

FACTORS AFFECTING ADHERENCE OF  
STAPHYLOCOCCUS EPIDERMIDIS IN  
PERITONEAL DIALYSIS SOLUTIONS

CENTRE FOR NEWFOUNDLAND STUDIES

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Factors Affecting Adherence of *Staphylococcus epidermidis*  
in Peritoneal Dialysis Solutions

BY

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A Thesis Submitted to the School of Graduate  
Studies in Partial Fulfillment of the  
Requirements for the Degree of Master of Science

Faculty of Medicine  
Memorial University of Newfoundland  
April 1987

St. John's

Newfoundland

Canada

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ISBN 0-315-37000-9

## Abstract

*Staphylococcus epidermidis* is the most frequent cause of peritonitis complicating continuous ambulatory peritoneal dialysis (CAPD). During CAPD, *S. epidermidis* presumably enters the peritoneal cavity through the surgical incision along the outer surface of the catheter tubing or less frequently through its lumen. The catheter is a foreign body and the peritoneal dialysis solution (PDS) may support bacterial attachment and growth.

Virulence mechanisms of *S. epidermidis* are poorly understood, but adherence properties and the presence of slime on some strains may contribute to this organism's ability to cause peritonitis.

Using an *in vitro* model for adherence, eight strains of *S. epidermidis* (two non-slime and six slime-producers) were tested in fresh and post-dialysis PDS. The effects of various additives (insulin, serum, albumin, and protamine sulfate) and antibiotics (gentamicin, penicillin, and cephalothin) on bacterial adherence to catheters were tested.

The presence of slime production did not enhance adherence to polyvinyl catheters used in this study. Compared to fresh PDS, there was a significant decrease in bacterial adherence in post-dialysis PDS for all strains tested. Insulin (5 units/mL), 5% serum, protamine sulfate (125 and 250 mg/L), gentamicin (0.06 and 0.12 mg/L), penicillin (0.06 and 0.12 mg/L), and cephalothin (0.12 and 0.25 mg/L) decreased adherence of bacteria to the catheter by at least four-fold compared to controls. A 5.0% concentration of albumin decreased adherence three-fold compared to controls. There was a 1.5-fold decrease in adherence with 0.625% serum, a four-fold decrease with 1.25% and 2.5% serum, and a six-fold decrease with 5.0% serum. The 10.0% serum additive produced nearly a ten-fold decrease in bacterial adherence to the catheter, compared to controls. Also, the 5% serum, heated at 60° C for one hour, decreased adherence two-fold. Scanning electron microscopy confirmed these results. Adherence was greatest in fresh PDS with no additive or antibiotic and least in post-dialysis PDS with an additive or an antibiotic.

These studies showed that certain therapeutic additives and antibiotics may inhibit pathogenetic mechanisms of *S. epidermidis*. *In vivo* studies are required to evaluate whether these results seen *in vitro* have relevance in the clinical situation.

## Acknowledgements

During the course of this thesis project, I have had much help and encouragement. I realize that it is too great a task to thank all the people who were involved in the organization and production of this work. However, I especially wish to thank Dr. Ian Bowmer, my supervisor, who has offered me much help and advice. Many thanks for helpful comments also go to Dr. P. Fardy, Dr. C. Michalski, and Dr. P. Parfrey, the other members of my supervisory committee, as well as Dr. D. Bryant, who provided statistical counsel. I also want to thank the many workers in the Clinical Microbiology Laboratories of the General Hospital who helped in various ways, and especially Miss Rosalind Ford for typing this thesis.

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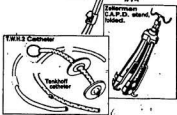
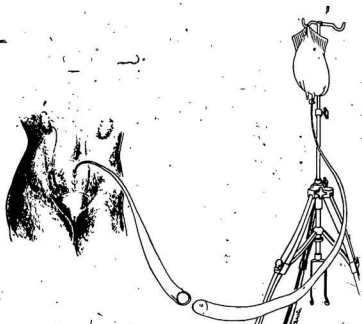
## Chapter 1

### Introduction

A process of continuous ambulatory peritoneal dialysis (CAPD) has proved to be effective in treating patients with renal diseases. CAPD uses the continuous presence of a peritoneal dialysis solution in the peritoneal cavity (Figure 1-1), transport across the peritoneal membrane from patient to dialysate occurring primarily by passive diffusion. The removal of water, and large and small molecular weight substances during peritoneal dialysis results from the addition of hypertonic glucose to the dialysate, creating an osmotic gradient. Periods of drainage and instillation of fresh dialysis solution are performed four or six times daily, using the CAPD catheter. After each drainage and fresh instillation, the patient is disconnected from all tubing, eliminating prolonged interruption from daily activities. Patients can perform dialysis alone. The CAPD bag and tubing are wearable and portable, and most patients appear to have an increased sense of well-being, increased energy and a good appetite.

The major limitation to the usefulness of this technique is the high incidence of infection. The incidence of infection from CAPD is 1.0 infection per patient per year, with a steady decrease from 1976 to 1980 (Gauntner, Feldman and Puschett, 1980). Peritonitis, an inflammation of the peritoneum usually caused by bacteria, is the most life threatening, while catheter exit site infections are the other serious complications of CAPD. The exit site is highly susceptible to infection from microorganisms on the skin. Disconnecting and connecting the coupling between the catheter and the dialysis tubing also cause peritoneal contamination with the patient's own skin organisms. Material defects may also promote infection. Surface irregularities in CAPD catheters have been associated with the presence of colonies in a study by Locci, Peters and Pulverer (1981).

**Figure 1-1: Diagrammatic Representation of the Usual  
CAPD Techniques, Showing the Dialysis Tubing  
Being Introduced into the Abdomen.  
Used with permission from Accurate Surgical  
Instruments Co., Toronto, Ontario.**



Intrinsic irregularities in the catheter appeared to provide convenient sites for organism attachment. Infections have also occurred with CAPD patients in whom the catheter surfaces were found to have breaks. In three cases of peritonitis associated with the development of a defect in the structure of the peritoneal dialysis catheter (Roxe and Santhanam, 1983), bacteria were discovered in these structural defects.

Laboratory studies have identified several species of microorganisms that have been associated with CAPD peritonitis. West *et al.* (1988) found that staphylococci are the most common organisms isolated and they account for the majority of infections (71.4%). These investigators further observed that enterococci and *Bacillus* sp. accounted for 2.6% and 1.3% of infections respectively, and that Gram negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* caused 10.4% of infections. Peritonitis resulting from *Candida* and other fungi caused 2.4% of infections (Vas, 1983; West *et al.*, 1988). Studies in children produced similar results, a study by Watson *et al.* (1986) revealing that 76% of the episodes of peritonitis were due to staphylococci. In the same study, *Streptococcus viridans* caused 18% of infectious episodes, *Escherichia coli* 4%, and  $\beta$ -hemolytic *Streptococcus* was responsible for 2% of these episodes of infection.

*Staphylococcus epidermidis* is the most common bacterial isolate from infected CAPD catheters (Keane and Peterson, 1984; Vas, 1983; Maki, Weige and Sarafin, 1977; Christensen *et al.*, 1982; Moyer, Edwards and Farley, 1983). Rubin *et al.* (1980) demonstrated that 31% of the episodes of peritonitis were due to *S. epidermidis*. Also, 75% of the patients with recurrent infections with the same bacterial species had *S. epidermidis* as the cause. There were 13 episodes of peritonitis during 136 patient weeks in the study by Popovich *et al.* (1978). Approximately 50% of these episodes were due to *S. epidermidis*. Approximately 25% of patients with skin exit-site infections had staphylococci as the predominant organisms (Khanna, 1981).

Over a three year period, an analysis of patients with CAPD catheters by Gauntner, Feldman and Puschett (1980) revealed that several factors contributed

to a reduction in the incidence of peritonitis. A transfer from the intensive care unit to an in-centre peritoneal dialysis wing reduced infection by providing a special care unit for the patients. Strict aseptic techniques by a well trained and supervised dialysis nursing staff were essential. The incidence of peritonitis has also declined with the improvement in dialysate delivery systems, including the utilization of plastic bags rather than glass bottles and improved connecting devices between catheter and extension tube (the titanium adapter). The early discontinuation of CAPD in patients with frequent infections has been helpful in controlling peritonitis. Continuous epidemiological screening for potential microorganisms causing infection helped in further encounters with these organisms. Also, the use of air and water-tight sterile surgical catheter dressings prevented peritonitis. Peritonitis prevention, early recognition, and prompt therapy are major goals for CAPD patients. Understanding the pathophysiology of the infectious process may also help in the prevention.

Peritonitis may occur after the *S. epidermidis* bacteria were introduced into the peritoneal cavity during fluid changes. It is also possible that the peritoneal catheter itself, acting as a foreign body, can provide a site of attachment and growth for the bacteria, introducing them into the peritoneal cavity.

Growth of *Staphylococcus epidermidis* varies depending on the type of peritoneal dialysis fluid to which the bacteria are exposed (Flournoy, Perryman and Qadri, 1983). When the fresh peritoneal dialysis fluid is instilled into the abdomen, there is a rapid equilibration that occurs. Experiments by MacDonald, Watts and Bowmer (1986) have shown that *S. epidermidis* does not grow in fresh dialysate. There was, however, significant promotion of growth in spent dialysate fluids. The main differences in unused and used fluids were: a more acid pH in fresh fluid, pH 5.25 (unused) versus 7.60-8.62 (used); a higher glucose concentration in fresh fluid, glucose 1500 and 2500 mg/mL (fresh) versus 407-1227 mg/mL (spent); and a higher protein concentration in spent fluid (Flournoy, Perryman and Qadri, 1983). None of the bacteria tested grew in unused fluid, but all grew in fluid which had been in the peritoneal cavity for as little as one and one-half hours (Flournoy, Perryman and Qadri, 1983; Verbrugh *et al.*, 1984).



Although the organisms did not grow in unused fluid, they were still viable. It is possible that the different solutions may affect the attachment as well as the colonization of the foreign body or peritoneal membrane.

Some strains of *Staphylococcus epidermidis* produce a mucopolysaccharide material called slime. This is a glycocalyx surrounding the cells (Costerton, Geesey and Cheng, 1978). The production of slime can be detected on the sides of test tubes which have contained cultures of this organism, by staining with a cationic dye, alcian blue (Shea, 1971). It has been postulated that the bacteria stick to any surface by means of this slimy material. Under experimental conditions, the glycocalyx also contributes to the bacteria's ability to resist surfactants, antibodies, phagocytes and certain antibiotics (Costerton, Geesey and Cheng, 1978; Gray *et al.*, 1984). Electron microscopy studies have shown that slime-producing *S. epidermidis* can adhere to and grow on catheter surfaces (Marrie, Noble and Costerton, 1983; Peters and Pulverer, 1984). Regular sampling of catheter specimens by Peters, Locci and Pulverer (1982) demonstrated adherence of the staphylococci to the catheter surface after 5-30 minutes, followed by cell proliferation after one hour, and pitting of the catheter surface after an incubation of bacteria and catheter for 48 hours. These pits, visualized by electron microscopy, were postulated to be associated with the production of slime covering the bacteria. In the study by Sheth *et al.* (1983), it was shown that after a two minute immersion of catheters in bacterial suspensions, some bacteria adhere to catheter surfaces and form colonies. Cultures of the final rinse solution were negative, therefore demonstrating that colonies grown after rolling the catheter on blood agar plates represent adherent bacteria. The number of colonies adherent per unit catheter surface area was greater for *S. epidermidis* than for *S. aureus* or *Escherichia coli*. Of the *S. epidermidis* strains tested, adherence was most marked for slime-producers.

