysis solution, without drainage of the instilled volume, on the response of renal failure mice to experimental S. epidermidis challenge was assessed.

ii. The influence of chronic renal failure on response of mice to S. epidermidis inoculation

The influence of chronic renal failure on response of mice to intraperitoneal S. epidermidis inoculation was investigated extensively thereby revealing a moderate, although significant, renal failure related immunosuppression. Time to 50% mortality following 10^9 cfu S. epidermidis inoculation was reduced in renal failure mice as was percent survival following 10^8 cfu challenge compared to sham-operated controls. Furthermore, S. epidermidis clearance of smaller inocula from the parietal peritoneum was signficantly delayed in renal failure mice compared to controls.

An increased prevalence of serious infections in end stage renal disease patients maintained with hemodialysis has been previously reported (134-136), however the relative importance of a number of clinical variable, including renal failure, remains unknown. In addition to differing underlying diseases, end stage renal disease patients may be maintained by several dialysis modalities and may be exposed to a variety of therapeutic agents. Whether the dialysis procedures themselves (hemodialysis requiring repeated vascular access and extracorporeal circulation, peritoneal dialysis requiring peritoneal access and repeated peritoneal instillation and drainage of dialysis solution) predispose the patient to infection, or whether the removal of uremic toxins by dialysis reduces the propensity toward infection has been the subject of conjecture. Many of these factors may interact to contribute to an increased prevalence of infection in this patient

population. However, the results of the current investigation in the mouse model of renal failure clearly demonstrated that uremia itself, in the absence of other confounding clinical and therapeutic variables, accounted for a signficant increase in the susceptibility to S. epidermidis infection.

Following intraperitoneal inoculation, S. epidermidis was quickly cleared from peritoneal washings of mice, while bacteria remained associated with the parietal peritoneum for a relatively prolonged period in all mice. These findings suggest that bacteria which are free floating in the peritoneal cavity are rapidly eliminated however, those that are associated with the peritoneal membrane may be more resistant to host defense mechanisms and thereby survive for a prolonged period of time.

Increased susceptibility of chronic renal failure mice to 10^6 cfu S. epidermidis intraperitoneal inoculation was associated with a markedly attentuated early local inflammatory response which was primarily characterized by diminished peritoneal polymorph counts. Previously reported studies have demonstrated that circulating leucocyte response of chronic renal failure rats was impaired during the first twenty four hours after intraperitoneal or intravenous Escherichia coli inoculation compared to controls (156). In addition, leucocyte emigration into subcutaneous implanted sponges of chronic renal failure rats was significantly impaired during the first six hours after implantation compared to sham-operated controls (155). In the current mouse model of renal failure, peritoneal polymorphonuclear leucocyte response to intraperitoneal thioglycolate injection was also diminished compared to

controls. An impairment of the early systemic and/or local leucocyte response to infection in the chronic renal failure host may significantly compromise host defense mechanisms, especially during the initial stages of bacterial colonization.

iii. The role of the peritoneal catheter implant in the progression of peritoneal infection in mice

Investigation of the influence of a peritoneal catheter implant on the response of mice to intraperitoneal inoculation demonstrated that bacterial infectivity was not apparently increased in the presence of the implant, however, following intracatheter challenge the catheter offered a surface for prolonged bacterial colonization. The outcome of intraperitoneal S. epidermidis challenge was unaffected by the peritoneal implant, regardless of the level of renal function of mice, and results compared favorably with those of previous experiments conducted in the absence of a catheter implant. The results of experimental intracatheter inoculation demonstrated that the peritoneal catheter was a preferred site for S. epidermidis persistence for as long as one month after bacterial challenge. Scanning electron microscopy of recovered catheters revealed progressive stages of biofilm formation on the catheter surface. Although S. epidermidis persisted at the catheter site, persistent colonization of other peritoneal structures did not occur.

One month after intracatheter inoculation of 10^8 cfu S. epidermidis, mice did not present evidence of peritoneal inflammatory response in spite of persistent bacterial

colonization of the peritoneal catheter. The growth of microorganisms within a biofilm has been proposed as a defensive mode of colonization which provides protection from host defense mechanisms and which elicits minimal host inflammatory response (213). Periodically, "swarmer" bacteria are released from the biofilm population. In this free floating state, bacteria elicit host immune response and are more efficiently eliminated. Recently, scanning electron microscopy studies have revealed bacterial biofilms on peritoneal catheters recovered from CAPD patients with and without clinical symptoms of peritonitis (168, 169). These clinical observations, and the results of the current investigation in the mouse model reveal the occult nature of catheter associated infections.

In addition to providing a surface for bacterial colonization, the influence of the peritoneal catheter implant, as a foreign body, must also be considered in the development and progression of peritoneal infection. Immunosuppression in the vicinity of a foreign body is well known and has been demonstrated clinically and in animal models of foreign body infection (52, 159-161). Foreign body infections are characterized by their slow evolution, site specific infection and persistence without removal of the foreign implant. Several of these features are also common to episodes of CAPD peritonitis and have been further demonstrated by the persistent and site specific S. epidermidis colonization of the catheter implant in the current mouse model.

The results of studies addressing the influence of chronic renal failure on the response of mice to intracatheter

peritoneal S. epidermidis challenge present a seeming paradox. In contrast to impaired early clearance of a small inoculum from the catheter site, the later stages of clearance of a larger inoculum were accelerated in renal failure mice. These findings may be explained by a number of hypotheses including a immunomodulating influence of the inoculum size such that the smaller inoculum did not sufficiently stimulate host defense mechanisms of renal failure mice while the larger inoculum elicited a significant response. Alternately, these findings may reflect a renal failure related impairment in the early stages of host response while later stages of host defense were efficient. Furthermore, these observations may reflect a complex interactive relationship between the renal failure host and the test microorganism. The immunosuppressive influence of chronic renal failure may be measurable only during the early phase of this infectious process. Thereafter, the growth conditions imposed by the uremic environment of the host may not be conducive to persistent catheter colonization. If slime production is an important determinant in catheter colonization by S. epidermidis, then this latter hypothesis may be supported by possible modulation of slime production by varying growth conditions (78, 213). These hypotheses may not be mutually exclusive and aspects of each may be operative in the infectious processes investigated.

Ongoing host bacterial interaction was evidenced by the emergence of S. epidermidis phenotypic colonial variants in specimens recovered from mice at least two weeks after intracatheter inoculation. Isolates recovered 1 week after the experimental challenge presented the parent phenotype only.

Phenotypic colonial variants recovered from mice 2 to 4 weeks after inoculation were slime producing and their antibiogram and biochemical properties did not differ from the parent phenotype (Appendix 3). Phenotypic colonial variants of staphylococci, representing encapsulated and nonencapsulated variants, have been previously described (64, 77, 78), and it has been proposed that animal passage may induce the elaboration of capsular material. In the current investigation the experimental S. epidermidis was a slime producing strain. Whether the variant isolates presented differing colony morphology as a result of varying degrees of slime production remains to be investigated.

It is noteworthy that following 10^8 cfu intracatheter inoculation, renal failure mice presented increased mortality in spite of accelerated bacterial clearance during the later stages of the experiment. These findings are currently without explanation however, interpretation of these results must be tempered by the fact that animals that succumbed to the inoculum were not assessed microbiologically at the time of death.

The interpretation of the relevance of the role of the peritoneal catheter implant in experimental peritoneal S. epidermidis infection of mice to the clinical practice of CAPD should be tempered by the limitations of the animal model. Unlike CAPD patients, renal failure mice were not dialyzed and, therefore, the potential influence of nondialyzed metabolic wastes and uremic toxins on host defense mechanisms and on bacterial growth conditions within the chronic renal failure mouse must be considered. Also in contrast to CAPD patients,

the catheter segment of mice was invariably surrounded by granulation tissue. This poorly vascularized fibroblast sheath may have impeded the direct communication of the catheter lumen with the peritoneal cavity however, following bacterial challenge there was a rapid and significant influx of polymorphonuclear leucocytes into the catheter lumen. Furthermore, since the catheter implant of mice did not have an open exit site to the skin, the kinetics of catheter colonization following intracatheter inoculation may differ from that of a functional peritoneal dialysis catheter. For example, the bacterial colonization of a CAPD catheter may include a number of different bacterial species and strains whose growth may be influenced by bacterial interference. The current mouse model, subjected to inoculation with a single S. epidermidis species, does not reproduce these conditions. The results of the current experiments, nevertheless, demonstrated that the catheter implant provided a preferred site for protracted S. epidermidis growth; a finding which may be relevant to the pathogenesis of the persistent and recurrent peritoneal infections that have been observed in CAPD patients.

iv. The influence of peritoneal exposure to dialysis solutions on response of mice to S. epidermidis challenge

The in vitro cytotoxicity of commercially available, hypertonic, acidic peritoneal dialysis solutions towards resident and elicited peritoneal leucocyte populations of mice was demonstrated in the current investigaion. Adjustment of the pH of the dialysis solutions to physiological levels

reduced subsequent cytotoxicity, but a significant residual loss of viability persisted which was independant of the osmolarity of the incubating solution. The residual cytotoxicity may be attributed to several constituents of commercial dialysis solutions such as platicizers, leached out of the plastic bags containing the dialysis solution, or glucose degredation products generated by heat sterilizaiton.

Previously, in vitro assessments have demonstrated the deleterious effects of fresh peritoneal dialysis solutions on leucocyte viability and function (173-175). Since peritoneal leucocytes, have an integral role in peritoneal defense against bacterial infection, the relationship between the in vitro deleterious influence of peritoneal dialysis solutions on host leucocytes and the clinical conditions of peritoneal dialysis is of obvious concern. Numerous characteristics of the peritoneal environment differ from the constant prevailing conditions of in vitro incubation. Following instillation into the peritoneal cavity, the pH and osmolarity of dialysis solutions achieved physiological levels after 1 hour, and 4 to 6 hours, respectively (22). Furthermore, soluble constituents present within the peritoneal cavity may protect host leucocytes from damage by inflowing dialysis solutions. Nevertheless, in vitro assessments have demonstrated that even after 2 hours of peritoneal dwell, effluent dialysis solutions from CAPD patients inhibited the phagocytic function of peritoneal polymorphs and macrophages (174).

In the current studies, in spite of the observed in vitro cytotoxicity of peritoneal dialysis solutions toward peritoneal leucocytes, repeated peritoneal instillation of dialysis

solution did not affect the outcome of intracatheter peritoneal S. epidermidis inoculation in mice. These findings may indicate that in vivo conditions interfere with the deleterious effects of the peritoneal dialysis solutions such that, if host leucocytes are damaged, the resulting deficit is not sufficient to impair host response to the bacterial challenge. Alternately, if the catheter associated bacterial biofilm protects the colonizing bacteria from host defense mechanisms, an impairment of leucocyte viability and function by inflowing dialysis solutions may not be measurable in terms of increased bacterial recovery. In this way the detrimental influence of dialysis solutions on host defense of the peritoneum could be masked.

Scanning electron microscopy of parietal peritoneum of mice revealed extensive morphological alteration as a consequence of repeated peritoneal instillation of dialysis solutions. In contrast to specimens from mice subjected to intracatheter S. epidermidis inoculation without infusion, those mice that underwent inoculation and peritoneal infusion presented evidence of peritoneal membrane degeneration which ranged in severity from attenuation of mesothelial microvilli to areas of complete denudation. Similar observations have been reported in scanning electron microscopy assessments of specimens of parietal peritoneum of CAPD patients as well as those of rats subjected to peritoneal instillation of peritoneal dialysis solutions (214, 215).

The animal preparation utilized for this series of experiments provided a system in which to assess the impact of repeated peritoneal instillation of commercially available

dialysis solutions on response of mice to intracatheter S. epidermidis inoculation. Peritoneal drainage of dialysis solutions and, in effect, dialysis was not conducted in the mouse model. Therefore, the impact of the actual practice of peritoneal dialysis, on host susceptibility to infection was not addressed in this investigation. CAPD imposes a continual dilution and loss of soluble and cellular immunoreactive substances from the peritoneum. Consequences of this dilution and loss may be reflected in the poor opsonizing capacity of effluent dialysate collected from CAPD patients (130, 131). Reduced opsonization of staphylococci would impair phagocytic uptake and may, therefore, be an important factor in the development of peritonitis. Furthermore, the outflow of spent dialysate, would regularly expose the catheter associated bacterial population to a potentially rich nutrient source which could contribute to the successful colonization of the peritoneal catheter. Even though the experimental animal model was not one of CAPD, the results of this investigation have demonstrated that repeated exposure of the peritoneal cavity to hypertonic, acidic dialysis solutions did not measurably affect the outcome of intracatheter S. epidermidis challenge. Nevertheless, in vitro assessments revealed a cytotoxic effect of the dialysis solutions towards peritoneal leucocytes and scanning electron microscopy of parietal peritoneum of inoculated infused mice demonstrated degenerative morphological alterations as a consequence of repeated peritoneal exposure to dialysis solutions.

Future Directions

The preceding investigations utilized a well characterized mouse model to address the relative roles of chronic renal failure and the defined CAPD associated treatment variables in the progression of peritoneal S. epidermidis infection. In considering the results of these studies and the characteristics of the developed animal models, a number of future directions for investigation are warranted.

i. Immunosuppression of chronic uremia

Previous attempts to demonstrate increased susceptibility to bacterial challenge in an animal model of chronic renal failure have been unsuccessful and therefore, the subsequent investigation of immunosuppressive mechanisms in chronic uremia has been precluded. In the current mouse model, increased susceptibility to S. epidermidis inoculation, in terms of survival and delayed bacterial clearance was demonstrated in renal failure animals compared to controls. Clearly, one aspect of future investigations would be to address the underlying mechanism of the observed immunosuppression. Before turning to in vitro assessments for the examination of discrete components of the immune system, additional in vivo experimentation may generate valuable new information.

The staphylococci are extracellular pathogens whose elimination from the host is effected by leucocyte phagocytosis. The use of other experimental organisms, whose elimination from the host requires alternate aspects of host defense mechanisms would be useful to further characterize the nature of the currently reported renal failure related

immunosuppression. Thus, an extensive study of the response of chronic renal failure and sham-operated mice to experimental challenge with an obligate intracellular pathogen, such as Listeria monocytogenes would more particularly address cell-mediated immune function in chronic uremia. The contribution of defined aspects of the immune response to the observed immunosuppression may also be investigated by utilizing inbred strains of immunodeficient mice (eg. T cell deficient mice) for the mouse model of renal failure.

Renal failure mice demonstrated an attenuated peritoneal leucocyte response to S. epidermidis challenge compared to control animals. Following intraperitoneal challenge, the peritoneal leucocyte count promptly decreased in all animals, however the depression was greatest in renal failure mice. The deficit was accounted for by a marked attenuation of peritoneal polymorph response and macrophages were also diminished. The specific nature of peritoneal leucocytes, in terms of their identity and function, should be further studied, over time following peritoneal inoculation, and compared in renal failure and control animals.

Another approach to the future investigation of immune response in chronic renal failure which may merit consideration is the use of immunomodulating agents as investigational tools. For example, an increased level of suppressor cell activity has been reported in chronic renal failure subjects (141-152). Investigation of the role of increased suppressor cell activity in the deficient response of renal failure mice to bacterial challenge could be

conducted by pretreating animals with cyclophosphamide to reduce suppressor cell activity.

Following intraperitoneal inoculation S. epidermidis was rapidly eliminated from peritoneal washings while it remained associated with the parietal peritoneum of a majority of animals up to 24 hours after inoculation. In spite of aggressive peritoneal washing, bacteria remained firmly associated with the peritoneal membrane. In order to better understand the nature of this association between S. epidermidis and the parietal peritoneum, future studies of peritoneal histology, including scanning electron microscopy, would be useful.

ii. The peritoneal catheter implant and associated bacterial biofilms

The mouse model of chronic renal failure having a peritoneal catheter implant is the only infection free animal model of this kind. The controlled in vivo development of the S. epidermidis biofilm will facilitate future assessments of therapeutic agents to be used for its elimination as well as those intended to prevent its development. Scanning electron microscopy of catheters removed from CAPD patients has demonstrated thick bacterial biofilms composed of multiple cell layers. It is believed that antimicrobial therapy is effective against the surface bacterial layers only, leaving the underlying population intact. Prolonged antibiotic therapy has been proposed however, whether antimicrobial treatment can eliminate an established CAPD catheter associated biofilm is currently unknown. Thus, this mouse model provides a valuable

tool for the assessment of different antimicrobial agents and varying treatment schedules that may be used to eliminate the catheter associated biofilm. Furthermore, this model may be used to evaluate S. epidermidis colonization and biofilm formation on the surface of catheters made of new, alternate materials. The role of catheter construction in terms of catheter size and shape may also be addressed.

The influence of an established catheter associated bacterial biofilm on host defense of the peritoneal cavity is another interesting area for future investigation. The response of renal failure and control mice, bearing peritoneal catheter implants with or without an established bacterial biofilm, to peritoneal inoculation would be compared. Experimental bacteria may be the homologuous catheter associate strain or other strains and species. In vitro assessments of immune function of leucocytes harvested from respective "biofilm" and "biofilm free" animals could also be conducted.

The current studies have demonstrated a role of the peritoneal catheter implant in the progression of the infectious process of S. epidermidis. Future investigations addressing foreign body infection in chronic renal failure mice might utilize other bacterial species presenting a variety of possible virulence determinants. The kinetics of bacterial colonization of the catheter implant with mixed bacterial species may also be addressed using this animal preparation.

iii. Slime production by S. epidermidis

Slime production by S. epidermidis may be an important virulence determinant of this microorganism. Future

investigations should include studies to compare the virulence of slime producing and non slime producing S. epidermidis strains. Renal failure and control mice, with and without peritoneal catheter implants, would be utilized in this assessment. These experiments would address the role of slime production in the progression of S. epidermidis infection as well as the contribution of this factor to the observed increased susceptibility of renal failure mice to S. epidermidis challenge. Future experiments should also address factors that may influence the elaboration of staphylococcal slime. For example, slime production by S. epidermidis recovered from (a) renal failure and sham-operated mice, (b) at varying times after inoculation, and (c) from varying isolation sites, should be compared. Furthermore, the ability of in vivo conditions to induce slime production in non slime producing strains of S. epidermidis should also be studied in renal failure and sham-operated mice.

The emergence of phenotypic colonial variants of the experimental S. epidermidis from catheter specimens recovered from mice at least two weeks after intracatheter S. epidermidis inoculation was representative of the ongoing interaction between S. epidermidis and the host. Future investigations should include studies to characterize the recovered variants particularly in terms of possible virulence determinants. In vitro assessment of slime production and in vivo determination of virulence in sham-operated and renal failure mice should be conducted for each of the recovered phenotypic colonial variants.

iv. The impact of peritoneal dialysis solutions on peritoneal defense mechanisms

The results of the current studies failed to demonstrate a measurable impairment of the response of mice, subjected to repeated peritoneal instillation of dialysis solutions, to S. epidermidis inoculation. Future investigations should include similar studies with a variety of test microorganisms and particularly a non slime producing strain of S. epidermidis. Therefore, the possibility that slime production masked the detrimental influence of the infusion procedure on response of mice to the bacterial challenge in the current studies would be addressed.

The investigation of the role of repeated peritoneal instillation of dialysis solutions on the response of mice to peritoneal S. epidermidis challenge utilized unmodified commercial peritoneal dialysis solutions. During the clinical practice of CAPD a number of therapeutic agents may be added to the inflowing dialysate (eg. heparin, insulin, streptokinase, desferrioxamine). The influence of such constituents of peritoneal dialysis solution on immune response may be addressed using the current mouse model.

Chapter 8

Statement of Originality

1. An animal model of peritoneal Staphylococcus epidermidis infection was developed in which the immunosuppressive effect of chronic renal failure was demonstrated. Increased susceptibility to S. epidermidis challenge in renal failure mice was associated with an impaired early peritoneal polymorphonuclear leucocyte response. Peritoneal polymorph response to intraperitoneal thioglycolate injection was similarly attenuated in renal failure mice compared to controls. The immunosuppressive influence of chronic uremia on response to bacterial challenge has never before been demonstrated in an intact animal model. Furthermore, attenuation of local inflammatory response may represent an important aspect of the underlying immunosuppressive mechanism of chronic renal failure.

2. An animal model of renal failure bearing a peritoneal catheter implant was developed which allowed repeated intracatheter peritoneal access without concomitant bacterial contamination of the catheter site.

3. The peritoneal catheter implant of renal failure and control mice presented a surface for persistent S. epidermidis colonization without overt signs and symptoms of infection. Scanning electron microscopy of catheters recovered from mice following intracatheter S. epidermidis inoculation revealed the progressive development of a bacterial biofilm.

The potential role of the peritoneal catheter in persistent or recurrent CAPD peritonitis has never before been systematically demonstrated in an intact animal model under controlled experimental conditions.

4. The emergence of phenotypic colonial variants of S. epidermidis from the peritoneal catheter of inoculated renal failure animals has not been previously reported. Although the significance of the phenotypic variant isolates is currently unknown, their emergence may represent the expression of characteristics which are advantageous for survival within the host.

5. The in vitro cytotoxicity of commercially available peritoneal dialysis solutions toward resident and elicited peritoneal leucocytes of mice was documented. The relative contributions of hypertonicity and acidic pH of the dialysis solutions to the observed cytotoxicity were delineated.

6. Repeated peritoneal instillation of dialysis solution did not measurably influence the susceptibility of mice to peritoneal S. epidermidis challenge. This is the first reported study which has extensively addressed the role of repeated peritoneal instillation of commercial peritoneal dialysis solution on the response of the intact renal failure host to peritoneal bacterial challenge. In this series of experiments the mouse model of chronic renal failure was successfully extended to include the peritoneal

catheter implant and repeated peritoneal infusion of dialysis solutions. The model thereby provided an infection free, thriving animal system in which to assess the influence of chronic renal failure and repeated peritoneal exposure to peritoneal dialysis solutions on response of mice to intracatheter peritoneal S. epidermidis inoculation.

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Appendix 1

Peritonitis in continuous ambulatory peritoneal dialysis: Retrospective analysis using computerized patient data files

Report prepared for Abbott Laboratories Limited, Canada

Submitted March 12, 1985.

Introduction

The confrontation between bacteria and the uremic host is of great interest to both the clinical nephrologist and the investigator, however, it has reached a degree of complexity unimagined by an earlier generation. The bacteria is a basic affront to the host defense mechanisms, but whether postulated defense mechanisms are effective in the eradication of bacteria in man or animals remains an unresolved topic of considerable interest. Effective or not, host defense mechanisms are clearly stimulated by bacteria. The essential biology of host defense against diverse exogenous microbial agents has evolved in higher organisms more specialized cells and the complex mediation pathways of the immune system, the reticuloendothelial system, the complement system, the basic inflammatory response, and the coagulation system are all integral components. For so long considered as different clinical specialties and investigative disciplines, a growing body of evidence indicates that these seemingly independent systems interact with one another and that all may be invoked to some degree upon introduction of a wide array of appropriate stimuli to the host.

Four general mechanisms exist by which bacteria may proliferate in the patient on continuous ambulatory peritoneal dialysis. The first mechanism is related to uremia itself and its purported immunosuppressive state (1,2). Although immunological deficits may be relatively severe in end-stage renal disease patients, we have reported that chronic experimental uremia is associated with only slight alterations of immune responses (3). This has been observed as well by the

group of Miller in New Zealand (4,5) and would suggest that the pathogenesis of the immunosuppression observed in patients may be attributed to factors other than uremia. Secondly, malnutrition which is often pronounced in CAPD patients as a result of nutrient losses in the dialysis effluent is associated with various immunological derangements affecting cell-mediated immunity particularly (6,7). The third proposed mechanism is that immunological impairment results from the local intraperitoneal effects of peritoneal dialysis. Immune reactants involved in local host defense mechanisms such as opsonizing antibodies and macrophages are literally washed out of the peritoneal cavity at the end of each dialysate dwell period. Finally the presence of the peritoneal catheter may further impair local immune responses (8,9).

One of the most common problems in continuous ambulatory peritoneal dialysis (CAPD) is peritonitis (10-12). This complication tends to limit the usefulness of CAPD and for this reason we have considered the study of this problem important. In June 1983, our unit acquired a computer whose software (CDS 2+, Clinical Computing Ltd) was developed for the care of end-stage renal disease patients (13-15). This report constitutes the first part of an analysis on the subject of peritonitis in our patient population carried out with this computer system.

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Demographic Data

The information compiled in this report refers to patients who were maintained on CAPD as of June 1983 and those who have entered the program since June 1983 to December 1984. This population was chosen because the computer arrived in our dialysis unit in June 1983 and we began to use it by entering the retrospective data from the hospital charts of the current patients. The endpoint of December 1984 was chosen arbitrarily in order to prepare this report. Those patients who were maintained on CAPD and terminated their CAPD practice for whatever reason prior to June 1983 are not as yet completely entered in the computer data set.