There is much controversy as to whether or not slime production contributes to the adhering ability of these bacteria. In a study by Mayberry-Carson *et al.* (1984), it was proposed that an extensive glycocalyx served a protective function for the bacteria and was important in bacterial adherence.

Another protective function of slime was that it provided an antiphagocytic barrier to the cellular and humoral defense systems, and perhaps a physical barrier to antibiotics. They speculated that this material could influence the survival of the organisms in the peritoneal cavity. Incubations of catheters with *S. epidermidis* strains daily for five days, produced macrocolonies on the surface of the catheter incubated with the slime-producing strain but not with a non-slime-producer (Christensen *et al.*, 1983). Scanning electron micrographs of catheters incubated in broth cultures showed the slime-producers matted onto the catheter surface, whereas the non-slime-producers, grown under identical conditions, did not show this accumulation. In animal models, three times as many infections occur with the slime-producers as occur with non-slime-producers. However, there are also studies in which adherence is not influenced by slime production. Ishak *et al.* (1985), using similar methods to those in this thesis, reported that neither adherence to catheters nor phagocytosis and killing of coagulase-negative staphylococci by polymorphonuclear leukocytes was significantly influenced by slime production. They could not show a significant relationship between catheter adherence and slime production.

The alleged effects of slime suggest that this substance may contribute to the extended course of *S. epidermidis* foreign body infections. If true, and if slime production occurs *in vivo* as it does *in vitro*, new strategies must be developed to prevent infections.

A number of variables affect bacterial growth. The addition of protamine sulfate to dialysate fluids decreased bacterial numbers (Sacchi *et al.*, 1982). They tested the *in vitro* antibacterial activity of protamine sulfate and suggested that it reduced infections in CAPD processes. Heparin also inhibits the antimicrobial effect of the protamine sulfate. Sodium acetate, has been utilized to reduce peritonitis in CAPD patients (Richardson and Borchardt, 1989). Peritoneal dialysis solutions which contained acetate had a greater antibacterial effect than those containing the previously used lactate. Serum factors have also been implicated in reducing CAPD infections. Leijh *et al.* (1979) suggested bacterial killing by complement.

Systemic and locally instilled antibiotics have been used to treat peritonitis in CAPD patients. Gentamicin was studied and used in patients undergoing continuous ambulatory peritoneal dialysis (Hyams *et al.*, 1971; Pancorbo and Comty, 1981). Staphylococcal peritonitis was successfully treated with gentamicin in approximately 60% of the patients. The concentrations of gentamicin in peritoneal effluent are variable and may not always exceed the minimum inhibitory concentration for a particular organism (Mandell, 1985). However, even subinhibitory concentrations of antibiotics may alter an organism's pathogenicity and may affect adherence or initial attachment. One-quarter and one-half the minimum inhibitory concentrations (MIC) of certain antibiotics such as clindamycin, ampicillin, and gentamicin have been shown to inhibit bacterial toxin production within 30 minutes of exposure to these antibiotics (Gemmell, 1985). Clindamycin was discovered to reduce the ability of staphylococci to cause bone invasion *in vitro* after a 48 hour incubation period, using sections of rabbit tibia (Mayberry-Carson *et al.*, 1986). In control preparations, in the absence of clindamycin, *S. aureus* colonized the bone surface and formed extensive glycocalyx-enclosed adherent microcolonies. At sub-bactericidal concentrations of clindamycin, the production of glycocalyx was reduced and there was less colonization of bone surfaces. At higher clindamycin concentrations (1 MIC), no adherence of bacterial cells to bone surface occurred. *Staphylococcus aureus* was grown overnight in the presence of one-third of the MIC of clindamycin in a study by Milatovic, Braveny and Verhoef (1983). Phagocytosis of the antibiotic-treated bacteria by human polymorphonuclear leukocytes was significantly enhanced compared with untreated controls. Inhibitory concentrations (1-30  $\mu\text{g}/\text{mL}$ ) of aminoglycoside antibiotics suppressed the adherence ability of bacteria to human epithelial cells in a study done by Ofek *et al.* (1979). The addition of sub-MICs of penicillin (27  $\mu\text{g}/\text{mL}$ ) resulted in the loss of the cell-adhering ability of *Streptococcus pyogenes* and *Escherichia coli* within a three hour period. Also, there was no adherence detected for bacteria grown with 10  $\mu\text{g}$  of streptomycin/mL. Daschner (1985) showed in his review that penicillins, cephalosporins, erythromycin, and clindamycin enhance killing of antibiotic-

altered bacteria by human polymorphonuclear leukocytes. Kaplan *et al.* (1985) noted that 75% of patients with staphylococcal peritonitis were successfully treated with the antibiotics gentamicin, vancomycin, and tobramycin. Peritonitis, caused by *Staphylococcus aureus*, has been successfully treated with intraperitoneal vancomycin (Nielsen, Sorensen and Hansen, 1979). Part of the effect of these antibiotics may be their ability to affect adherence as well as their efficacy in killing the infecting organisms.

Despite effective therapies, peritonitis still remains a major complication of CAPD. Understanding the pathogenetic mechanisms and their control may reduce both colonization and subsequent disease states in these patients. This thesis investigates the effect of various additives and antibiotics on bacterial adherence to the CAPD catheters in both fresh and spent peritoneal dialysis fluids.

## Chapter 2

# Materials and Methods

This work was performed during the interval of September 1985 to February 1987 at the Health Sciences Centre, St. John's, Newfoundland. During this time period, strains of *Staphylococcus epidermidis* were examined for their ability to adhere to peritoneal dialysis catheters suspended in peritoneal dialysis solutions, with or without additives.

### 2.1. Collection and Maintenance of Strains

Eight strains of *S. epidermidis*, isolated from patients with peritonitis, were obtained from the Microbiology Laboratory of the General Hospital. Each isolate was considered to be a separate strain if it originated from a different patient. Preliminary identification was made on the basis of Gram-stain morphology, negative coagulase test, and positive catalase test (Kloos and Jorgensen, 1985). Final identification was established by means of the STAPH-IDENT system (API Laboratory Products Ltd, St. Laurent, Quebec) which has been shown to be a reliable method for the identification of staphylococcal species (Almeida and Jorgensen, 1983).

All isolates were maintained on Mueller-Hinton II agar [Baltimore Biologicals Laboratory (BBL), Cockeysville, Maryland] and in Mueller-Hinton broth (Oxoid, Basingstoke, Hampshire, England) at 4-8° C. Purity of the cultures was checked periodically by plating on this agar. Test inocula were prepared from overnight cultures in Mueller-Hinton broth (Oxoid, Basingstoke, Hampshire, England).

## 2.2. Test for Slime Production

Some strains of *S. epidermidis* produce a glycocalyx, a polysaccharide, slimy material surrounding the cells. This material may help them adhere to catheter surfaces (Costerton, Geesey and Cheng, 1978). The cationic dye, 1% alcian blue in 3% acetic acid was utilized to test for slime production (Shea, 1971). A few drops of this dye were added to a test tube containing about 1 mL of an overnight *S. epidermidis* culture strain. After 1-2 minutes, the bacteria-dye mixture was poured out and the test tube incubated for 1-2 hours. The tube was then examined for the presence or absence of a blue, slimy film lining the walls of the test tube. Formation of a ring at the liquid-air interface was not considered a positive test (Christensen *et al.*, 1982). Experiments were performed on strains discovered as being slime-producers and non-slime-producers (Table 2-1).

## 2.3. Dialysis Solutions

Two types of peritoneal dialysis solutions were utilized in the experiments. These were unused or fresh peritoneal dialysate (Baxter-Travenol, Toronto) and used or spent peritoneal dialysate which had been in uninfected patients at the Health Sciences Centre, St. John's for 6-8 hours. The main differences noted in unused and used fluids were, respectively, pH 5.25 and 7.60-8.62, glucose 1500 and 2500 mg/mL, and 407-1227 mg/mL, potassium 0 and 2.0-4.2 mEq/L, phosphorous 0 and 2.5-5.5 mg/dL (Popovich *et al.*, 1978). Used PDS was filtered through a 0.22  $\mu$ m millipore filter. The dialysis solutions were dispensed in sterile test tubes and refrigerated until used. For convenience the fresh and spent fluids were predispensed in 9.9 mL and 9.0 mL volumes in screw cap 16 x 125 mm test tubes and stored in the refrigerator. Each test tube contained 9.9 mL of the fresh dialysate or 9.9 mL, or later 9.0 mL, of spent dialysate. The use of 9.0 mL of spent dialysate did not alter final calculations of bacterial colony counts.

**Table 2-1: Strains of *Staphylococcus epidermidis*  
Tested for Slime Production.**

**STRAINS**

<b>Slime - Producers</b>	<b>Non-Slime - Producers</b>
A	G
B	H
C	
D	
E	
F	

## 2.4. Enumeration of Bacteria

### 2.4.1. Turbidimetric Methods

A standard inoculum density used in CAPD experiments is approximately  $10^6$  colony forming units (CFU)/mL (Flournoy, Perryman and Qadri, 1983). Preliminary studies indicated that dilution of overnight Mueller-Hinton broth cultures, so as to yield a 75% transmittance (Spectronic 20, Bausch and Lomb) (Wilson and Miles, 1975; Wistreich and Lechtman, 1976) at a wavelength of 550 nm, resulted in numbers of CFU within a desirable range of 30-300 per plate. The final cell concentration of the inoculum was  $10^8$  organisms. A 1.0 mL inoculum was then added to the spent dialysate, while the fresh dialysate received a 0.1 mL inoculum. Preliminary testing indicated that a 10-fold inoculum was required for experiments involving immersion of catheter segments in spent dialysate so as to obtain catheter plate counts within, or near, the 30-300 colony range.

### 2.4.2. Viable Plate Counts

#### a) Suspending Fluids

Ten-fold serial dilutions to 1:1000 of each fluid were prepared in 10-mL volumes using sterile distilled water as diluent. One-tenth mL samples of the 1:100 and 1:1000 dilutions were transferred to the surface of Mueller-Hinton agar in 100 x 15-mm Petri dishes (Fisher) and spread thoroughly with a disposable inoculating loop. The plates were allowed to dry at room temperature and were then incubated at 37° C overnight. As far as possible, plates with 30 to 300 colonies were selected for counting and subsequent calculation of CFU/mL in the relevant suspending fluid.