This population as defined above consisted of 29 patients at the beginning of the study in June 1983 and of 35 patients at the end of the study in December 1984. During these 18 months a number of patients left the population (9) and a number (6) entered. Of these 35 patients at the end, 14 were men and 21 were women. The mean (±SD) age of these men was 56.4±16.3 years and that of the women was 59.1±12.9 years. This study represents 1026 total patient months of CAPD experience. Six diabetics have entered the program five of which developed chronic renal failure secondary to diabetes.

Of the 35 patients in the program as of the time frame above 9 have left the program to date for the following reasons:

- 3 patients died while on CAPD, one from failure to thrive and two from peritonitis, (one with septic shock, one fungal in nature).
- 4 patients received transplants. Of these four patients one received an unsuccessful transplant and following

subsequent complications was maintained on hemodialysis until his death.

- 2 patients changed modality of dialysis to become permanently maintained on hemodialysis. Their medical profile suggested a failure to thrive on CAPD prior to transfer to hemodialysis. One of them later died of a cardiovascular event.

List of Tables

Comments on the Tables

Table 1

The primary renal disease of patients in the MGH - Abbott study group is compared to the total Canadian experience. The 1984 Canadian experience has not yet been published and so could not be included in this Table. We have included the 1982 data as well as that of 1983 because it represents the entire Canadian experience published to date and because it shows the consistency of the data from one year to the next. This comment also applies to Tables 4 and 7.

The high figure for the group "chronic renal failure, etiology unknown" is possibly the result of the fact that at the MGH we are conservative in our indications to perform kidney biopsies. This results in a larger proportion of our patients not having a kidney biopsy and therefore labelled "etiology unknown" in instances where the etiology is not obtained from other tests.

Chronic glomerulonephritis is generally considered as the major cause of end-stage renal disease in North America. Our results as well as the Canadian experience differ in that respect. Even when assuming that the category "etiology unknown" is made up entirely of patients suffering from chronic glomerulonephritis, this disease entity is still responsible for less than 40% of the cases.

It is noteworthy that a sizeable number of patients, 11.4% in our population, with polycystic kidney disease who usually suffer from extensive abdominal distention can be successfully treated with CAPD.

Table 2

This Table depicts clinical features of the 35 patients. It emphasizes the relatively advanced age of the study group for a mean age of 58.1±14.2, ranging from 28 to 86 years old. This is in keeping with the experience that older patients who will not otherwise adapt to hemodialysis will do well on CAPD. This is often due to their compromised cardiovascular status which cannot tolerate the pronounced hemodynamic changes taking place during hemodialysis. In addition the Table shows the 3:2 female sex predominance.

The Table demonstrates that CAPD was the mode of treatment elected from the start, if we exclude an initial period of hemodialysis during which the definite form of renal replacement therapy is chosen and training for CAPD is done. Only 7 patients had spent more than 3 months on hemodialysis before starting on CAPD. Conversely, 3 patients maintained on hemodialysis for over a year switched to CAPD. Finally, a CAPD population is not static as evidenced by the fact that 9 patients changed status during our 18 months observation period, 6 of them to move to another therapeutic modality.

Table 3

This Table attempts to differentiate between patients who remained in the program and those who left. A first point to make is that the CAPD population is a fluctuating one. A second point to make is that peritonitis can be serious condition; indeed 2 of the patients who died had peritonitis,

one with septic shock, the other a fungal peritonitis. A third point to make concerns the patients who left to be transplanted because a suitable cadaver kidney was found. 3 of those patients were much younger than the others.

Table 4.

In this Table, since we have only 9 patients in our sample as compared to more than 100 for the Canadian experience, the figures given may lack statistical significance. In addition the high percentage in our group under the heading "inadequate dialysis" may reflect the difficulty in characterizing the concept of adequacy of dialysis. Note also that 2 patients died of peritonitis which signifies that peritonitis may be a much more serious condition than is usually assumed.

Table 5

Table 5 presents clinical data as it relates to peritonitis episodes for each of the patients under study. The patients had been on CAPD for close to 2½ years for a mean duration of 29.3±18.3 months. The number of peritonitis episodes varied greatly, although the moderate offenders between 5-10 episodes were not infrequent (10 patients) and the severe offenders of 10 and more episodes were rare (2 patients). In accordance with reported experience a third of patients (12 or 35 patients had 5 episodes or more) are responsible for more than 2/3 of the peritonitis episodes (85 out of 123, for 69.1%). The time on dialysis bore no relationship to the total number of episodes except for the

extreme offenders. There was no difference in the number of episodes between the beginning on CAPD and the end of the study, thereby suggesting that technical experience in doing CAPD is not a factor in our unit. There was no definite pattern in the incidence of peritonitis; 1) high incidence throughout; 2) low incidence throughout; 3) decreasing incidence; 4) increasing incidence; and 5) fluctuating incidence.

Tables 6 and 7

A general comment on Tables 6 through 9: the definition of peritonitis has changed over the years and is still changing. To help explain Table 6, consider the column for 1983. During that year there were a total of 44 episodes of peritonitis in 29 patients (who were in our study group at that time) representing 312 patient months. The peritonitis rates were therefore of 0.14 episodes per patient month and of 1.69 per patient year. The breakdown of peritonitis episodes was the following: 8 patients had no episodes, 8 patients had one episode, 6 patients had 2 episodes, 5 patients had 3 episodes, one patient had 4 episodes and one patient had five episodes, again for a grand total of 44 episodes in 29 patients.

We have no explanation for the bad figures for the year 1983 for the MGH-Abbott study group in terms of incidence of peritonitis. Note however that unlike other centers we continue CAPD in patients having more than 3 episodes of peritonitis and this undoubtedly makes the statistics look worse. In 1983 for instance 9 or the 44 episodes of peritonitis occurred in just 2 patients.

Tables 8 and 9

The breakdown by year of causative organisms of CAPD peritonitis episodes is presented in Tables 8 and 9. A majority of episodes were caused by single organisms, the predominant pathogen being Staphylococcus epidermidis. In some cases the peritoneal effluent of patients otherwise presenting symptoms of peritonitis was culture negative and were reported as no bacteria identified. We have no explanation for the large proportion of such instances in 1984. A small proportion of peritonitis episodes were caused by multiple microorganisms and Staphylococcus epidermidis was again identified in a majority of such cases.

Table 10

Serum albumin prior to entry into the program was normal (i.e. equal or over 3.4 g/dl) in 16 patients and was abnormal (under 3.4 g/dl) in 17 patients. No information is available for two patients who were transferred to the MGH after being maintained on CAPD at another center. No correlation could be found between the level of serum albumin and the peritonitis rate. As explained in the legend this Table compares values of several parameters including albumin at several times during the study. Note that the first recorded values for each parameter were obtained three months after entry into the program rather than at entry in order to collect all data when the patients were in a stable state.

Tables 11 and 12

The CAPD systems in use by patients during the study period are reported in Tables 11 and 12.

Conclusion

This study has 3 main conclusions:

1) that peritonitis in CAPD patients remains a frequent, serious (in some cases even fatal) condition which sometimes makes necessary a change of therapy. Our patients had between one and 2 episodes of peritonitis per year. While the majority of patients have few episodes and are doing well on this form of therapy, a minority, 2 of our 35 patients, had such serious episodes with failure to thrive that it became necessary to change their form of therapy and 3 patients actually died during (2) or after (1) severe episodes. The seriousness of this condition and the fact that it can be fatal has not been previously stressed in the literature.

2) that the main organism responsible for those episodes is Staphylococcus epidermidis. An organism was recovered in more than 80% of the cases, allowing positive identification of this cause. This rate of organism recovery covers not only the period of the study but also the previous history of the patients in the study, which goes in some cases as far as 1978. Furthermore, despite improvements in sterile technique and tubing connections, Staphylococcus epidermidis originating from the patient's skin remains the prevailing infective organism.

3) that the rate of infection of our study population has not changed significantly since the beginning of our CAPD program in 1978 (whereas the rate of infection for our entire patient population has decreased significantly during the same period). During this entire period, a minority of patients were responsible for a majority of the episodes: this is partly

because here we keep patients in our program longer than other centres do. Although it might be expected that the rate of infections might decrease with increased centre experience with this form of treatment, this has not happened in our study population and in fact 1983 was one of our worst years.

We must be careful to treat the results of this study with some caution. The study is not a controlled experiment but a retrospective analysis with its known drawbacks in particular data which can now be seen to be useful was not recorded. In addition, the criteria for diagnosing peritonitis have changed over the years. Furthermore, the study examined only the data current and past of the patients included in an 18 month period. The second part of this study which will be prospective in nature should be more useful.

Nevertheless this study showed the value of a computer for an in depth analysis of this sort. It also gave us the opportunity to characterize our patient population in terms of peritonitis and has helped determine the best direction of future research in this field. In particular it indicates that a clinical study of this kind does not reveal why CAPD patients are prone to develop peritonitis with Staphylococcus epidermidis and therefore that the most fruitful direction for future research is the development of an animal model. Such a model of acute Staphylococcus epidermidis peritonitis in the chronically uremic mouse is presently under development in our unit.

Table 1. Primary renal disease of patients.

Comparison between Montreal General Hospital and Canadian experience.

	MGH-Abbott study group		Canada - New patients			
			1982*		1983**	
	No. of patients	%	No. of patients	%	No. of patients	%
Total	35	100	1231	100	1313	100
Glomerulonephritis	8	22.9	327	26.6	347	26.4
Chronic renal failure, etiology unknown	6	17.1	142	11.5	132	10.1
Pyelonephritis+	7	17.1	121	9.8	151	11.5
Diabetic nephropathy	5	14.3	174	14.1	222	16.9
Renal vascular disease	4	11.4	168	13.6	151	11.5
Polycystic kidney disease	4	11.4	88	7.1	94	7.2
Scleroderma	1	2.9	4	0.3	6	0.5
Other diseases	0	0	207	14.9	210	16.0

* Canadian Renal Failure Register, 1982, p59, Table 23 and p60, Table 24.

** Canadian Renal Failure Register, 1983, p53, Table 16 and p54, Table 17.

\+ Includes obstructive uropathy with infection.

Table 2. Clinical data on patients in MGH-Abbott study group.

Patient number	Age	Sex	Date of first CAPD (mo/day/yr)	Duration dialysis treatment (mo)	Duration CAPD (mo)	Current status
1-CA	67	M	10/04/82	20	17	died
2-GA	86	M	01/04/83	24	24	CAPD
3-SB	39	F	09/26/79	48	48	transplant
4-WC	67	M	04/19/80	57	56	CAPD
5-DC	66	M	08/19/82	29	28	CAPD
6-LC	54	F	03/12/84	10	9	CAPD
7-LC	52	F	06/21/82	31	30	CAPD
8-WC	34	M	05/17/82	31	31	CAPD
9-GC	68	F	03/24/82	33	32	transpl/HD/died
10-SC	66	F	02/05/81	46	46	CAPD
11-BC	66	F	04/03/78	72	67	changed/HD/died
12-LC	76	F	05/26/83	30	19	CAPD
13-BD	28	F	05/02/83	7	5	transplant
14-AG	60	M	09/21/82	28	28	CAPD
15-BG	53	M	04/26/82	46	32	CAPD
16-TH	56	F	02/12/80	59	56	CAPD
17-DL	28	M	01/26/81	161	48	CAPD
18-FM	69	F	08/11/80	54	52	CAPD
19-VM	36	M	01/09/84*	33+	12#	CAPD
20-RM	55	F	11/12/84	1	1	CAPD
21-MM	73	F	04/07/83	24	17	changed/HD
22-DM	54	M	02/05/83	23	22	CAPD
23-PM	62	F	05/10/82	31	31	CAPD
24-TN	70	F	01/16/81	39	36	died
25-MP	32	F	07/16/81	42	41	CAPD
26-CP	72	M	02/26/79	70	70	CAPD
27-HR	70	F	06/11/84	6	6	CAPD
28-DR	62	M	03/26/82	33	33	CAPD
29-ER	61	F	06/01/84	6	6	CAPD
30-MR	65	F	10/16/84	2	2	CAPD
31-BR	67	F	01/13/83	24	24	CAPD
32-JS	63	M	06/21/82	19	19	died
33-AS	53	F	02/07/81	48	46	CAPD
34-SW	42	M	08/01/82*	45+	16#	transplant
35-YY	60	F	08/08/83	17	16	CAPD

* date of transfer to MGH
\+ total duration of dialysis (prior to and after transfer to MGH)
\# CAPD duration after transfer to MGH

Table 3. Comparison of CAPD patients who have discontinued treatment with those who have not

Patients who discontinued CAPD due to	Age	Sex	Duration dialysis treatment (mo)	Duration CAPD treatment (mo)	Initial albumin (g/dl)	Date 1st peritonitis episode (mo/day/yr)	No. days on CAPD prior to 1st peritonitis episode
Death							
1 CA	67	M	20	17	2.6	11/18/82	44
24 TN	70	F	39	36	3.7	09/03/82	532
32 JS	63	M	19	19	4.0	NA#	NA
Transplant							
3 SB	39	F	48	48	4.2	02/18/80	172
9 GG	68	F	33	32	3.5	05/03/83	404
13 BD	28	F	7	5	3.2	NA	NA
34 SW	42	M	45	41*	ND+	ND	ND
Change to hemodialysis							
11 BC	66	F	72	67	3.8	04/28/78	25
21 MM	73	F	24	17	3.1	10/29/83	202

Patients who remain on CAPD (n=26)	Age	Sex	Duration dialysis treatment (mo)	Duration CAPD treatment (mo)	Initial albumin (n=25)	No. days on CAPD prior to 1st peritonitis episode (n=19)
Mean	58.3	15F, 11M	36.2	29.6	3.6	242.4
SD	13.7		31.2	18.3	0.6	230.0

* 16 months at MGH
\# not applicable
\+ not determined

Table 4. Reasons for discontinuation of CAPD.
Comparison between MGH and Canadian experience.