#### b) Catheter Segments

Dialysis fluids were removed from each test tube. Each catheter was washed using sterile distilled water (approximately 10 mL). The tube was then vortexed to allow an adequate rinsing of the catheter. Washings were performed a total of 10 times for each catheter piece. After washing, the catheter piece was



removed, aseptically from the test tube using a glass pipet inserted into the lumen of the catheter segment. The catheter was then rolled up and down three times on a plate, forming an "n"-shaped inoculation. These "catheter plates" were then incubated overnight with the other plates.

## 2.5. Additives and Antibiotics

Both the additives and the antibiotics used in these experiments were added to the test tubes of fresh and spent peritoneal dialysate. The additives utilized were a 5% heat-inactivated solution of fetal bovine serum (Grand Island Biological Company, New York), 5% Toronto beef and pork insulin (Nova Laboratories, Willowdale, Ontario), and protein protamine sulfate (Eli Lilly Canada Inc., Toronto). The antibiotics used were gentamicin (Roussel Canada Inc., Quebec), cephalothin (Eli Lilly Canada Inc., Toronto), and penicillin (Glaxo Laboratories, Toronto).

Further experiments involved incubation in fresh PDS with additives 5% human serum albumin (Canadian Red Cross Society, St. John's), and 0.6%, 1.3%, 2.5%, 5.0%, and 10.0% fetal bovine serum (Grand Island Biological Company, New York). A 5.0% solution of fetal bovine serum, heated (60° C) for 1 hour was also utilized. The slime-producing strain F and the non-slime-producing strain G were tested.

The minimum inhibitory concentrations (MIC) of the protamine sulfate and of each antibiotic were determined for each strain of *S. epidermidis* (Table 2-2). The MIC value for protamine sulfate was established manually by serial two-fold dilutions of the product in Mueller-Hinton broth. The end-point was read as the lowest concentration which inhibited growth. The MIC values of the antibiotics were determined using predisposed microtitration plates ("Sensititre", Gibco, Scotland). The experiments evaluated the effect of one-quarter and one-half MIC of each of the antibiotics and protamine sulfate, on each strain. Stock solutions of the antibiotics, at concentrations of 1000 mg/L were prepared and stored in a Revco freezer (Revco, Inc., South Carolina) at -70° C to prevent deterioration of the antibiotics. A concentration of 1000 mg/L was also prepared for protamine

sulfate, as needed. In order to obtain the appropriate minimum inhibitory concentration, for a particular strain, dilutions were done in 0.9% normal saline.

## 2.6. Catheters

The catheters utilized in these experiments were of the type used for continuous ambulatory peritoneal dialysis patients. They were polyvinyl catheters of the Oreopoulos-Zellerman, Toronto Western Hospital design (Accurate Surgical Instruments, Toronto). A catheter was cut into 1-cm portions, and sterilized by autoclaving.

## 2.7. Procedure

Overnight Mueller-Hinton broth cultures of *S. epidermidis* from peritoneal dialysis patients were tested. Three tubes of refrigerated fresh and spent peritoneal dialysate were collected. Testing in triplicate ensured being within 5% confidence limits. Fresh and spent dialysate each with and without additives or antibiotics were utilized, making a total of twelve test tubes of dialysate. Therefore, each test tube (16 x 125 mm) of dialysate, contained bacteria alone, bacteria and additive, or bacteria and antibiotic. A 1.0-cm piece of sterile catheter was added, aseptically, to each test tube. As mixing was shown by microscopy to disperse cell clusters, the suspension was agitated well with a vortex mixer (Scientific Industries Inc., Springfield, Massachusetts). The twelve test-tubes were then placed in an ice bath to prevent bacterial growth.

Nine test-tubes each containing 9-mL sterile distilled water were prepared. Viable plate counts were performed on the suspending fluids as previously described. The three test-tubes containing dialysis fluid and additives were placed in a 37° C incubator for 10 minutes.

Preliminary observations revealed no observable differences in bacterial numbers among 10, 20 or 60 minute incubation periods. Therefore, the shorter incubation time of 10 minutes was chosen. Colony counts in the suspension fluids at 10 minutes were also not observed to be significantly different from counts at 0 minutes. After 10 minutes, the test tubes containing dialysis fluid were removed

**Table 2-2: Minimum Inhibitory Concentrations  
of Several Additives for the Staphylococcal  
Strains.**

**MINIMUM INHIBITORY CONCENTRATIONS ( $\mu\text{g}/\text{mL}$ )**

<b>Bacterial Strain</b>	<b>Gentamicin</b>	<b>Penicillin</b>	<b>Cephalothin</b>	<b>Protamine Sulfate</b>
<b>A</b>	0.25	0.25	0.50	500.00
<b>B</b>	0.12	0.25	0.50	500.00
<b>C</b>	0.25	0.25	1.00	500.00
<b>D</b>	0.12	0.12	0.50	500.00
<b>E</b>	0.12	0.25	0.50	500.00
<b>F</b>	0.25	0.25	0.50	500.00
<b>G</b>	0.50	0.25	1.00	500.00
<b>H</b>	0.25	0.25	1.00	500.00

from the incubator and plate counts were initiated as above in order to ensure that appreciable bacterial population changes did not occur during the holding periods. After the "10 minute plates" were inoculated and allowed to dry, they were incubated overnight at 37° C.

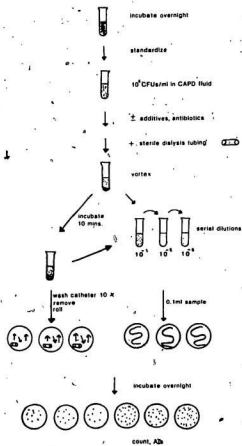
The three test tubes containing dialysis fluids, with their respective catheter pieces, were kept in ice baths until dilutions and plating of the suspension liquid were performed. Catheters were then removed and plate counts performed as previously described.

After the overnight incubation, a Quebec colony counter was used to count the colonies on each plate. The dilution plate with the highest number of CFU within the 30 to 300 limits was selected. The number of colonies was recorded for the 0 and 10 minute plates, as well as for the catheter plates. This was done for the fresh and spent dialysis solutions with and without additive or antibiotic. Data from all bacterial strains were collected and recorded. This procedure followed that adopted by MacDonald, Watts and Bowmer (1986). A summary of the laboratory method utilized is presented in Figure 2-1. Adherence indices were calculated for each replicate, as previously described.

To determine whether the presence of serum would affect bacterial adherence the following experiments were performed in fresh PDS. Firstly, the catheter pieces were preincubated in serum for 20 minutes at 37° C. Identical experimental procedures were then performed, as previously undertaken, with controls involving no preincubation. Secondly, the bacterial cells were immersed in serum in the following manner. The cells were pelleted by centrifugation (IEC Model HN-S Centrifuge, Massachusetts) at 35,000 RPM for 20 minutes. The Mueller-Hinton broth was then removed, and 3 mL of serum was added to the test tube. The mixture was vortexed and incubated for 20 minutes. After incubation, 7 mL of fresh PDS was added. A further quantity of fresh dialysate was added until a 75% transmittance at a wavelength of 550 nm was reached. Then, normal experimental procedures were followed as before, with inoculation into fresh PDS. The organisms, in this experiment, were preincubated in serum. As before, controls with no preincubation were prepared.

**Figure 2-1: Diagramatic Representation of the Experimental Procedures Utilized.**

## Flow Diagram for Experimental Method



The constituents of serum include albumin and complement. In order to ascertain if a component of serum could inhibit adherence, a 5% solution of albumin was prepared and added to fresh dialysis solutions, as in the previous experiments. Also, various percentages of serum were tried for their inhibiting properties. Solutions of 0.6%, 1.3%, 2.5%, 5.0%, 5.0% (60° C), and 10.0% serum were added to fresh PDS in the same manner as above. Colony counts for all these experiments were recorded and adherence indices were calculated.

## 2.8. Adherence Indices

External catheter surface areas were measured via a micrometer and calculated according to the following formula: surface area =  $\pi \times \text{diameter} \times \text{length}$ . Final adherence indices (AI) were calculated and expressed as roll culture colonies/cm<sup>2</sup> of catheter external surface area per 10<sup>6</sup> CFU/mL of broth culture (Franson *et al.*, 1984; Sheth *et al.*, 1983). Adherence indices were calculated after the 10 minute holding time. The catheter surface area (SA) used in these experiments was as follows: SA =  $\pi \times 0.51 \text{ cm} \times 1.0 \text{ cm} = 1.60 \text{ cm}^2$ .

## 2.9. Statistical Methods

Data from bacterial colony counts and adherence studies were compared for statistical significance using several tests with  $P < 0.05$  chosen to be the significance level. The statistical design was analyzed by two-way and three-way analysis of variance. As a result of having the three replicates, the degrees of freedom from residual error were sufficient (MacDonald, Watts and Bowmer, 1986). Therefore, the mean square due to error, or the variance, was obtained with precision from the analysis of variance. This mean square due to error could then be utilized in other statistical tests. This mean square due to error is the unexplained variation; and it is the variance of the adherence indices that is used to calculate confidence intervals or to compare averages. The student's *t*-test, or the least significant difference ( $\text{lsd} = t\text{-value} \times \text{standard error}$ ) (Steel and Torrie, 1980), was used wherever two arithmetic averages were being compared independently. For example, the lsd test compared control results with

experimental results, within the same bacterial strain, and experimental results for the same additive over two strains. Tukey's  $w$  procedure for multiple comparisons, utilizing a critical difference value  $w$  (Steel and Torrie, 1980), was used wherever three or more averages were being compared in all possible combinations. Tukey's  $w$  test analyzed differences between the controls of additives or antibiotics for the same bacterial strain or over different strains. The analysis of variance was done using the computer and the SPSS-X package (SPSS Inc.). The  $l_{sd}$  and Tukey's  $w$  values varied from strain to strain (Table 2-3).

### 2.10. Electron Microscopy

The peritoneal dialysis catheters were examined by scanning electron microscopy (SEM) to study the adherence ability of the *S. epidermidis* strains. Both fresh and spent conditions were considered, as well as the effects of the additives and antibiotics. The following catheters were prepared for microscopy: a control, treatments 5% serum and one-half the MIC of gentamicin. SEM was performed on catheters tested in fresh dialysate and spent dialysate, containing these additives.

Using aseptic techniques, catheter pieces were collected and placed in small vials containing a fixative. This fixative was a combined formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy (Karnovsky, 1965), pH 7.4. The fixative was allowed to react with the specimens for a minimal time of 1 hour or until processing could be done. The fixative was then removed from the catheter pieces, and replaced with 0.1 M sodium cacodylate buffer, pH 7.4, for 30 minutes, under continuous rotation to allow solution penetration. Samples were then placed in 2% osmium tetroxide and rotated for 1-2 hours. Specimens were further buffered in sodium cacodylate buffer before dehydration. Fixed samples were dehydrated for SEM in a graduated ethanol-water series of 40%- 70%- 95%- absolute ethanol, and were critical point dried from liquid carbon dioxide in a Polaron E3000 critical point drier. The critical point dried specimens were then mounted on aluminum stubs and sputter coated with gold in an Edwards SI50A sputter coater. Specimens



**Table 2-3:** Statistical Values from the lsd and Tukey's w Tests for Bacterial Strains A to H at a 95% Confidence Level.