	MGH-Abbott study group		Canada 1982*		Canada 1983**	
	No. of patients	%	No. of patients	%	No. of patients	%
Total	9	100	147	100	320	100
Reasons						
Transplanted	4	44.4	58	39.5	111	34.6
Inadequate dialysis	2	22.2	18#	12.2	14	4.4
Other	1+	11.1	39	26.5	69	21.6
Peritonitis	2+	22.2	20	13.6	84	26.2
Unable to cope	0	0	-	-	22	6.9
Other abdominal complications	0	0	12	8.2	20	6.3

* Canadian Renal Failure Register, 1982, p69, Table 30 and p70, Figure 21

** Canadian Renal Failure Register, 1983, p58, Table 20 and Figure 20.

\+ Three patients who died (failure to thrive, peritonitis with septic shock, fungal peritonitis).

\# Includes indeterminate numbers of patients unable to cope.

Table 5. Peritonitis during CAPD: Clinical data

Patient No & Initial	Sex	Age	Status (mo/yr)	Duration CAPD Months	Date CAPD Began	Date of 1st Peritonitis (mo/day/yr)	Peritonitis Episodes Total	Episodes Per Patient Month	Episodes During 1st 6 months	Episodes During Last 6 months	Episodes per patient month by year						
											1978	1979	1980	1981	1982	1983	1984
1-CA	M	67	Dead(5/84)	17	10/04/82	11/18/82	4	0.24	2	1	-	-	-	-	0.50	0.20	0.20
2-GA	M	86	CAPD	24	01/04/83	11/24/84	1	0.04	0	1	-	-	-	-	-	0	0.08
3-SB	F	39	Trans(9/83)	48	09/26/79	02/18/80	5	0.10	1	0	-	0	0.17	0.17	0.08	0	NA
4-WC	M	67	CAPD	56	04/19/80	05/20/80	7	0.13	2	1	-	-	0.25	0.17	0.17	0	0.08
5-DC	M	66	CAPD	28	08/19/82	12/10/82	4	0.14	1	1	-	-	-	-	0.25	0.08	0.17
6-LC	F	54	CAPD	9	03/12/84	NA+	0	0	0	NA	-	-	-	-	-	-	0
7-LC	F	52	CAPD	30	06/21/82	04/06/83	5	0.17	0	1	-	-	-	-	0	0.17	0.25
8-WC	M	34	CAPD	31	05/17/82	05/31/82	5	0.16	2	2	-	-	-	-	0.29	0.08	0.08
9-GC	F	68	HD(11/84)	32	03/24/82	05/03/83	5	0.32	0	0	-	-	-	-	0	0.25	0.18
10-SC	F	66	CAPD	46	02/05/81	05/10/82	6	0.13	0	1	0.38	0.33	0.17	0	0.17	0.17	0.17
11-BC	F	66	Dead(2/84)	67	04/03/78	04/28/78	17	0.25	2	2	-	-	-	0.33	0.08	0.22	0.50
12-LC	F	76	CAPD	19	05/26/83	NA	0	0	0	0	-	-	-	-	-	0	0
13-BD	F	28	Trans(11/83)	5	05/02/83	NA	0	0	0	NA	-	-	-	-	-	0	NA
14-AG	M	60	CAPD	28	09/21/82	08/05/83	3	0.11	0	0	-	-	-	-	0	0.17	0.08
15-BG	M	53	CAPD	32	04/26/82	03/08/83	3	0.09	0	0	-	-	-	-	0	0.25	0
16-TH	F	56	CAPD	56	02/12/80	02/20/80	10	0.18	1	0	-	-	0.33	0.20	0.08	0.30	0
17-DL	M	28	CAPD	48	01/26/81	07/01/82	3	0.06	0	1	-	-	-	0	0.08	0.08	0.08
18-FM	F	69	CAPD	52	08/11/80	11/21/80	7	0.13	2	0	-	-	0.25	0.25	0.17	0.08	0
19-VM	M	36	CAPD	12	01/09/84*	02/24/84#	2	0.17	NA	1	-	-	-	-	-	NA	0.17
20-RM	F	55	CAPD	1	11/12/84	NA	0	0	NA	NA	-	-	-	-	-	-	0
21-MM	F	73	HD(9/84)	17	04/07/83	10/29/83	2	0.12	1	1	-	-	-	-	-	0.13	0.11
22-DM	M	54	CAPD	22	02/05/83	06/07/83	7	0.32	2	3	-	-	-	-	-	0.40	0.25
23-PM	F	62	CAPD	31	05/10/82	06/10/82	3	0.10	1	0	-	-	-	-	0.14	0.08	0.08
24-TN	F	70	Dead(3/84)	36	01/16/81	09/30/82	3	0.08	0	1	-	-	-	0	0.08	0.17	0
25-MP	F	32	CAPD	41	07/16/81	09/16/81	6	0.15	1	0	-	0	0.08	0.20	0.08	0.25	0.08
26-CP	M	72	CAPD	70	02/26/79	01/30/80	1	0.01	0	0	-	-	-	0	0	0	0
27-HR	F	70	CAPD	6	06/11/84	NA	0	0	0	NA	-	-	-	-	-	-	0
28-DR	M	62	CAPD	33	03/26/82	06/10/82	4	0.12	1	0	-	-	-	-	0.33	0.08	0
29-FR	F	61	CAPD	6	06/01/84	08/09/84	1	0.17	1	NA	-	-	-	-	-	-	0.17
30-HR	F	65	CAPD	2	10/16/84	NA	0	0	NA	NA	-	-	-	-	-	-	0
31-BR	F	67	CAPD	24	01/13/83	08/08/83	1	0.04	0	0	-	-	-	-	-	0.08	0
32-JS	M	63	Dead(1/84)	19	06/21/82	NA	0	0	0	0	-	-	-	-	0	0	0
33-AS	F	53	CAPD	46	02/07/81	03/01/83	5	0.11	0	0	-	-	-	0	0	0.42	0
34-SW	M	42	Trans(3/84)	16	08/01/82*	02/20/83#	3	0.19	NA+	2	-	-	-	-	-	0.25	NA
35-YY	F	60	CAPD	16	08/08/83	NA	0	0	0	0	-	-	-	-	-	0	0
Mean				29.3				0.11	0.65	0.66	NA	NA	0.21	0.12	0.11	0.13	0.09
SD				18.3				0.1	0.80	0.80			0.09	0.12	0.13	0.12	0.11

+ Not applicable because 1st 6 months experience was at another center.
* Date of tranfer to MGH.
# Date of 1st peritonitis episode at MGH after transfer from another hospital.

Table 6. Peritonitis rates (1978-1984)*

	Total	1978	1979	1980	1981	1982	1983	1984
Episodes	123	3	4	12	14	20	44	26
Patient Mos.	1026	8	25	60	116	198	312	307
Episodes per patient month Mean±SD	0.12±0.1	NA+	NA	0.20±0.09	0.12±0.12	0.10±0.13	0.14±0.12	0.08±0.11
Episodes per patient year	1.44	NA	NA	2.4	1.45	1.21	1.69	1.02
No patients	35	1	3	6	11	22	29	32
No patients								
0 episodes	8		2		5	8	8	15
1 "	4		1	2	1	9	8	10
2 "	2			3	3	4	6	5
3 "	6	1	1		1	1	5	2
4 "	3			1	1		1	
5 "	5						1	
6 "	2							
7 "	3							
10 "	1							
17 "	1							

* Based on experience of patients practicing CAPD or entering the CAPD program between June 1983 and December 1984.
\+ Not applicable.

Table 7. Episodes of peritonitis.
Comparison between MGH and Canadian experience.

	MGH-Abbott study group				Canada			
	1983		1984		1982*		1983**	
	No. of patients	%	No. of patients	%	No. of patients	%	No. of patients	%
Total	29	100	32	100	803++	100	1124	100
No. of episodes								
0	8	27.6	15	46.9	419	52.2	532	47.3
1	8	27.6	10	31.3	207	25.8	289	25.7
2	6	20.7	4	12.5	84	10.5	133	11.8
3	5	17.2	3	9.4	93+	11.6	87	7.7
4	1	3.4	0	0	-	-	39	3.4
5	1	3.4	0	0	-	-	44‡	3.9

* Canadian Renal Failure Register, 1982, p65, Table 29 and p70, Figure 20.
** Canadian Renal Failure Register, 1983, p57, Table 19 and Figure 19.
++ Documentation on peritonitis episodes only available on 803 patients out of 816.
\+ Three peritonitis episodes and more.
‡ More than four peritonitis episodes.

Table 8. Microbiological distribution of peritonitis episodes (absolute numbers)

Year	Total number by year							
	Total	1978	1979	1980	1981	1982	1983	1984
Episodes	123	3	4	12	14	20	44	26
1 Single organism	86	0	4	11	13	16	25	17
Staphylococcus epidermidis	51	0	4	7	6	11	12	11
Staphylococcus aureus	8	0	0	1	2	1	3	1
Coliform(s)	12	0	0	1	2	2	4	3
Enterococcus	5	0	0	1	0	0	4	0
Diphtheroids	1	0	0	0	0	0	1	0
Non hemolytic streptococci	1	0	0	1	0	0	0	0
Hemolytic streptococci	2	0	0	0	2	0	0	0
Streptococcus viridans	5	0	0	0	1	1	1	2
Bacillus subtilis	1	0	0	0	0	1	0	0
2 More than one organism	16	1	0	1	0	1	11	2
S. epidermidis & coliforms	3	1	0	0	0	0	2	0
S. epidermidis & Streptococcus viridans	2	0	0	0	0	0	2	0
S. epidermidis anaerobic cocci	1	0	0	1	0	0	0	0
S. epidermidis & diphtheroids	3	0	0	0	0	0	3	0
S. epidermidis, aureus & Streptococcus viridans	1	0	0	0	0	0	1	0
S. aureus & hemolytic streptococci	1	0	0	0	0	0	0	1
S. aureus, Citrobacter & Bacteriodes	1	0	0	0	0	0	1	0
Coliforms & Streptococcus viridans	1	0	0	0	0	0	0	1
Acinetobacter & Propionibacterium	1	0	0	0	0	0	1	0
Gram negative & positive	1	0	0	0	0	0	1	0
Gram negative, positive, diphtheroids	1	0	0	0	0	1	0	0
3 No bacteria identified	21	2	0	0	1	3	8	7

Table 9. Microbiological distribution of peritonitis episodes (%)

	1978	1979	1980	1981	1982	1983	1984	Overall
Total episodes	3	4	12	14	20	44	26	123
Percentage caused by								
Single organism	0	100	91.7	92.9	80.0	56.8	65.4	69.9
Multiple organism	33.3	0	8.3	0	5.0	25.0	7.7	13.0
No bacteria identified	66.6	0	0	7.1	15.0	18.2	26.9	17.1
Percentage single organism Peritonitis caused by								
Staphylococcus epidermidis	NA*	100	63.6	46.2	68.8	48.0	64.7	59.3
Staphylococcus aureus	NA	0	9.1	15.4	6.3	12.0	5.9	9.3
Coliforms	NA	0	9.1	15.4	12.5	16.0	17.6	13.6
Enterococci	NA	0	9.1	0	0	16.0	0	5.8
Diphtheroids	NA	0	0	0	0	4.0	0	1.2
Nonhemolytic streptococci	NA	0	9.1	0	0	0	0	1.2
Hemolytic streptococci	NA	0	0	15.4	0	0	0	2.3
Streptococci viridans	NA	0	0	7.7	6.3	4.0	11.8	5.8
Bacillus subtilis	NA	0	0	0	6.3	0	0	1.2
Percentage multiple organism Peritonitis caused by								
Staphylococcus epidermidis,other	100	NA	100	NA	0	72.7	0	62.5
Other multiple organisms	0	NA	0	NA	100	18.2	100	37.5

* Not applicable.