Strains	lsd	Tukey's w
A	4.2	9.6
B	1.8	4.1
C	1.7	3.9
D	0.5	1.0
E	1.2	2.8
F	0.4	0.8
G	1.5	3.5
H	0.8	1.8

were then examined with a Hitachi S570 scanning electron microscope operating at an accelerating voltage of 20 kv. Scanning electron micrographs were produced with Polaroid 665 black and white film. These were then examined for the presence of bacterial clusters.

## Chapter 3

### Results

#### 3.1. Controls

It was necessary to do a separate control for each treatment over all eight strains (Tables 3-1 and 3-2) as there was variation in control values. Statistically significant differences existed among the control results of the ten experiments.

##### 3.1.1. Intra Strain Variation

Control values in fresh fluid for Strain A (Table 3-1) were significantly different when values in Experiment 1 were compared to the other nine experiments ( $w=0.6$ ,  $P<0.05$ ). The spent fluid had lower adherence indices than the fresh solution, and thus significance with the smaller control values from spent fluids did not occur as frequently. Similar results were evident for the other slime-producers, as well as the non-slime-producers (Table 3-2).

##### 3.1.2. Fresh Versus Spent Dialysate

Control adherence index results for fresh fluids were always significantly higher than those of the spent fluids. For example, Table 3-1 shows approximately a 30-fold increase between fresh and spent solutions for strains D, E, and F ( $l_{sd}=0.5$ ,  $l_{sd}=1.2$ ,  $l_{sd}=0.4$  respectively,  $P<0.05$ ). Both the slime-producers and the non-slime-producers showed this pattern.

**Table 3-1: Adherence Indices from Control Results  
for the Ten Experiments for Strains A to F  
in Fresh and Spent Fluids.**

Strain A		Strain B		Strain C	
Fresh	Spent	Fresh	Spent	Fresh	Spent
508	10	47	7	154	8
954	10	88	20	155	5
617	18	82	8	125	16
879	20	157	13	173	9
507	38	135	5	102	3
994	17	115	7	187	8
552	18	122	7	233	9
531	19	321	7	138	4
1302	18	141	5	101	2
805	19	201	9	143	4

Strain D		Strain E		Strain F	
Fresh	Spent	Fresh	Spent	Fresh	Spent
218	8	175	12	232	8
236	8	294	8	229	7
236	5	270	6	224	5
232	4	298	9	207	5
184	8	318	5	277	7
227	7	298	8	288	6
225	9	243	9	218	9
204	8	247	8	217	6
230	9	262	6	250	8
241	6	272	4	272	8

**Table 3-2: Adherence Indices from Control Results for Strains G and H in Fresh and Spent Fluids for the Ten Treatments.**

Strain G		Strain H	
Fresh	Spent	Fresh	Spent
212	6	284	9
128	5	208	9
151	9	216	5
287	6	204	6
238	6	213	4
148	3	234	4
153	7	197	7
351	13	197	6
205	6	270	7
200	9	259	15

### 3.1.3. Inter Strain Variation

Significant differences for the control results also existed from strain to strain. All eight adherence indices were statistically significantly different from each other ( $w=4.5, P<0.05$ ). The spent fluids had lower adherence indices, and therefore did not show as many significant results.

### 3.2. Additives and Antibiotics

The various additives and antibiotics utilized in this thesis inhibited adherence of the *Staphylococcus epidermidis* strains.

#### 3.2.1. Serum

Control results were always significantly different from experimental results for all bacterial strains when the additive serum was utilized (Table 3-3). Except for strain C in spent fluids, experimental results had significantly lower adherence indices than did control results. Table 2-3 contains the significance values used. Experiments performed in fresh solutions were always significantly different from those in spent fluids. Among strains A to H, experimental results were significantly different, especially in fresh fluids ( $w=2.8, P<0.05$ ).

#### 3.2.2. Insulin

Adherence indices were significantly lower than control values when insulin was added to fresh fluids (Table 3-4). Values in spent solutions were smaller and were, therefore, significant in five out of the eight strains tested. Experiments done in fresh fluids were significantly different from those in spent solutions. Strain to strain variations occurred when the adherence indices were calculated ( $w=4.5, P<0.05$ ).

**Table 3-3: Adherence Indices for  
Bacterial Strains in Fresh and  
Spent Fluids with Serum.  
Significance is at the  $P < 0.05$  level.**

Additive Serum

ADHERENCE INDICES

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	507.6	45.9 (S)	10.1	3.5 (S)
B	48.6	6.4 (S)	6.8	1.8 (S)
C	154.4	35.0 (S)	8.1	12.7 (S)
D	218.2	10.3 (S)	6.4	3.2 (S)
E	175.2	26.1 (S)	11.9	2.7 (S)
F	231.7	32.1 (S)	8.2	2.5 (S)
<b>non-slime</b>				
G	212.1	52.6 (S)	5.7	2.2 (S)
H	284.2	34.1 (S)	9.0	2.7 (S)

(S) indicates significance between control and experimental.

**Table 3-4: Adherence Indices for  
Bacterial Strains in Fresh and  
Spent Fluids with Insulin.  
Significance is at the  $P < 0.05$  level.**

Additive Insulin

Strain	ADHERENCE INDICES			
	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	953.7	126.9 (S)	10.0	10.4 (NS)
B	85.6	39.4 (S)	20.4	8.0 (S)
C	154.5	19.2 (S)	4.5	3.9 (NS)
D	235.9	58.6 (S)	7.6	5.6 (S)
E	293.5	18.8 (S)	7.5	2.2 (S)
F	228.7	53.7 (S)	7.1	4.7 (S)
<b>non-slime</b>				
G	127.6	9.3 (S)	5.1	4.1 (NS)
H	206.4	51.1 (S)	9.1	3.5 (S)

(S) indicates significance, (NS) indicates non-significance between control and experimental.



### 3.2.3. Gentamicin

Except for two strains in sixteen experiments, 1/4 and 1/2 the minimum inhibitory concentrations of gentamicin lowered bacterial adherence values in the fresh fluids (Table 3-5). Likewise, in spent fluids, significant differences existed between control and experimental values for all strains except one. Experimental results obtained when using fresh solutions were significantly higher than if spent fluids were used ( $w=2.8$ ,  $w=3.8$ ,  $P<0.05$  for 1/4 and 1/2 MIC respectively). Again with this antibiotic, strain to strain variations existed with experimental results.

### 3.2.4. Cephalothin

One-quarter the minimum inhibitory concentration of cephalothin lowered bacterial adherence in fresh fluids for six of the eight strains, but one-half the MIC of cephalothin lowered adherence in all strains, using fresh fluid (Table 3-6). In the spent fluids, however, 4/8 of the strains were inhibited by 1/4 MIC cephalothin; while 6/8 of the strains were inhibited from adhering by 1/2 MIC cephalothin. The fresh solutions gave significantly higher adherence indices than did the spent fluids ( $w=4.4$ ,  $w=7.7$ ,  $P<0.05$  for 1/4 and 1/2 MIC respectively). Adherence results were significantly different for strains among both antibiotic concentrations.

### 3.2.5. Penicillin

All but one bacterial strain was significantly inhibited from adhering to catheters when 1/4 the MIC of penicillin, and also when 1/2 the MIC were used as compared with control values. This occurred in both the fresh and the spent solutions (Table 3-7). Both 1/4 and 1/2 the minimum inhibitory concentrations had significantly higher adherence indices in fresh fluids as compared with spent solutions ( $w=2.9$ ,  $w=3.2$  respectively,  $P<0.05$ ). Again with this antibiotic, all strains showed variations for experimental results, especially in fresh dialysis fluids.

Table 3-5: Adherence Indices for Bacterial Strains  
in Fresh and Spent Fluids with 1/4 MIC and  
1/2 MIC Gentamicin.

Significance is at the  $P < 0.05$  level.

ADHERENCE INDICES

Additive 1/4 MIC Gentamicin

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	616.5	542.6 (S)	16.0	9.2 (S)
B	62.2	66.5 (S)	7.9	5.2 (S)
C	125.3	44.5 (S)	15.8	13.9 (S)
D	235.8	142.0 (S)	4.8	2.8 (S)
E	269.8	198.4 (S)	5.7	3.3 (S)
F	224.4	140.0 (S)	5.0	1.8 (S)
<b>non-slime</b>				
G	150.7	81.0 (S)	9.1	5.1 (S)
H	215.9	69.8 (S)	4.6	4.5 (NS)

Additive 1/2 MIC Gentamicin

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	879.3	900.1 (S)	19.9	14.7 (S)
B	157.3	52.7 (S)	12.7	4.1 (S)
C	173.2	67.4 (S)	8.5	2.8 (S)
D	232.0	67.7 (S)	3.8	2.6 (S)
E	298.4	216.4 (S)	9.4	2.8 (S)
F	206.8	40.0 (S)	5.0	2.3 (S)
<b>non-slime</b>				
G	286.8	111.3 (S)	6.3	3.6 (S)
H	203.9	46.8 (S)	6.2	3.1 (S)

(S) indicates significance, (NS) indicates non-significance  
between control and experimental.

**Table 3-6: Adherence Indices for Bacterial Strains in Fresh and Spent Fluids with 1/4 and 1/2 MIC Cephalothin.**

Significance is at the  $P < 0.05$  level.

**ADHERENCE INDICES**

**Additive 1/4 MIC Cephalothin**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	507.2	566.6 (S)	37.8	64.5 (S)
B	134.6	46.3 (S)	4.7	5.1 (NS)
C	102.0	72.4 (S)	2.7	2.2 (NS)
D	184.0	112.9 (S)	7.7	5.3 (S)
E	318.2	166.5 (S)	5.4	3.0 (S)
F	277.0	143.8 (S)	6.8	4.3 (S)
<b>non-slime</b>				
G	237.7	268.7 (S)	5.5	3.5 (S)
H	212.9	140.8 (S)	3.8	3.1 (NS)

**Additive 1/2 MIC Cephalothin**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	993.8	623.3 (S)	16.5	9.9 (S)
B	115.3	67.5 (S)	7.0	7.4 (NS)
C	166.6	117.2 (S)	8.1	3.0 (S)
D	227.1	55.8 (S)	7.2	3.3 (S)
E	297.9	210.4 (S)	8.4	5.2 (S)
F	287.6	131.2 (S)	5.5	2.8 (S)
<b>non-slime</b>				
G	148.0	63.6 (S)	3.1	3.0 (NS)
H	233.7	79.6 (S)	3.5	1.4 (S)

(S) indicates significance, (NS) indicates non-significance between control and experimental.

**Table 3-7: Adherence Indices for Bacterial Strains  
in Fresh and Spent Fluids with 1/4 and  
1/2 MIC Penicillin.**

Significance is at the  $P < 0.05$  level.