Table 10. Biochemical data at beginning of CAPD and at most recent assessment

	Start Date (mo/day/yr)	Glu (mg/dl)	BUN (mg/dl)	Creat (mg/dl)	Alb (g/dl)	Chol (mg/dl)	End Date (mo/day/yr)	Glu (mg/dl)	BUN (mg/dl)	Creat (mg/dl)	Alb (g/dl)	Chol (mg/dl)
1-CA	10/04/82	97.00*	55.66	9.46	3.46	258.00	05/02/84	103.33**	70.76	9.53	3.83	285.50
2-GA	01/04/83	277.60	44.00	9.60	3.20	235.30	12/10/84	139.33	55.00	10.93	3.16	211.66
3-SB	09/26/79	84.33	63.00	10.83	4.03	290.33	09/20/83	95.33	47.00	11.50	3.90	316.66
4-WC	04/19/80	105.66	69.66	14.63	3.50	171.00	12/10/84	94.33	61.33	13.63	3.40	157.33
5-DC	08/19/82	108.33	85.66	8.26	3.73	159.66	12/10/84	91.33	69.66	9.10	3.73	152.33
6-LC	03/12/84	117.66	75.33	10.90	3.93	318.66	12/10/84	119.66	75.00	13.56	4.13	320.00
7-LC	06/21/82	136.66	69.66	7.76	3.10	355.33	12/10/84	148.00	53.66	11.43	3.20	248.33
8-WC	05/17/82	56.00	58.66	8.66	3.33	208.33	12/10/84	190.00	69.66	12.00	3.83	210.00
9-GC	02/24/82	109.33	72.66	9.16	3.33	125.00	11/16/84	132.66	61.00	12.20	3.12	108.66
10-SC	02/05/81	141.66	26.66	6.50	3.36	255.00	12/10/84	233.30	28.66	8.46	3.70	322.00
11-BC	04/03/78	142.67	46.33	9.86	3.43	321.30	02/20/84	114.66	31.66	7.83	3.20	118.66
12-LC	05/26/83	122.66	47.66	8.86	2.90	286.33	12/10/84	120.33	47.66	8.80	3.43	268.66
13-BD	05/02/83	99.66	65.66	9.50	3.50	63.66	10/19/83					
14-AG	08/31/82	78.66	58.66	10.20	3.26	215.00	12/10/84	405.00	49.00	8.33	3.53	212.66
15-DG	04/26/82	78.66	62.00	10.20	3.26	284.66	10/10/84	86.66	56.66	9.93	3.26	268.00
16-TH	01/10/80	164.00	57.67	7.10	3.70	190.66	12/10/84	169.00	61.00	11.20	3.36	195.33
17-DL	02/26/81	88.00	60.00	13.83	3.35	145.00	12/10/84	124.33	103.66	18.00	3.70	186.33
18-HM	08/11/80	130.66	56.33	6.96	3.40	188.66	12/10/84	128.00	53.33	11.50	3.76	244.66
19-VM	01/09/84	129.33	79.66	15.70	4.20	214.00	12/10/84	119.00	80.66	15.60	3.90	195.33
20-RM	11/12/84						12/10/84					
21-MM	04/07/83	107.33	64.33	8.33	3.23	228.67	09/17/84	128.33	44.00	9.60	3.10	218.00
22-JM	02/05/83	108.66	69.66	12.83	3.50	251.00	12/10/84	89.33	60.66	12.83	4.10	267.33
23-PM	05/10/82	84.66	52.33	8.46	3.50	404.00	12/10/84	100.33	62.00	12.06	4.03	489.00
24-JN	01/16/81	212.00	44.33	4.46	3.06	285.00	03/24/84	223.33	49.00	7.36	2.93	291.33
25-MP	07/16/81	78.00	78.33	10.90	4.10	186.66	12/10/84	94.33	56.33	10.86	3.53	160.00
26-CP	02/26/79	131.33	50.00	9.80	3.33	199.33	12/10/84	124.00	45.00	8.36	2.90	169.00
27-HR	06/11/84	102.66	23.66	6.60	2.20	127.00	12/10/84					
28-DR	03/26/82	342.00	72.00	9.03	2.86	183.33	12/10/84	193.66	70.00	14.83	3.90	192.66
29-ER	06/01/84	105.33	76.66	8.37	2.67	239.00	12/10/84					
30-MR	10/16/84						12/10/84					
31-BR	01/13/83	114.67	52.00	10.50	3.30	239.33	12/10/84	168.33	64.00	13.83	4.07	300.00
32-JS	06/21/82	84.33	63.33	7.60	3.66	218.66	02/07/84	144.66	56.66	9.96	4.00	316.66
33-AS	02/07/81	79.00	79.66	8.13	3.13	172.00	12/10/84	98.00	50.00	10.60	3.33	143.33
34-SW	08/01/82	75.33	98.66	17.90	4.13	257.66	12/20/83	119.00	53.33	13.73	3.16	252.66
35-YY	08/08/83	106.67	55.67	7.30	3.43	269.33	12/10/84	103.33	60.33	9.80	3.80	249.67

* Mean of the first 3 values obtained immediately following the first 3 months on CAPD.
** Mean of the last 3 values obtained immediately prior to the last 3 months on CAPD.

Table 11. CAPD system in use as of December 1984*

CAPD system	No. of patients
Abbott 1	3
Abbott 111	19
Baxter 1	0
Baxter 2	2
O-Z connector	2
Beta Cap	0
Total	26

* 26 patients were active on dialysis as of December 1984.

Table 12. CAPD system in use during the study period*

CAPD system	No. of patients
Only Abbott 1 and 111	19
Only Baxter	3
Baxter to Abbott	11
Abbott to Baxter	2
Total	35

* 35 patients were treated in the program during the specified time window.

Appendix 2

Characterization of a mouse model of chronic renal failure

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Abstract

A mouse model of renal failure, which is induced by the sequential electrocoagulation of the right renal cortex and left nephrectomy, was examined for the capacity to reproduce the characteristics of chronic uremia. Assessment was conducted six weeks after the second surgical procedure in 13 week old female C57BL/6 inbred mice with renal failure and in normal and sham-operated controls. The surgery, which was well tolerated, was free of local and systemic signs of inflammation or infection. Growth was significantly delayed in all animals post surgery however renal failure mice presented the most severe growth retardation. Biochemical analysis of plasma revealed multiple abnormalities with commensurate elevations of urea and creatinine. In addition to the expected hyperphosphatemia, hyperkalemia and acidosis a significant increase in cholesterol was present. Furthermore, in contrast to controls, renal failure mice produced large volumes of urine which contained significant levels of protein. Renal failure mice presented profound hematological changes in the red cell series in which anemia was evident. Changes in plasma biochemistry and in bone histology revealed the presence of severe secondary hyperparathyroidism. It was therefore concluded that the described mouse model of chronic renal failure presented characteristics consistent with those observed clinically in end-stage renal disease.

Introduction

The pathophysiology of experimental chronic renal failure remains to be clearly delineated. Animal studies using

surgical models have received the most attention and most investigators have utilized the rat as the test species. Three basic models have been described in which renal lesions are created surgically with the purpose of inducing chronic renal failure without initiating concomitant infectious or inflammatory processes. The first is the remnant kidney model in which a subtotal nephrectomy is performed (1, 2). The second model involves ligation of renal arteries (3). Finally, papillectomy can be achieved successfully in some defined rat strains (4, 5).

Recently, we have described a model of chronic renal failure (6), in which mice are subjected to electrocoagulation of the surface of one kidney with subsequent contralateral nephrectomy according to a modification of a technique originally applied to rats (7). The present model is of considerable practical as well as theoretical interest. The mouse is particularly well suited for immunological studies since its immunogenetic background and the function of its various immunocompetent cells and immunoglobulin classes have been extensively investigated. Accordingly, we initiated a study to examine the characteristics of this mouse model of chronic renal failure, particularly those related to extrarenal abnormalities secondary to uremia. Most studies were done approximately 6 weeks after the onset of renal failure, a time at which we had previously performed several immunological investigations (8-12). These experiments were conducted in female C57BL/6 inbred mice in which a number of biological features are well established (13-15). The ability of chronic renal failure mice to thrive following nephrectomy was assessed

by survival and growth rate. Routine biochemical and hematological studies were done to evaluate the systemic consequences of uremia and blood pressure determinations were conducted. Structural alterations in bone were also assessed.

Methods

Animals. Experiments were performed in 5 week old female C57BL/6 mice (Canadian Breeding, St. Constant, Qué. and Kingston NY, USA) left to acclimatize for one week in our animal facilities prior to use. All animals were fed a commercially available mouse diet (Ralston Purina Co., St. Louis, Mo, USA) containing approximately 20% protein by weight and provided in pellet form. Drinking water was untreated tap water. Food and water were available at libitum.

Production of renal failure. Renal failure was induced by a two-step procedure involving electrocoagulation of the surface of the surgically exposed right kidney and left nephrectomy. Details of this method have been reported previously (6). Briefly, electrocoagulation of the entire surface of the right kidney except for a 2 mm margin of intact tissue around the hilum was followed by left nephrectomy twelve to fifteen days later. In sham-operated animals, the right kidney was electrocoagulated and the left kidney was temporarily exposed in a similar fashion to that used for nephrectomy but it was not manipulated. All animals were subjected to electrocoagulation of the renal surface, nephrectomy or sham-surgery which were conducted under controlled ether anesthesia through small bilateral flank incisions leaving the intestines and the upper abdominal

contents undisturbed. Renal electrocoagulation was performed using a foot-operated single point cauterizer angled at 30° (Hyfrecator, Model X-712, The Birtcher Corp., Los Angeles, Calif., USA). The kidney was freed from perirenal fat and the adrenal gland prior to electrocoagulation and special care was taken not to manipulate the ureter. After electrocoagulation, the kidney was replaced into the renal fossa and completely covered by the tissues of the abdominal wall and skin. After either surgical procedure, the incisions were closed in layers with clips applied to the skin. The duration of surgery from skin-to-skin never exceeded 10 min. Unless stated otherwise, the animals were studied 6 weeks after the second operation. The degree of renal failure was defined by the blood urea nitrogen (BUN) concentration as measured on sacrifice day.

Body weight. Mice were weighed at the time of induction of renal failure, ear-clipped for future identification and weighed weekly until sacrifice.

Blood pressure measurement: Systemic blood pressure was measured in conscious mice by a tail-cuff method. For two weeks prior to assessment mice underwent daily acclimatization to the holding chambers required for blood pressure determinations. All measurements were made at the same time of day under standardized conditions.

Blood tests. At the time of sacrifice blood was collected by cardiac puncture into plastic syringes coated with a 3:10 dilution of heparin (Hepalean, Harris Laboratories, Toronto, Canada). Blood urea nitrogen concentration was measured by autoanalyzer method either separately (IL9 Autoanalyzer, Instrumentation Laboratory Inc., Lexington, Mass., USA) or as

part of SMAC-16 blood testing (Technicon Instruments Corporation, Montreal, Canada). Routine hematological assessment was done by Coulter Counter (Model 2B1, Coulter Diagnostics Inc., Hialeah, Fla., USA). In a separate experiment, platelet counts were also performed in parallel by direct phase-contrast microscopy (16). Differential leucocyte counts were performed on the basis of 100 cells per slide on Wright-stained blood smears. In a limited number of mice reticulocyte counts were carried out on fresh and unstained thick blood smears.

Urine biochemistry. Eighteen-hour urine collection was conducted in modified metabolic cages. Urinary protein content was measured with a Technicon RA 1000 Autoanalyzer (Technicon Instruments Corp., Montreal, Canada). Osmolarity was measured on frozen samples with an Advanced Cryomatic Osmometer (Model 3C2; Advanced Instruments Inc., Needham Heights, Mass., USA).

Bone analysis. The bone was fixed in a 0.5% sucrose formalin buffer, then processed as previously described following embedding in glycol methacrylate, staining for acid phosphatase and counter-staining with Harris hematoxylin. 2 undecalcified sections of the proximal tibia were examined.

Statistical analysis. All results are expressed as mean SD and single comparisons were made with Student's t test.

Results

Survival

Except for the occasional death occurring during surgery and ascribed to anesthesia, this model of chronic renal failure (mean BUN values of 100 mg/dl) was associated with a mortality rate

which varied from 4 to 15% in separate experiments according to the degree of electrocoagulation applied to the right renal cortex. Deaths in renal failure mice occurred on the second to fourth day following left nephrectomy. Plasma biochemistry performed at that time in animals where death seemed imminent revealed values of BUN above 250 mg/dl and of potassium above 8 mmol/l. All animals that expired were excluded from analysis. In select experiments where severe renal failure (BUN above 180 mg/dl) was induced by excessive cautery of the right kidney, increased mortality during the days following nephrectomy was observed (Figure 1). Thereafter during the six week follow-up, no significant animal loss occurred. No post surgical fatalities were encountered in sham-operated control animals.

Growth

The effects of renal failure on growth were readily observed and growth curves of the three mice groups are presented in Figure 2 where measurement began after the second surgical procedure. Following the second operation, there was a significant growth impairment in all animals. This impairment was most severe in renal failure mice in which significant weight loss was recorded one week after nephrectomy. Sham-operated animals recovered more quickly from the operation than renal failure mice and both groups had significant growth retardation at sacrifice six weeks later.

General observations

In this study, none of the renal failure mice having survived the first week post nephrectomy demonstrated signs of spontaneous bleeding or gross neurological impairment. Furthermore, the nature and level of activity of renal failure

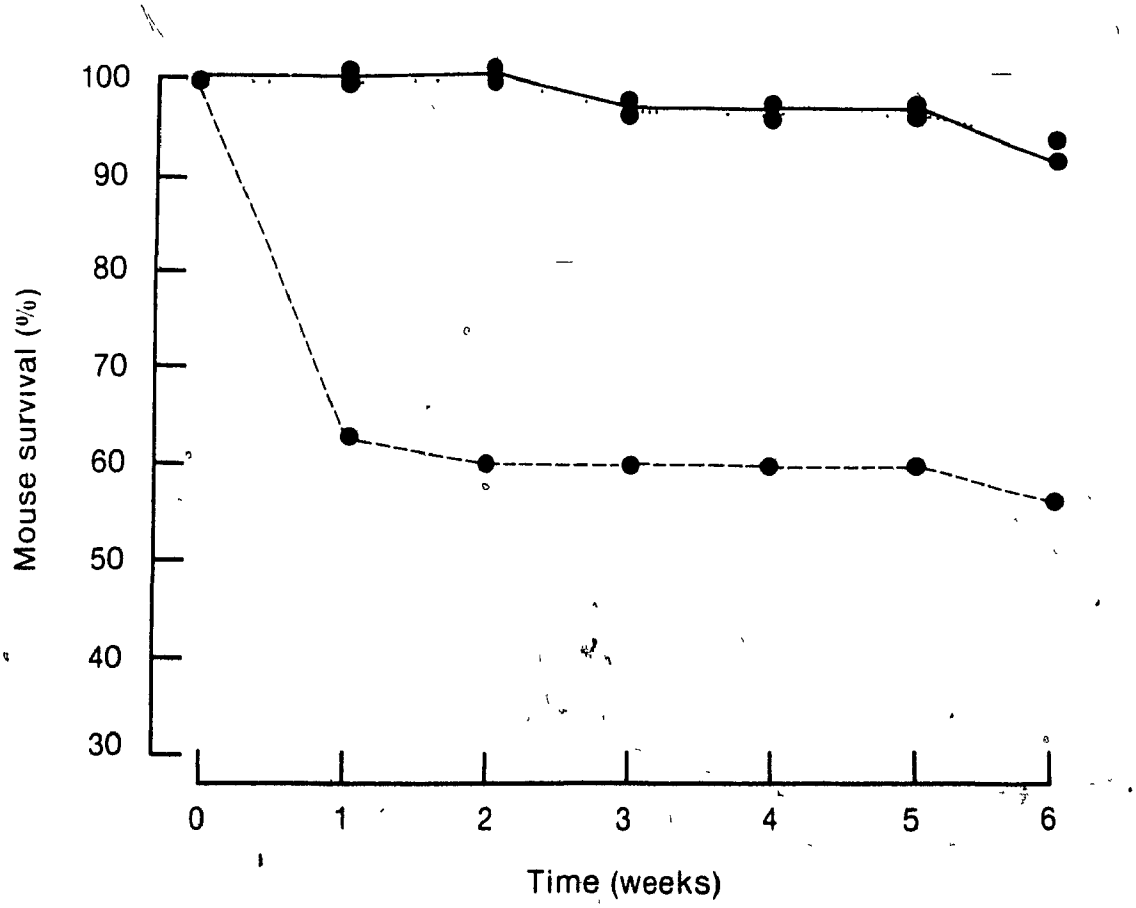

Figure 1. Survival curve of mice during the course of renal failure (dashed line, N=48 at the start) and of sham-operated (dotted line, N=35) and normal (solid line, N=36) controls. Observations made in C57BL/6 female inbred mice during three separate experiments. All deaths past the first week were due to accidental overanesthesia.

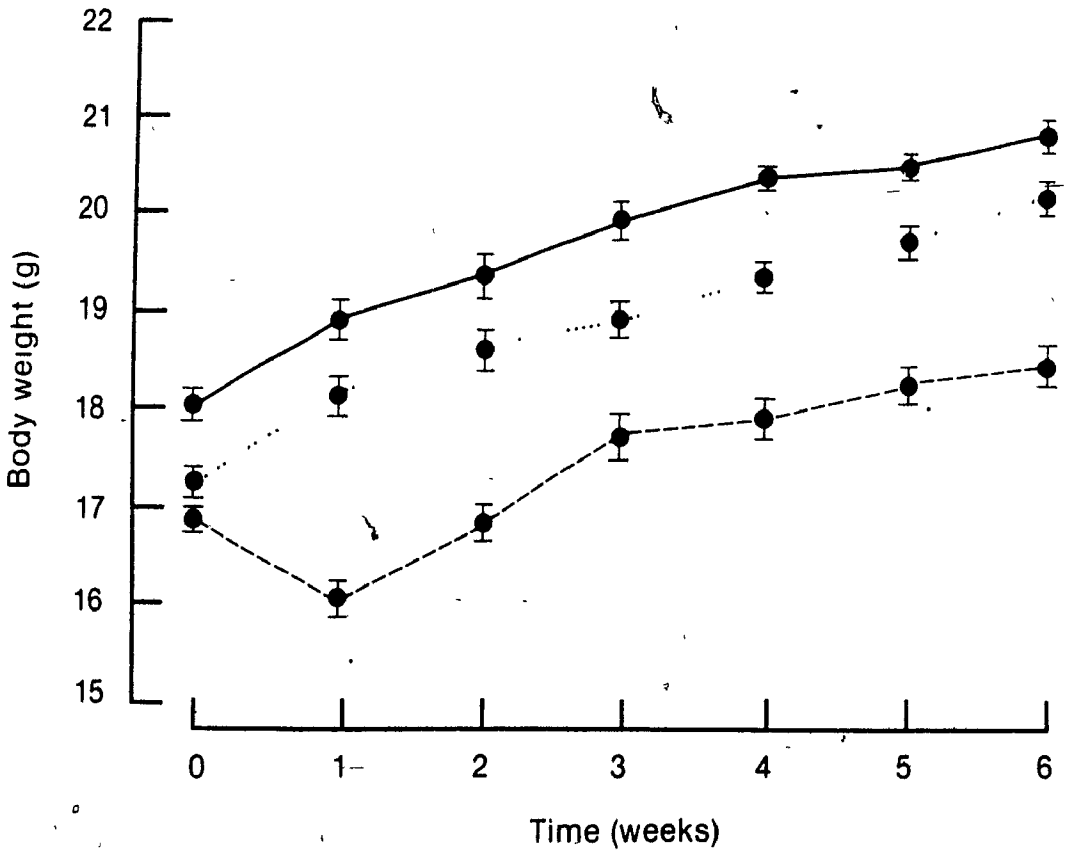

Figure 2. Growth curve in mice surviving during the course of renal failure (dashed line) and in sham-operated (dotted line) and normal (solid line) controls. Points and brackets represent the mean±SD of at least 93 determinations obtained during the same experiments reported in Figure 1.

mice as well as their overall appearance was comparable to control animals.

Local findings

This surgical mode of induction of renal failure was free of local complications. The skin clips were removed one week after each of the two surgical procedures. On both occasions, in sham-operated and renal failure mice alike, the flank wounds were completely healed, and no signs of inflammation were detectable. Examination of the peritoneal cavity six weeks after the second surgery revealed no abnormalities outside of the kidneys. Again, there were no signs of inflammation or infection. The intraperitoneal side of the surgical wounds had healed completely. The general appearance of the electrocoagulated kidneys of renal failure mice was markedly altered. The outer surface was pale, irregular and distended with translucent areas through which urine could be seen. When punctured the kidney rapidly emptied of urine and collapsed to a thin-walled pouch. In contrast, the electrocoagulated kidney of sham-operated animals had markedly atrophied and was generally difficult to locate, appearing as a very small mass of nondescript tissue adhering firmly to the liver. In these animals marked hypertrophy of the contralateral kidney was always present.

Biochemical evaluation

The biochemical data of mice with renal failure and sham-operated and normal controls are shown in Table 1. A number of species differences between mice and man can be appreciated, the most striking involving creatinine, bicarbonate, phosphate, cholesterol, and the enzymes GOT, GGTR and LDH. The expected retention of nitrogenous compounds was

observed in renal failure mice. Commensurate increases of urea and creatinine occurred averaging fivefold and two to threefold, respectively. In sham-operated animals, values of urea and creatinine did not change significantly, indicative of the degree of contralateral hypertrophy that followed the extensive destruction of the right kidney by electrocoagulation. In contrast to control animals, renal failure mice presented moderately, although significantly, elevated mean values of plasma potassium, chloride, calcium and phophate as well as significantly reduced bicarbonate concentrations. Furthermore, renal failure mice always presented biochemical evidence of secondary hyperparathyroidism with hyperphosphatemia and elevated alkaline phosphatase in the absence of hypocalcemia. An unexpected finding in this study was the presence of hypophosphatemia in the sham-operated animals. Interestingly, renal failure mice presented significantly elevated levels of plasma cholesterol compared to controls. The other biochemical parameters tested were similar across the three groups of mice.

Urinary characteristics of specimens collected from mice during an 18 hour collection period are presented in Table 2. Renal failure mice were not oliguric and in fact produced the greatest volumes of urine and sham-operated mice also produced increased urine volumes compared to normal controls. Significant levels of protein were recovered from urine specimens collected from renal failure mice while urine osmolarity was not significantly different between renal failure and control animals.

Hematological assessment

After six weeks of renal failure, there was a marked

Table 1. Plasma concentrations of selected blood constituents in mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

Status of animals	Normal n=32	Sham-operated n=32	Renal failure n=30	Normal range in man
Glucose (mg/dl)	223±31	218±48	198±26	60-115
Urea nitrogen (mg/dl)	19±3	24±5	105±36[c,d]	8-25
Creatinine (mg/dl)	0.4±0.1	0.4±0.1	0.9±0.3[c,d]	0.5-1.5
Sodium (mmol/l)	147±6	140±13	148±6	136-147
Potassium (mmol/l)	4.2±0.7	4.4±0.9	5.5±0.9[c,d]	3.5-5.0
Bicarbonate (mmol/l)	12±2	11±3	9±3[c,d]	21-30
Chloride (mmol/l)	109±4	102±8[e]	119±6[c,d]	97-109
Uric acid (mg/dl)	3.0±0.6	3.6±1.2[e]	2.6±1.2[c,d]	2.5-8.5
Calcium (mg/dl)	9.3±0.7	9.8±0.9[e]	10.6±1.0[c,d]	9.0-10.6
Phosphate (mg/dl)	7.4±1.0	6.6±0.9[e]	8.8±1.6[c,d]	2.4-4.5
Total protein (g/dl)	5.2±0.4	5.1±0.7	5.2±0.3	6.0-8.0
Albumin (g/dl)	3.0±0.3	3.0±0.4	3.0±0.3	3.5-5.2
Cholesterol (mg/dl)	110±16	119±21	188±40[c,d]	110-250
Bilirubin (mg/dl)	0.1±0.1	0.1±0.1	0.1±0.1	0.2-1.2
Alkaline phosphatase (U/l)	112±25	124±20[e]	307±106[c,d]	30-110
GOT (U/l)[b]	221±125	201±85	226±107	8-40
GPT (U/l)[b]	58±51	35±24[e]	30±28[c]	0-40
GGTR (U/l)[b]	3±2	3±3	2±1[c]	7-55
LDH (U/l)[b]	393±198	382±134	366±181	100-216
Globulins (g/dl)	2.2±0.4	2.1±0.6	2.2±0.3	2.3-3.3

[a]Determinations performed at sacrifice in 13 week old female inbred C57BL/6 mice and expressed as mean±SD.