**ADHERENCE INDICES**

**Additive 1/4 MIC Penicillin**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	551.9	527.6 (S)	18.4	7.5 (S)
B	121.9	131.2 (S)	6.8	6.7 (NS)
C	233.0	139.3 (S)	9.2	3.9 (S)
D	224.9	133.7 (S)	8.7	2.7 (S)
E	242.5	156.4 (S)	8.8	3.6 (S)
F	216.3	102.3 (S)	8.8	4.8 (S)
<b>non-slime</b>				
G	152.5	70.3 (S)	6.6	2.1 (S)
H	197.4	92.1 (S)	7.4	3.6 (S)

**Additive 1/2 MIC Penicillin**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	531.1	612.9 (S)	18.8	8.5 (S)
B	320.5	84.6 (S)	7.0	4.7 (S)
C	137.5	96.4 (S)	3.5	2.7 (NS)
D	203.5	97.8 (S)	8.4	3.8 (S)
E	247.3	103.0 (S)	8.2	4.0 (S)
F	216.9	79.9 (S)	6.3	2.6 (S)
<b>non-slime</b>				
G	350.6	241.2 (S)	13.0	11.5 (NS)
H	197.3	124.8 (S)	5.7	3.2 (S)

(S) indicates significance, (NS) indicates non-significance between control and experimental.

### 3.2.6. Protamine Sulfate

Five out of the eight strains of *S. epidermidis* fresh fluids were significantly inhibited from adhering to catheters with 1/4 MIC protamine sulfate, as compared with control results. Seven out of the eight bacterial strains were inhibited from catheter adherence in the spent solutions (Table 3-8). One-half the minimum inhibitory concentration of protamine sulfate, in both fresh and spent dialysis solutions, significantly inhibited staphylococcal adherence for seven of the eight strains compared with control results (Table 3-8). Adherence indices were always significantly lower in spent fluids than in fresh solutions for both 1/4 and 1/2 the MIC of protamine sulfate ( $w=4.5$ ,  $w=4.4$  respectively,  $P<0.05$ ). Strain to strain variations existed among experimental results with this protein, especially in fresh dialysis fluids.

### 3.2.7. Serum Concentrations and Components

The presence of various concentrations of serum and serum components reduced adherence of *Staphylococcus epidermidis*. For example, for the slime-producing strain F, catheters or bacterial cells immersed in serum significantly inhibited adherence ( $lsd=0.4$ ,  $P<0.05$ ) (Table 3-9). Also, a 5.0% solution of albumin, heated serum (60° C), and various percentages of serum significantly reduced adherence in the fresh dialysis solution ( $lsd=0.4$ ,  $P<0.05$ ).

The non-slime-producing strain G was also significantly inhibited by the additives (Table 3-10). All nine additives tested significantly reduced adherence of the *S. epidermidis* strain ( $lsd=1.5$ ,  $P<0.05$ ) in the fresh dialysis solution. The 0.625% serum additive, with strains F and G, (Tables 3-9 and 3-10) did not reduce adherence as much as the higher concentrations, but results were still significant.

**Table 3-8: Adherence Indices for Bacterial Strains in Fresh and Spent Fluids with 1/4 and 1/2 MIC Protamine Sulfate.**  
Significance is at the  $P < 0.05$  level.

**ADHERENCE INDICES**

**Additive 1/4 MIC Protamine Sulfate**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	1301.6	306.3 (S)	16.1	8.1 (S)
B	140.7	167.2 (S)	4.6	2.3 (S)
C	100.5	149.1 (S)	2.3	2.2 (NS)
D	229.6	113.0 (S)	9.4	4.0 (S)
E	262.0	197.5 (S)	6.1	3.2 (S)
F	249.6	73.2 (S)	7.8	2.3 (S)
<b>non-slime</b>				
G	204.9	203.7 (NS)	6.3	1.8 (S)
H	270.3	104.3 (S)	6.5	2.8 (S)

**Additive 1/2 MIC Protamine Sulfate**

Strain	Fresh		Spent	
	Control	Experimental	Control	Experimental
<b>slime</b>				
A	805.3	337.1 (S)	18.7	14.9 (NS)
B	201.4	126.7 (S)	8.7	4.0 (S)
C	142.7	156.6 (S)	3.6	2.4 (NS)
D	241.4	113.3 (S)	5.7	2.5 (S)
E	272.1	152.4 (S)	4.0	1.5 (S)
F	271.9	86.2 (S)	8.2	3.3 (S)
<b>non-slime</b>				
G	200.0	93.6 (S)	9.1	1.5 (S)
H	258.6	81.8 (S)	14.8	2.8 (S)

(S) indicates significance, (NS) indicates non-significance between control and experimental.

**Table 3-9: Adherence Indices for  
Strain F in Fresh Dialysis  
Fluid Utilizing Various Additives.  
Significance is at the  $P < 0.05$  level.**

Strain F

ADHERENCE INDICES

Additive	Control	Experimental
	Fresh	Fresh
Serum-Catheter	213.8	31.5 (S)
Serum-Bacteria	244.5	53.4 (S)
Albumin (5.0%)	223.8	76.4 (S)
Serum (0.625%)	230.0	217.6 (S)
Serum (1.25%)	241.9	79.9 (S)
Serum (2.5%)	236.6	94.7 (S)
Serum (5.0%)	231.7	32.1 (S)
Serum (10.0%)	238.2	26.8 (S)
Serum (60° C)	229.7	117.4 (S)

(S) indicates significance between control and experimental.

Table 3-10: Adherence Indices for  
Strain G in Fresh Dialysis  
Fluid Utilizing Various Additives.  
Significance is at the  $P < 0.05$  level.

Strain G

ADHERENCE INDICES

Additive	Control	Experimental
	Fresh	Fresh
Serum-Catheter	239.3	115.5 (S)
Serum-Bacteria	186.3	90.3 (S)
Albumin (5.0%)	217.1	104.3 (S)
Serum (0.625%)	229.0	207.8 (S)
Serum (1.25%)	234.4	76.3 (S)
Serum (2.5%)	237.1	99.5 (S)
Serum (5.0%)	212.1	52.5 (S)
Serum 10.0%	258.4	26.9 (S)
Serum (60° C)	228.3	123.5 (S)

(S) indicates significance between control  
and experimental.



### 3.3. Slime Production Versus Non-Slime Production

Significant differences in adherence to catheters existed for all eight strains of *S. epidermidis* for the treatments. This included both the slime-producing (A-F) and the non-slime-producing (G, H) strains. Table 3-11 shows the proportion of slime-producing and non-slime-producing strains which have adherence significantly reduced by the additives or the antibiotics, as compared to control results. A higher proportion of strains had adherence reduced in fresh fluids than in spent solutions for a treatment.

Graphic representation of the adherence indices for a slime-producing strain and a non-slime-producing strain are presented in histograms (Figures 3-1 to 3-4). The adherence of the slime-producing strain F in fresh PDS (Figure 3-1) was significantly inhibited by all additives and antibiotics, compared with control results. The adherence of this strain in spent PDS (Figure 3-2) was also significantly inhibited by all treatments utilized, compared with control results.

The adherence of the non-slime-producing strain G in fresh PDS (Figure 3-3) was significantly inhibited by 8/10 of the treatments, compared with control results. Results from 1/4 MIC of cephalothin and 1/4 MIC protamine sulfate were not significantly less than control results. In spent fluids, all additives except insulin, 1/2 MIC cephalothin, and 1/2 MIC penicillin reduced adherence of strain G (Figure 3-4).

### 3.4. Electron Microscopy

The electron micrographs represent the control, serum, and 1/2 MIC gentamicin results of Tables 3-3 and 3-5. Figure 3-5 shows numerous bacteria which have adhered to the catheter suspended in fresh PDS and in the absence of any additive. There was a decrease in bacterial numbers on the spent PDS control catheter (Figure 3-6). The serum and gentamicin additives further decreased adherence to the catheter pieces, especially in fresh dialysis fluid (Figures 3-7 and 3-8 respectively), but not as large a decrease was observed in the corresponding spent fluids (Figures 3-9 and 3-10 respectively).

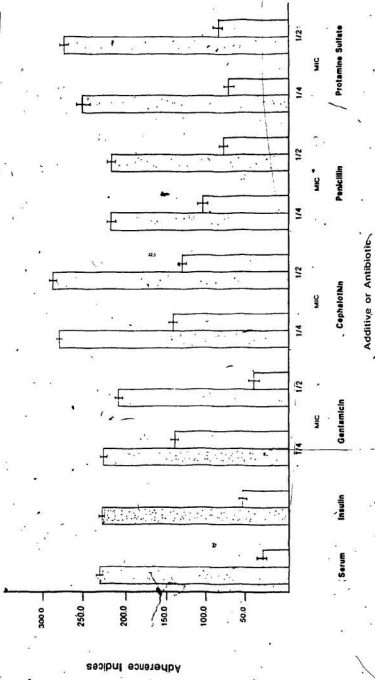
The *Staphylococcus epidermidis* strain used for scanning electron

**Table 3-11: Proportion of Slime-Producing or Non-slime-Producing Strains of *S. epidermidis* in which Adherence is Significantly Reduced by a Treatment.  $P < 0.05$**

Adherence Significantly Reduced By:	Slime		Non-slime	
	Fresh	Spent	Fresh	Spent
Serum	6/6	5/6	2/2	2/2
Insulin	6/6	4/6	2/2	1/2
1/4 MIC Gentamicin	5/6	6/6	2/2	1/2
1/2 MIC Gentamicin	5/6	6/6	2/2	2/2
1/4 MIC Cephalothin	5/6	3/6	1/2	1/2
1/2 MIC Cephalothin	6/6	5/6	2/2	1/2
1/4 MIC Penicillin	5/6	5/6	2/2	2/2
1/2 MIC Penicillin	5/6	5/6	2/2	1/2
1/4 MIC Protamine Sulfate	4/6	5/6	1/2	2/2
1/2 MIC Protamine Sulfate	5/6	4/6	2/2	2/2

**Figure 3-1: Adherence Indices for  
Control and Experimental Data,  
of Bacterial Strain F in Fresh  
PDS. Values Plotted Represent  
Mean  $\pm$  1 Standard Error.**

Control  
Experimental

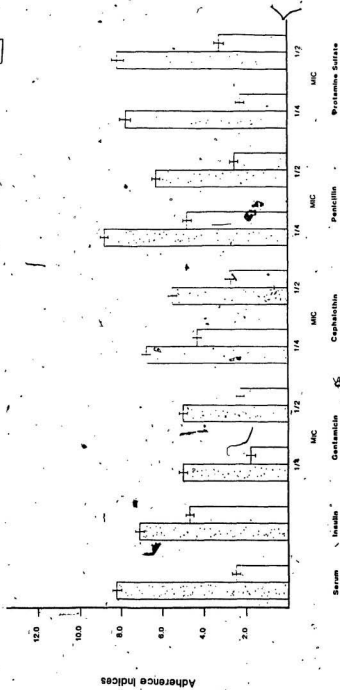


Adherence Indices

Additive or Antibiotic

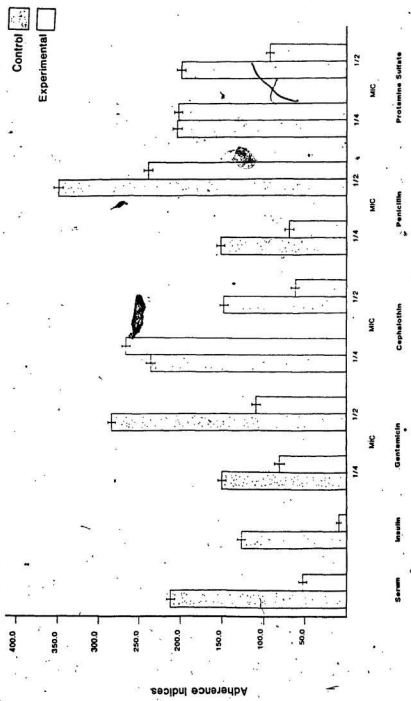
**Figure 3-2: Adherence Indices for  
Control and Experimental Data,  
of Bacterial Strain F in Spent  
PDS. Values Plotted Represent  
Mean  $\pm$  1 Standard Error.**

Control  
Experimental



1 Additive or Antibiotic

**Figure 3-3: Adherence Indices for Control and Experimental Data, of Bacterial Strain G in Fresh PDS. Values Plotted Represent Mean  $\pm$  1 Standard Error.**

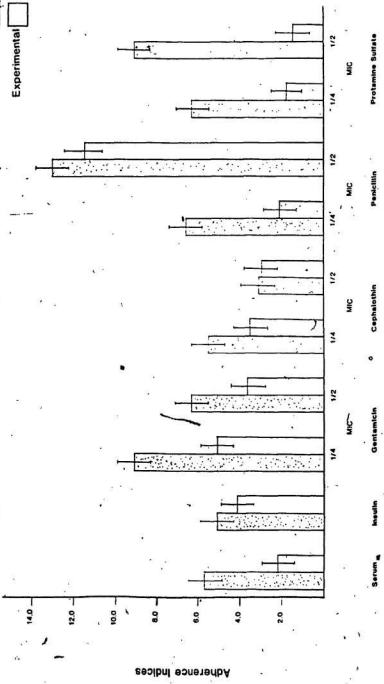


Additive or Antibiotic



**Figure 3-4: Adherence Indices for  
Control and Experimental Data,  
of Bacterial Strain G in Spent  
PDS. Values Plotted Represent  
Mean  $\pm$  1 Standard Error.**

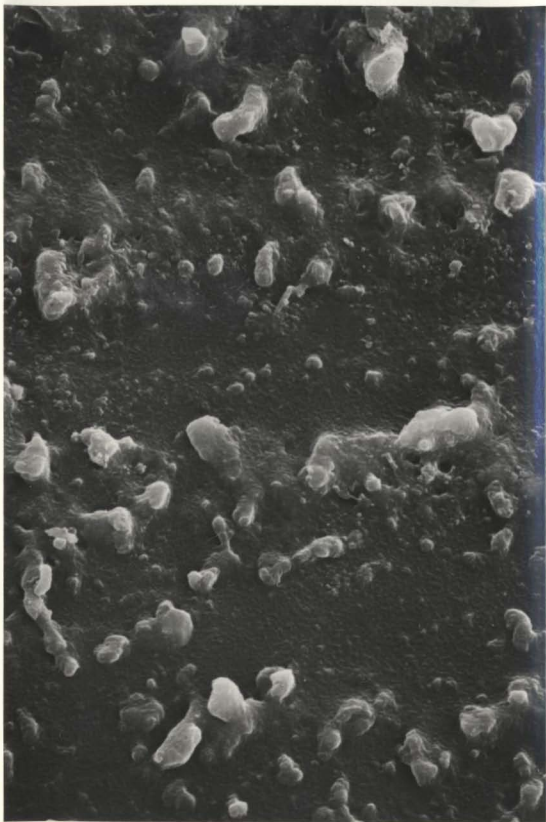
Control  
Experimental



Additive or Antibiotic

microscopy was a slime-producer. Slime is present covering the organism, or in waves on the catheter surface (Figure 3-11). Several bacterial cells immersed in the slimy material are portrayed in Figures 3-12 to 3-14.

**Figure 3-5: *S. epidermidis* on a CAPD Catheter from  
Fresh Dialysis Solution without Additives or Antibiotics.  
Magnification 3960 x.**

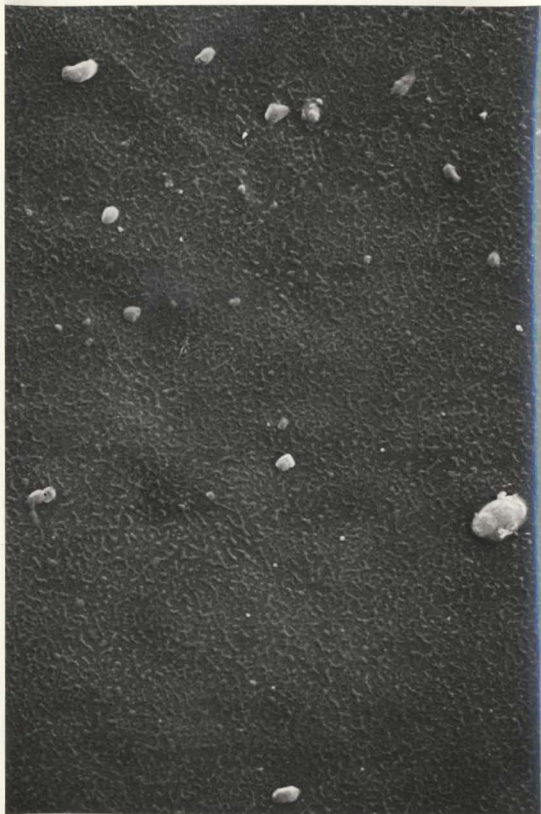


**Figure 3-6:** *S. epidermidis* on a CAPD Catheter from  
Spent Dialysis Solution without Additives or Antibiotics.  
Magnification 3060 x.

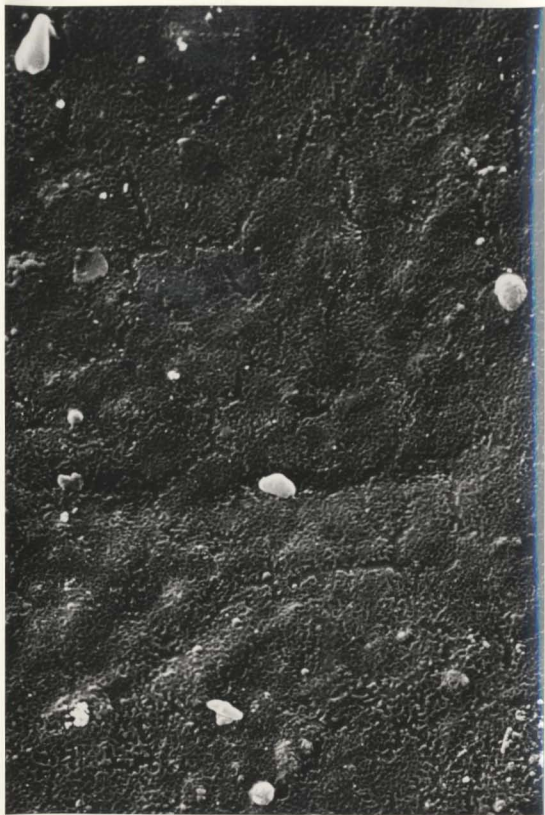


**Figure 3-7: *S. epidermidis* on a CAPD Catheter  
from Fresh Dialysis Solution with the Additive Serum (5.0%).  
Magnification 3960 x.**



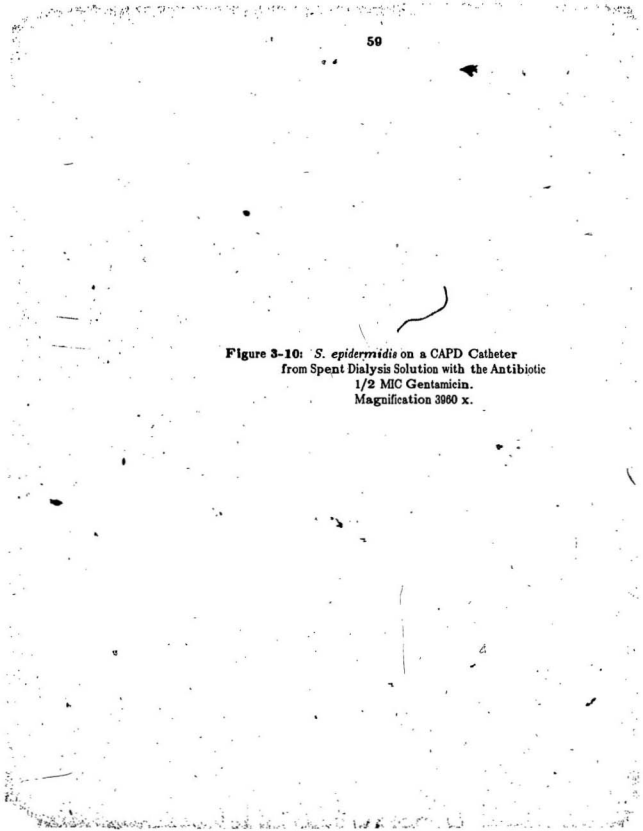


**Figure 3-8:** *S. epidermidis* on a CAPD Catheter  
from Fresh Dialysis Solution with the Antibiotic  
1/2 MIC Gentamicin.  
Magnification 3960 x.

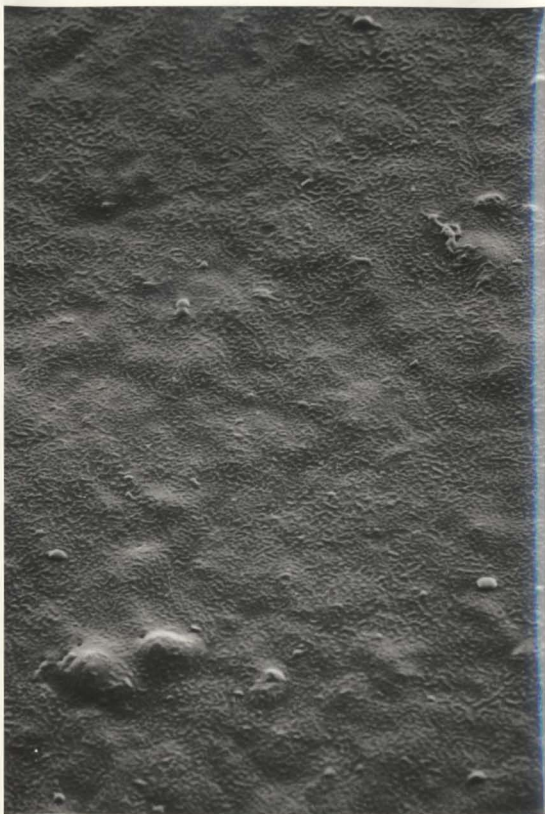


**Figure 3-9: *S. epidermidis* on a CAPD Catheter  
from Spent Dialysis Solution with the Additive Serum (5.0%).  
Magnification 3960 x.**



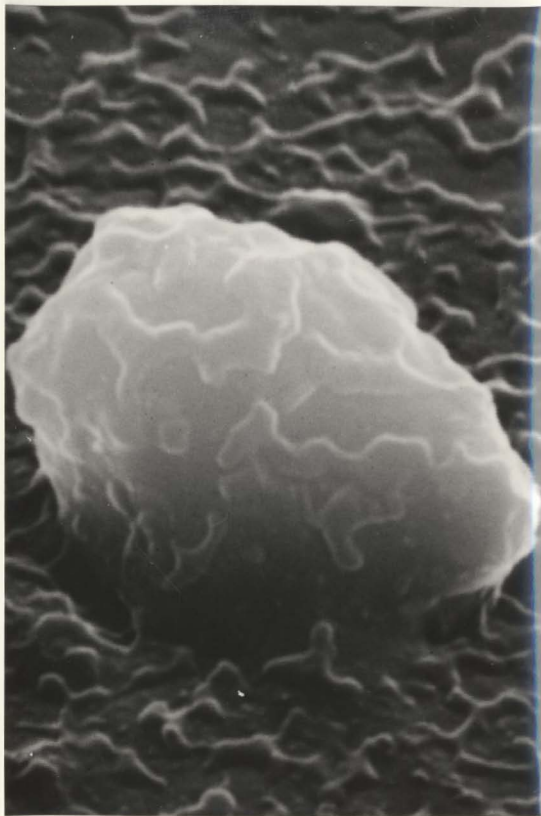


**Figure 3-10:** *S. epidermidis* on a CAPD Catheter  
from Spent Dialysis Solution with the Antibiotic  
1/2 MIC Gentamicin.  
Magnification 3960 x.