[b]Abbreviations used are: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; GGTR, gamma glutamyl transferase; LDH, lactate dehydrogenase.

[c-d]Indicates a significant difference ($p<0.05$) between renal failure mice and (c) normal mice and (d) sham-operated mice.

[e]Indicates a significant difference ($p<0.05$) between sham-operated and normal mice.

Table 2. Urine biochemistry in mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

Status of animals	Normal n=14	Sham-operated n=14	Renal failure n=11
BUN (mg/dl)	26.1± 5.0	25.3± 3.5	119.3± 21.6[b,c]
Volume (ml)	1.5±0.8	2.2±0.8	3.0± 1.3[b]
Osmolarity (mOsm/kgH_2O)	417.6± 163.9	307.5±86.7	338.5± 107.0
Protein (mg/l)	0.12±0.05	0.13±0.05	0.47± 0.4[b,c]

[a]Eighteen hour urine collection performed the day before sacrifice in 13 week old female inbred C57BL/6 mice; values given are means±SD.

[b]Indicates a significant difference ($p < 0.05$) between renal failure and normal control mice.

[c]Indicates a significant difference ($p < 0.05$) between renal failure and sham-operated mice.

decrease in hemoglobin concentration (Table 3) as well as erythrocyte number and hematocrit (data not shown). A close relationship was observed between the fall in hemoglobin and the severity of the renal failure (Figure 3). Erythrocyte indices were only moderately, although significantly, affected by renal failure where mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were reduced compared to control mice. It is noteworthy that normal values of MCV and MCH in the mouse are approximately half that observed in man. Reticulocyte counts indicative of the extent of bone marrow response to the anemia tended to be reduced in renal failure mice and when corrected for the degree of anemia reticulocytosis was even more indicative of an inadequate bone marrow response.

Examination of the total and differential leucocyte counts and platelet numbers, revealed no differences between renal failure mice and controls (Table 3). Circulating leucocytes in the mouse are predominantly lymphocytes and polymorphs constitute a small minority, approximately 5%, of circulating leucocytes while monocytes are rarely seen (17). Platelet numbers are consistently higher than in man. The standard method to ascertain platelet counts is to use the autoanalyzer counter. However, the counter is set for human blood in which erythrocytes are considerably larger than platelets but in the mouse erythrocytes are small and in fact close to the size of platelets. Therefore the validity of mouse platelet counts based on assessment by the autoanalyzer method was confirmed by conventional counting of platelets employing visual means (Figure 4).

Table 3. Hematological features of mice six weeks after the onset of renal failure and in sham-operated and normal controls[a]

Status of animals	Normal n=68	Sham-operated n=64	Renal failure n=56	Normal range in man
BUN (mg/dl)[b]	25±6	29±6	108±33[d,e]	8-25
Leucocytes/mm^3	5334±2937	6125±2269	5188±3278	4800-10800
Neutrophils (%)	5.3±3.6	6.1±3.6	7.3±5.8[d]	-----
Neutrophils/mm^3	295±328	379±277	341±272	-----
Lymphocytes (%)	94.3±4.0	93.5±3.6	92.3±6.0[d]	-----
Lymphocytes/mm^3	4910±2723	5725±2144	4891±3144	-----
Platelets ($x10^3/mm^3$)	813±222	804±193	807±208	130-400
Hemoglobin (g/dl)	13.4±0.9	13.3±0.8	8.4±1.4[d,e]	14-18
MCV (μm^3)[b]	45.7±1.0	45.4±1.1	44.1±1.0[d,e]	82-100
MCH (pg)[b]	16.4±0.7	16.5±0.7	15.6±0.6[d,e]	27-31
MCHC (g/dl)[b]	35.8±1.4	36.4±1.9	35.3±1.6[e]	32-36
Reticulocytes (%)	2.5±0.9	2.7±0.6	1.8±0.7[e]	-----

[a]Determinations performed at sacrifice in 13 week old female inbred C57BL/6 mice and expressed as mean±SD.

[b]Abbreviations used are: BUN, blood urea nitrogen; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

[c]Reticulocyte counts were performed in only eight animals of each group and are not corrected for the degree of anemia.

[d-e]Indicates a significant difference ($p<0.05$) between renal failure and (d) normal mice and (e) sham-operated mice.

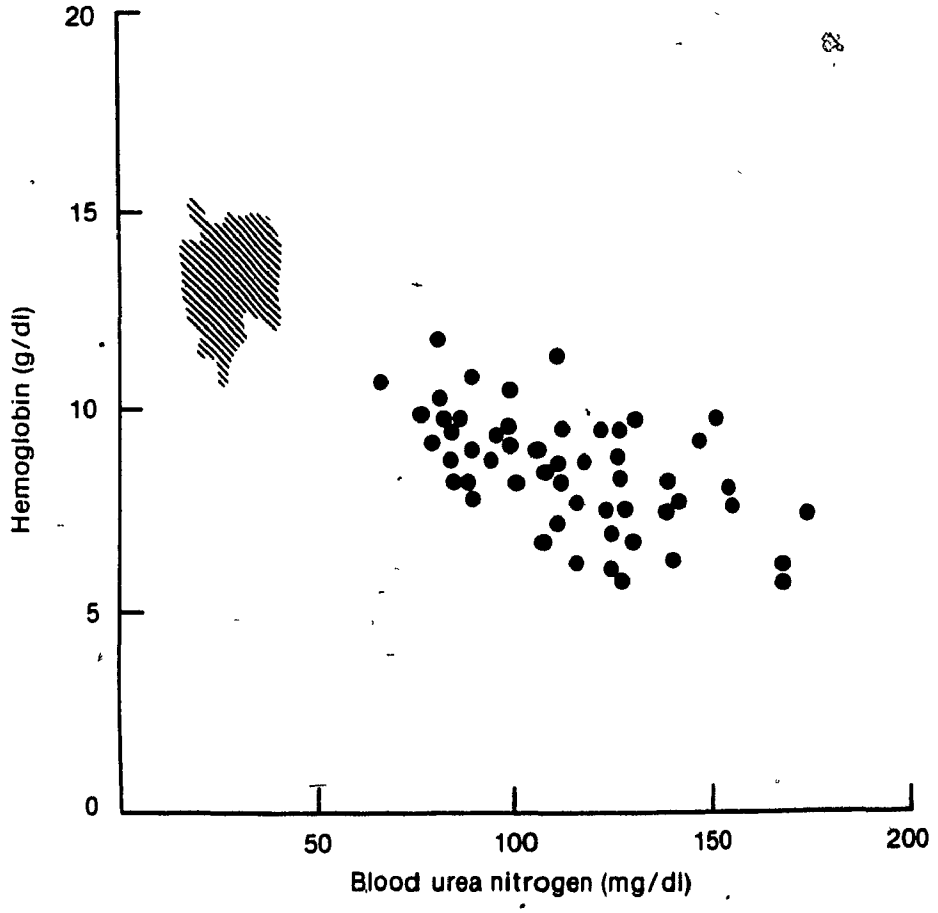

Figure 3. Correlation between concentrations of blood urea nitrogen and hemoglobin ($y = -0.03x + 12.07$, $r = -0.608$, $p<0.01$) of 53 mice 6 weeks after the onset of renal failure. Values for sham-operated and normal controls are contained within the shaded area. Other hematological features of the mice are presented in Table 2.

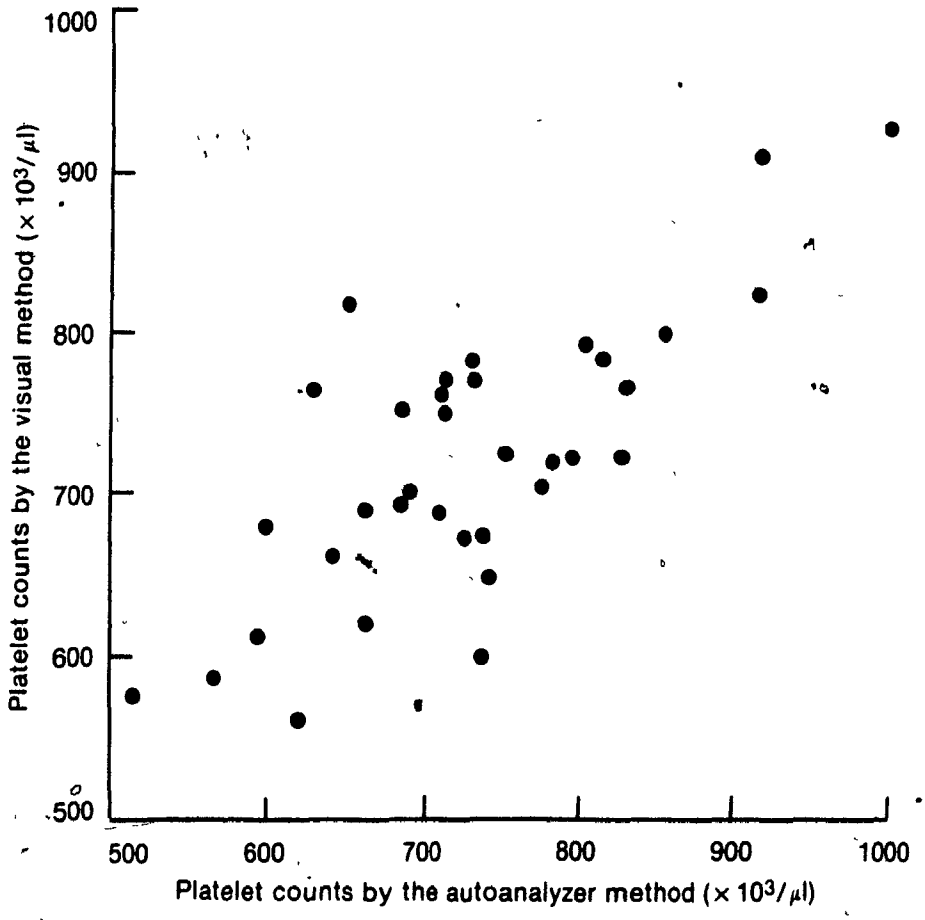

Figure 4. Correlation between circulating platelet numbers of 35 normal 6 week old C57BL/6 female inbred mice as determined by the visual and autoanalyzer methods. ($y = 72x + 90$, $r = 0.852$, $p < 0.01$).