**Figure 3-11: *S. epidermidis* on a CAPD Catheter.**  
Slime material covers the organism as well as  
the surrounding catheter.  
Magnification 42900 x.

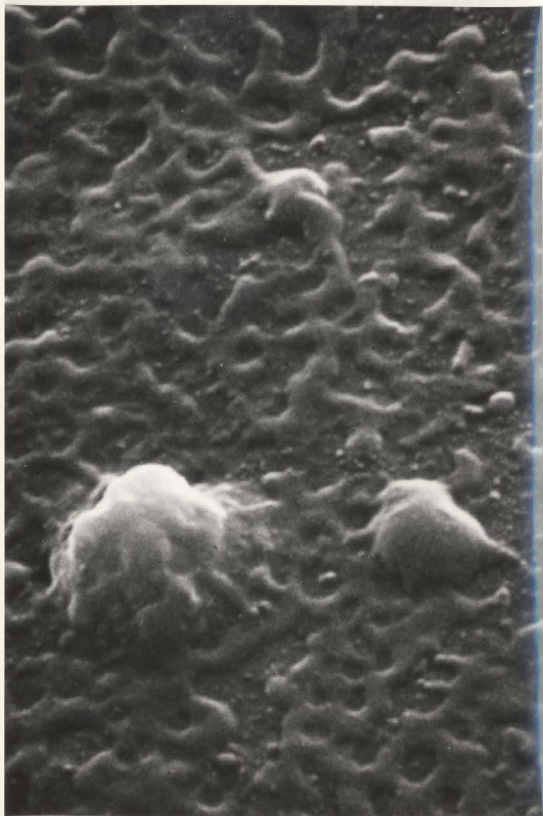




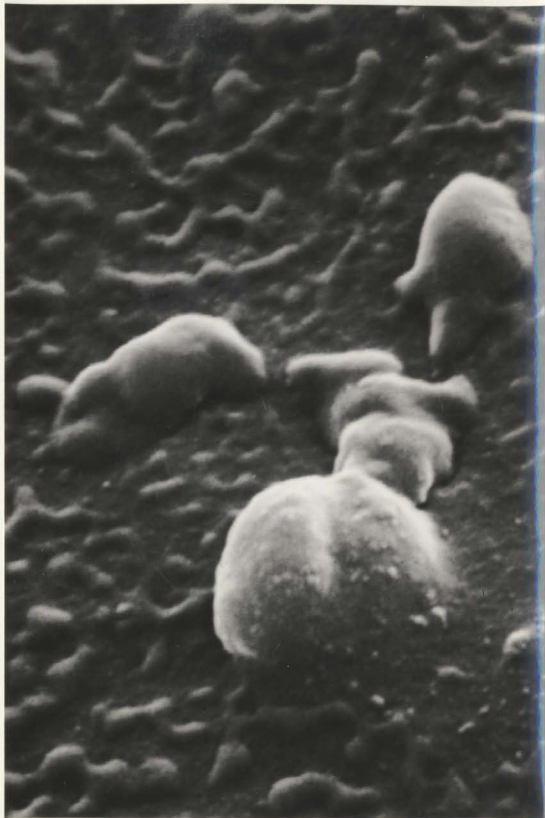
**Figure 3-12:** *S. epidermidis* on a CAPD Catheter.  
Organisms are well embedded in slime.  
Magnification 33000 x.



**Figure 3-13: *S. epidermidis* on a CARD Catheter,  
Showing Slime Production Surrounding the Colonies.  
Magnification 36300 x.**



**Figure 3-14:** *S. epidermidis* Embedded  
in Waves of Slimy Material.  
Magnification 36300 x.



## Chapter 4

### Discussion

Continuous ambulatory peritoneal dialysis (CAPD) is an accepted treatment for renal failure, and offers an effective alternative to the more established technique of hemodialysis.

The major disadvantage, however, and also the major limitation to long-term CAPD therapy, remains recurrent peritonitis. It occurs in 5 to 50 per cent of patients, with an incidence of about one episode per patient year (Moriarty, 1985). The portal of entry for infection is most frequently the peritoneal catheter or the tunnel through which the catheter breaks the normal skin barrier and enters the peritoneal cavity. Contamination of the dialysate delivery system during bag changes or catheter disconnections are less common causes of infections. Other causes of infection, important but less common, include perforation of the bowel, bladder, or vessels.

*Staphylococcus epidermidis*, the most common cause of peritonitis (Pinella *et al.*, 1983), is part of the skin's normal flora. The skin provides a natural and effective barrier system which, once broken by the catheter, can allow these organisms of low pathogenicity to penetrate and cause infection. Dunne and Franson (1986) reported that the colonization of foreign bodies by *S. epidermidis* is the first step leading to serious infection.

In order for a microorganism to survive in the environment, it must first attach to a substrate, resist host defense mechanisms, grow, and colonize the surface. Studies by Christensen *et al.* (1983) suggested that the ability to attach to a foreign body and colonize this body was important in the pathogenesis of foreign body infections with *S. epidermidis*. There may be a considerable period of time necessary for bacterial colonization, and up to a two week period was



assigned by these researchers. Results from this thesis examined the initial attachment of *S. epidermidis* to catheters during the first 10 minutes.

A number of mechanisms for the attachment of bacteria to surfaces, such as catheters, have been suggested. Ludwicka *et al.* (1984) noted that there are specific binding mechanisms to cell membranes. These include attachment factors where the surface of bacteria (adhesins) presumably adheres to host cell membrane receptors. They observed a stronger adhesion when extracellular polymers produced by bacteria interact with the surface. There was a 25% increase in bacterial attachment when slime, produced by the *S. epidermidis*, coated the polyethylene. Electrostatic forces were also postulated to be adherence factors (Ludwicka *et al.*, 1984). The net charge between bacterial cells and solid surfaces create forces for attachment. The hydrophobicity of the surface of bacteria and solid surfaces also create forces between them for attachment.

Staphylococcal growth is greater in spent dialysis fluids than in fresh solutions (Flournoy, Perryman and Qadri, 1983; MacDonald, Watts and Bowmer, 1986). Although studies have been performed on both staphylococcal growth in fresh and spent fluids, and adherence to CAPD catheters by *S. epidermidis*, staphylococcal adherence to catheters in different types of dialysis solutions has not been previously studied. There are differences in bacterial adherence to catheters in fresh and spent solutions. There is significantly lower adherence of all staphylococcal strains in spent dialysis fluid when compared to fresh PDS (Tables 3-1 and 3-2). Adherence to catheters in spent fluids is possibly inhibited by the chemical or physical changes that have occurred in the fluid during dialysis. Some identifiable changes include increases in urea, amino acid, and electrolyte concentrations, as well as pH changes from acidic to neutral. Therefore, these experiments demonstrate significant differences in attachment in the first ten minutes. It can be concluded that initial attachment occurs within this short period. These experiments did not examine longer incubation periods.

#### 4.1. Slime Production Versus Non-Slime Production

Bacterial strains used in this thesis were categorized on the basis of the presence or the absence of slime production. Slime production, as tested at intervals during the experiments, remained stable throughout. The data of Christensen *et al.* (1983) supported these results in that they demonstrated that the production or non-production of slime by a particular strain was stable during the testing period of approximately one year. Davenport *et al.* (1986) also showed that slime production is a stable trait of strains of *S. epidermidis*.

Studies by Costerton, Geesey and Cheng (1978), Mayberry-Carson *et al.* (1984); Peters, Locci and Pulverer (1981); Peters, Locci and Pulverer (1982); Peters and Pulverer (1984) have included electron micrographs of bacterial adhesion to catheters. Slime production by staphylococci has been observed, and has been postulated to promote adherence. The electron micrographs shown in Figures 3-11 to 3-14 demonstrate *S. epidermidis* surrounded by slime, and this may be considered a factor in adherence. However, the results of this thesis do not support that early attachment is dependent on slime. No difference was seen between slime-producers and non-slime-producers.

Slime may have adhesion functions for the staphylococcal bacteria. However, the results of the experiments presented in this thesis, especially those presented in Table 3-11, indicate that adherence within the first ten minutes is independent of slime production. In addition, this initial adherence was inhibited by additives such as serum, insulin, and protamine sulfate in both slime-producing and non-slime-producing strains. Antibiotics such as gentamicin, cephalothin, and penicillin, reduced adherence in both slime-producing and non-slime-producing strains. There was strain to strain variations in adherence but these were independent of slime production.

There has been a divergence of opinion in the literature as to whether or not slime enhances adhesion to catheters and prolongs infections. Franson *et al.* (1986) discovered that there were no differences in the adherence of organisms producing slime when compared to the adherence by non-slime-producing strains of *S. epidermidis* during 96 hours of incubation. Both strain types had equal

affinity for the catheter. Their observations suggested that slime production did not contribute to the increased adherence or pathogenicity of coagulase-negative staphylococci. There was no association between adherence, polysaccharide production, and infection after overnight incubation of staphylococcal cultures with plastic devices (West *et al.*, 1986). Studies by Ishak *et al.* (1985) showed that the presence or absence of slime did not correlate with adherence ability when catheter segments were immersed in suspending fluid for 30 minutes. Slime-producing strains adhered to catheters in approximately the same number as did non-slime-producing strains. *In vivo* studies by several researchers have shown that slime production does not enhance infection. Kristinsson and Spencer (1986) examined patients who had peritonitis caused by *S. epidermidis* and who were undergoing CAPD. They did not find slime production to be a prominent feature in organisms isolated from patients with peritonitis. Likewise, Kristinsson, Spencer and Brown (1986) found that there was no increase in the length or severity of peritonitis when slime-producing strains of staphylococci were isolated. A study by Cheesbrough, Finch and Burden (1986) indicated that the production of slime by strains of *S. epidermidis*, which correlated with the presence of an extracellular glycocalyx, was not the cause of peritonitis with this organism. This finding was confirmed in a series of studies by the same authors.