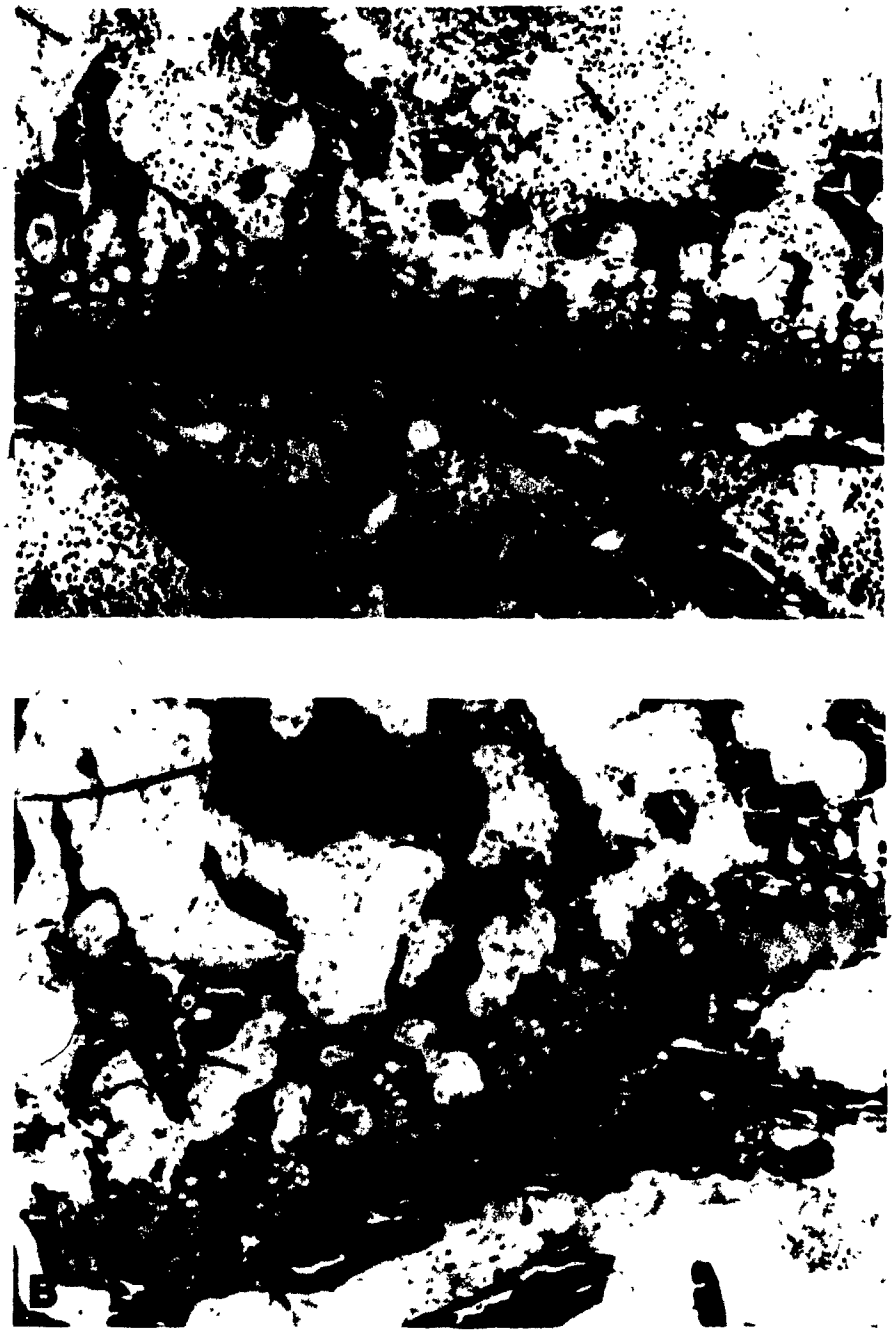

Figure 5. Examples of bone sections (upper metaphyseal plate of tibia) in normal mice (A) and in mice six weeks after the onset of renal failure (B). The prominence of osteoclasts, bone remodeling and marrow fibrosis is visible during renal failure (Magnification x 170) (Courtesy of Dr. Michael Kaye).

Bone studies

Renal osteodystrophy was fully established 6 weeks after the onset of renal failure. Changes in bone histology consistent with severe hyperparathyroidism were observed with an increase in the number of osteoclasts, extensive areas of increased resorption and widespread marrow fibrosis. Differences in bone histology between normal and renal failure mice can be appreciated in Figure 5.

Blood pressure

Systolic blood pressure determinations (tail cuff technique) were conducted in a small number (4 animals in each group) of renal failure mice (112±13 mmHg) (mean±SD) and their sham-operated (94±9) and normal (103±4) controls. No significant differences in systolic blood pressure between the three animal groups were recorded.

Discussion

This report describes abnormalities in mice with chronic renal failure which resemble those observed in humans with end-stage renal disease. Significant growth retardation, severe anemia, major alterations in blood chemistry and striking secondary hyperparathyroidism were observed in female C57BL/6 inbred mice after six weeks of renal failure. The same abnormalities are found in humans and it was concluded that this mouse preparation is a suitable animal model of chronic uremia. The results of this study confirm our earlier findings (8-12) in that a significant reduction in renal function is consistently achieved with the ensuing retention of nitrogenous products and severe consequences of the renal failure readily follow. Previous reports (18-21) support these data,

indicating a substantial increase in blood urea nitrogen for prolonged periods in similar experimental mouse models following controlled injury to both kidneys. In the present model we have previously shown that the severity of the renal failure is greatest if the electrocoagulation is limited to one kidney with a subsequent contralateral nephrectomy (6).

It was our goal to develop a surgical preparation with a consistent and reproducible degree of renal failure which could be readily induced in a large number of mice that could then be maintained under standard conditions of animal husbandry. After an initial animal loss during the first week post nephrectomy due to excessive renal failure in a small proportion of mice, no significant mortality was observed during a six week follow-up period. According to previous observations six weeks after the second surgical procedure, renal failure and sham-operated mice did not present evidence of infection or inflammation consequent to the surgical preparation (11, 22). Peritoneal leucocytes collected from renal failure mice were similar to a resident population and peritoneal structures were sterile as evidenced by microbiological assessment.

This mouse preparation of renal failure was originally developed for the study of immune reponses in chronic experimental uremia. In that context, sham-operated animals were subjected to electrocoagulation of the right kidney at the time of the first surgery and mobilization rather than removal of the contralateral kidney two weeks later. Therefore the potential immunomodulating influence of thermal injury to the kidney as well as the surgical trauma inherent to the model was

accounted for in the sham-operated control animal (23, 24). The present study demonstrated that the level of nitrogenous waste products of sham-operated and normal mice were not significantly different.

Postoperatively, growth of uremic mice was significantly impaired and six weeks after the induction of renal failure mean body weights had decreased to 80% of normal mice. Although growth rates were accelerated 2 and 3 weeks after surgery, renal failure mice were smaller and actually gained less weight than did sham-operated controls. In previous experiments we have shown that sham-operated mice demonstrated catch-up growth whereas renal failure animals continued to present growth retardation up to 15 weeks after the induction of renal failure (25).

The remarkable difference in the appearance of the electrocoagulated kidney between renal failure mice and sham-operated controls might find an explanation in the phenomenon of renal counterbalance (26). It has been known for a long time that the effect of unilateral renal damage on the function of the affected kidney depends on the function of the contralateral kidney. The exact mechanism of renal counterbalance is less clear. Recent studies suggest that this occurs as a result of either increases in vasodilatory substances or decreases in vasoconstrictor compounds. Alternately this response may be mediated by the accumulation of naturally occurring cytoprotective agents. Evidence for the physiological importance of renal counterbalance was brought about by a number of experimental observations, particularly by unilateral ureteral ligation or renal artery clamping in the rat. Instead, the electrocoagulation technique that we used in

our study produces an nonhomogenous renal injury leaving a rim of renal cortex around the hilum intact and the deep juxtamedullary nephrons which may have escaped the thermal injury.

When compared to adult man, we found that differences in normal blood constituents were common in thirteen week old female C57BL/6 inbred mice. These differences included an increase in inorganic phosphate, the enzymes alkaline phosphatase, GOT and LDH, and in the number of platelets and circulating lymphocytes. As well a decrease in creatinine, bicarbonate, cholesterol, bilirubin, the enzyme GGTR, the erythrocyte indices MCV and MCH and in polymorphonuclear counts was demonstrated. Elevations in blood glucose could have resulted from the scheduling of our evaluation in the early morning in mice with nocturnal eating habits. The sodium containing heparin preparation used for the blood collection might explain the observed hypernatremia relative to man.

Normal blood levels of creatinine in mice comparable to that of man have been observed by several groups (13-15). Meyer and colleagues (1985) have demonstrated the overestimation of the picric acid method in measuring serum creatinine levels in mice (27). In studies of normal C57BL/6 female mice, we have observed a similar fourfold difference between two automated methods of determination of plasma creatinine of normal mice, using the SMAC equipment in the present report and the 1L-1 elsewhere (11). One difficulty with the former method is that the volume required by that technique is large for mice. In addition, even the latter method is still insensitive to very low levels of creatinine. For these

reasons the measurement of blood urea nitrogen constituted our routine index of evaluation of renal function in mice.

Biochemistry assessments of renal failure and control mice revealed several interesting manifestations of uremia. Renal failure mice presented evidence of secondary hyperparathyroidism, with elevated plasma levels of inorganic phosphate and alkaline phosphatase. Hypocalcemia, described as a complication of chronic renal failure and attributed to the rise of serum phosphate and vitamin D deficiency was not observed in these chronically uremic mice. Although we did not measure ionized calcium in the present study, it is very likely that increments in total serum calcium in chronically uremic mice reflect a rise in ionized calcium. It is currently not feasible to perform measurement of parathyroid hormone in the mouse and therefore no conclusions regarding such measurements can be drawn from the current data.

In contrast to the evident secondary hyperparathyroidism observed in the chronically uremic mice, investigations of sham-operated mice unexpectedly revealed hypophosphatemia, hypercalcemia and high alkaline phosphatase. These biochemical findings are characteristic of primary hyperparathyroidism yet sham animals had undergone renal electrocoagulation yielding a reduction of renal parenchyma. Therefore the observed hyperparathyroidism must in fact be of a secondary nature. We do not have a satisfactory explanation for the signs of early secondary hyperparathyroidism observed in the sham-operated mice.

Much like in man, the hematological consequences of chronic renal failure in mice affected predominantly the red

cell series (28). Six weeks after the onset of renal failure mice invariably presented severe anemia with evidence of relative bone marrow unresponsiveness. We have demonstrated previously the progressive development of the anemia from the time of onset of the renal failure (25). In view of the prominence of the bone disease the anemia could result in part from excess parathyroid hormone through at least three pathways (29). These include inhibition of erythropoiesis, shortening erythrocytes survival and inducing fibrosis of the bone marrow cavity. Thus the overall effects of the multiple consequences of uremia on hematopoiesis may result in prevailing functional alterations which may also be accompanied by structural changes. The observations made in this study would suggest that bone marrow fibrosis might play an important contributing role in the anemia of severe renal failure.

Our results confirm and extend the observations of others who have developed animal models of experimental uremia through surgical reduction of renal parenchyma and have assessed renal failure induced systemic changes. However, our model is singular in that it provides the means for studying the role of renal failure in modulating immune responses. The availability of immunodeficient and immunomodified strains, and the volume of knowledge on immune cell populations and membrane markers favor the mouse in studies of the potential influence of renal failure on immune function.

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Appendix 3: Characteristics of the experimental Staphylococcus epidermidis, clinical isolate 29260

The S. epidermidis, reference number 29260, that was used for all experimental inoculations was an isolate recovered from the peritoneal fluid of a CAPD patient presenting peritonitis. This isolate was kindly supplied to us by Dr. S.I. Vas of the Toronto Western Hospital.

In addition to assessment by gram stain (positive), catalase (positive) and coagulase (negative) production, strain 29260 also underwent identification by the API Staph-Ident System (Analytab Products, NY, USA) and the Vitek System (McDononnel Douglas Health Systems Company, MO, USA). The results of these assessments are presented in Tables 1 and 2 respectively.

Slime production by 29260 was investigated according to standard methods and was compared to reference slime-producing S. epidermidis strains that were kindly supplied by Dr. G.D. Christensen (University of Tennessee, Tenn, USA). Slime-production by 29260 compared favorably to that of prototypic slime-producing reference S. epidermidis.

Strain 29260 presented a characteristic antibiotic sensitivity profile. A panel of seven of the tested antibiotics were selected for routine positive identification of recovered isolates from inoculated mice. The antibiotic sensitivity characteristics of 29260 are presented in Table 3.

Table 1: API Staph-Ident System: Identification of *Staphylococcus epidermidis* 29260

Microcapule	Test	Positive	Negative
1	Phosphatase	X	
2	Urea utilization	X	
3	β-Glucosidase		X
4	Mannose utilization		X
5	Mannitol utilization		X
6	Trihalose utilization		X
7	Salicin utilization		X
8	β-Glucuronidase		X
9	Arginine utilization		X
10	β-Galactosidase		X

Table 2: Vitek Systems: Identification of Staphylococcus epidermidis 29260

Well No.	Test	Positive	Negative
1	Peptone Base growth	X	
2	Bacitracin resistance	X	
3	Optochin resistance	X	
4	Hemicellulase utilization		X
5	Tolerance to high NaCl concentration	X	
6 and 7	Tolerance to high level of bile	X	
8	Esculen hydrolysis		X
9 and 10	Argenine hydrolysis		X
11	Urease	X	
12	Tetrazolium red reduction	X	
13	Novobiocin resistance		X
14	Dextrose utilization	X	
15	Lactose utilization	X	
16	Mannitol utilization		X
17	Raffinose utilization		X
18	Salicin utilization		X
19	Sorbitol utilization		X
20	Sucrose utilization	X	
21	Trehalose utilization		X
22	Arabinose utilization		X
23	Pyruvate utilization		X
24	Pullulan utilization		X
25	Inulin utilization		X
26	Melibiose utilization		X
27	Melezitose utilization		X
28	Cellobiose utilization		X
29	Ribose utilization		X
30	Xylose utilization		X

Table 3: Antibiotic sensitivity characteristics of *Staphylococcus epidermidis* 29260

Staphylococcus epidermidis 29260 response	Antibiotic tested
Sensitive	Tetracycline, Bactrim Vancomycin, Cefamandole nafate, Cefazolin
Resistant	Erythromycin, Clindamycin, Penicillin, Tobramycin, Methicillin