However, some authors did report that slime production enhanced adherence and infection. Sheth, Franson and Sohale (1985) observed that slime-producing strains survived better and had increased adherence to catheters when compared to non-slime-producing strains. They also showed that after a 2 hour incubation, there seemed to be an increase in slime production, and noted that it was nearly impossible to eradicate a slime-producing organism from an implanted device. However, these workers incubated their catheters in trypticase soy broth rather than dialysis solutions as used in the present study. *In vitro* studies have demonstrated increased adherence to catheters by slime-producing isolates. Peters, Locci and Pulverer (1981), using scanning electron microscopy, detected colonies of *S. epidermidis* closely packed in a slime matrix. Peters and Pulverer (1984) proposed that slime may protect *S. epidermidis* against antimicrobial

agents as well as natural host defense mechanisms. These latter two groups of researchers did not state the length of time for organism and catheter incubation. Dunne and Franson (1986), in their review, reported that the ability of all species of staphylococci to adhere to and multiply on catheters correlated directly with virulence. Approximately 60% of all clinically significant staphylococci were slime-producing adherent strains. Davies and Stone (1986), in their review, suggested that staphylococcal strains which produced slime were able to adhere to catheter surfaces *in vivo*, and that the slime protected the organisms against antibody and phagocytic activity. Non-slime-producers were not as adherent, and were more easily eradicated with antibiotics. Findings of Davenport *et al.* (1986) showed that repeated isolation of a slime-positive organism was four times more likely to represent infection than isolation of a non-slime-producer. Their findings noted that slime production was important in the colonization of catheters and acted as a virulence factor in infections with staphylococci. These results were obtained from *in vivo* studies and did not involve incubations of catheters and bacteria. Treatment of these infections by antibiotics alone was successful in only 30% of the cases involving a slime-positive organism. Christensen *et al.* (1983) showed in an experimental peritonitis model that a slime-producing strain of *S. epidermidis* produced three times as many infections as a non-slime-producing strain in mice, after 10 days.

## 4.2. Additives and Antibiotics

Results from the experiments presented in this thesis and the literature show that there are additives and antibiotics which can inhibit the initial or early bacterial adherence to catheters.

### 4.2.1. Additives

Additives such as serum, insulin, albumin, and protamine sulfate used in this report were successful in significantly inhibiting adherence of *S. epidermidis*, compared with control adherence indices. This was especially true in fresh fluids and less so in spent solutions. Although inter strain variations existed, most

strains were inhibited by the additives. Heating the serum (60° C, 1 hour) served to ensure the inactivation of any complement that may have been present in the original fetal calf serum. As adherence indices were reduced with heated serum, it is unlikely that complement was involved in the reduction of adherence. Increasing concentrations of fetal calf serum proportionately decreased adherence indices (Tables 3-9 and 3-10). The least difference between control and experimental groups occurred with the lowest concentration of serum used in these experiments, 0.625% serum, while the greatest difference between controls and additives existed with a 10.0% serum concentration.

Serum may coat the bacteria or coat receptor sites on the catheter. Adherence was significantly reduced when serum was incubated initially with the catheters, then added to fresh solution containing bacteria (Tables 3-9 and 3-10). This also occurred when bacteria were initially incubated with the serum, and added to fresh fluids containing catheters. Therefore, serum contains factors which specifically inhibit the immediate adherence to catheters. Fleer, Verhoef and Hernandez (1986) discovered that, *in vitro* adherence of *S. epidermidis* to catheters proved to be a rapid process (less than 4 hours), which was easily inhibited by preincubating either the catheter or the bacteria in serum. Fletcher (1980) noted that the presence of an adsorbed serum or albumin protein film on glass or plastic surfaces inhibited the attachment of bacteria by converting a favourable surface to one that had a protective coating. Fletcher (1980) speculated that this film prevented bacterial attachment and adherence. Ofek *et al.* (1979) proposed that the use of inhibitors may reach the surface of epithelial cells and that this coating prevents bacterial attachment. Hynes (1986) discussed a cell-surface protein present in serum, fibronectin. He noted that the fibronectin could specifically bind to bacteria, block bacterial binding sites, or prevent adhesion by acting as a layer on the catheter. It is possible that a fibronectin, present in the serum incubated with the staphylococci used in experiments presented in this thesis, could have specifically blocked binding sites, and inhibited adherence.

The literature suggests many additives that inhibit the adherence of

bacteria. Bovine serum inhibited attachment of bacteria to catheters in a study by Fletcher (1980). Successful eradication of intravenous catheter adherent *S. aureus* occurred when serum was added (Rahal, Chan and Johnson, 1986). Albumin non-specifically inhibited bacterial adherence to polystyrene and glass surfaces (Fletcher, 1980). Ofek *et al.* (1986) concluded that albumin specifically inhibited the adherence of *Streptococcus pyogenes* to epithelial cells. Binding of these bacteria to plastic plates was also prevented. Albumin may have been the factor in serum which prevented adherence of *S. epidermidis* in the experiments of this thesis. However, more experiments are required to verify this.

Previous studies of the effect of insulin on bacterial adherence have not been done. Studies presented in this thesis, however, suggest that insulin is also an inhibitor of bacterial adherence to catheters. Insulin is a charged molecule and may interfere with electrostatic forces that have been postulated to play a role in attachment.

The results presented in this thesis utilizing protamine sulfate to inhibit adherence supported findings of several other researchers. Sacchi *et al.* (1982) discovered that protamine sulfate reduced staphylococcal infections in CAPD patients. *In vivo* studies, using an animal model of peritonitis, have suggested that protamine sulfate (100 µg/mL) was effective prophylaxis against bacterial peritonitis (Beam *et al.*, 1984).

#### 4.2.2. Antibiotics

Results showed that the antibiotics gentamicin, cephalothin, and penicillin reduced adherence indices of most strains of *S. epidermidis*. Significant differences between control and cephalothin results were not as apparent in spent fluids as in fresh solutions, especially for 1/2 MIC cephalothin, strain G (Table 3-6). Likewise, significant differences between control and 1/2 MIC penicillin with strain C (Table 3-7) and strain G (Figure 3-4) in spent fluids was not as apparent as in fresh solutions. Figures 3-1 to 3-4 show that there was not a consistent decrease in adherence with the higher concentrations of cephalothin and penicillin. A reason for there being no significant differences between these

previously mentioned results of controls and antibiotics in spent PDS could be the lower adherence indices which resulted from experiments in the spent fluids.

The use of cephalosporins is controversial and discrepancies exist between *in vivo* and *in vitro* studies. Beam (1982) and Fass *et al.* (1986) noted that cephalosporins did not always inhibit bacterial growth. Both *in vitro* and *in vivo* evidence supported the limited usefulness of the cephalosporins. *S. epidermidis* bacteremia did not respond well to treatment with cephalosporins. Also, 10 mg/L serum levels of cephalosporins were not effective in eradicating staphylococci *in vivo* (Keane and Cafferkey, 1984). Davies (1985) noted that *S. epidermidis* and *S. aureus* were multiresistant to cephalosporins *in vivo*, but were sensitive *in vitro* to these antibiotics. Sabath and Mokhbat (1983) noted that there were conditions in patients, such as the presence of a foreign body, that prevented bactericidal antibiotics from being bactericidal. Archer (1985) noted that up to 80% of the *S. epidermidis* bacteria causing nosocomial infections were resistant to cephalosporins. Not only are there discrepancies between *in vitro* and *in vivo* cephalosporin activity, but in this thesis' results, the cephalosporins have a variable effect on adherence.

Cephalothins and penicillin were chosen because literature studies have noted that they are effective antibiotics against staphylococcal bacteria. Antimicrobial-induced lysis and killing *in vitro* of staphylococcal colonies occurred with a 32 mg/L concentration of cephalosporins, and a 32 mg/L concentration of penicillin (Stratton *et al.*, 1986). Penicillin reduced the adhering ability of bacterial cultures to epithelial cells (Ofek *et al.*, 1979). Many strains of *S. epidermidis* studied by Fass *et al.* (1986) were cephalothin susceptible. Archer (1978) noted that 70% of *S. epidermidis* isolates were killed by a 3.1 mg/L concentration of cephalothin. Ninety percent of the episodes of peritonitis due to *Staphylococcus epidermidis* were successfully treated with cephalothin in a study of Watson *et al.* (1986). Cephalothin was successfully used in the treatment of staphylococcal peritonitis in a study by Vas (1983). The effect of antibiotics used in this thesis was measured after 10 minutes. It is unlikely that any effects observed in these experiments were due to lysis or bactericidal activity of *S.*

*epidermidis*. However, these antibiotics affect cell membranes and are charged. So, immediate effects on adherence may be due to these factors.

Results from the literature show that gentamicin is an effective therapy against staphylococcal infections. Shibl (1985) demonstrated smaller lesions and reduced toxin production in mice treated with subinhibitory concentrations (one-eighth MIC) of gentamicin for *S. aureus* infections. *S. epidermidis* organisms isolated from CAPD catheters by Irwin, Hart and Martin (1973) and Kaplan *et al.* (1985) were sensitive to gentamicin. Gentamicin, given in dosages as low as one-eighth the MIC, resulted in significantly less episodes of peritonitis.

The previous studies indicate that, in most instances, additives serum, albumin, insulin and protamine sulfate, and antibiotics gentamicin, cephalothin and penicillin reduce bacterial adherence and/or infections. Experimental results from this thesis showed that adherence of *S. epidermidis* was inhibited by concentrations lower than required to inhibit bacterial growth. The clinical relevance of these studies, especially with regards to the different cephalosporin concentrations, remains to be further investigated.

#### 4.3. Statistical Significance or Biological Significance

Adherence indices for the control and experimental data of additives serum and insulin were different from each other. That is, they were statistically significant at  $P < 0.05$ , and also could be categorized as biologically significant, meaning they could, theoretically, inhibit bacterial adherence to catheters *in vivo*. Although significant differences between control and experimental adherence indices existed for many of the antibiotics tested in this thesis, it was difficult to accept some of these as being biologically significant. For example, the difference between control and 1/2 MIC gentamicin adherence indices for strain E in fresh fluids was not great (Table 3-5). Therefore, even though these were statistically significant, their biological significance could be questioned. Also, biological significance may not be accepted for the control and experimental results of strain A in 1/4 MIC penicillin for fresh PDS (Table 3-7). Therefore, antibiotics used in this report were statistically significant based on a chosen  $P < 0.05$  level of



significance between control and experimental data. However, whether or not some of these were significant biologically remains unclear.

#### 4.4. Variations from Expected Findings

Compared to the other strains, adherence indices for strain B in fresh PDS with serum, insulin, and 1/4 MIC gentamicin (Tables 3-3, 3-4, and 3-5 respectively) were lower. For an unknown reason, over these three days of testing, bacterial adherence to the catheters was lower than normal. This could have been due to such possibilities as poor overnight growth of the strain, or a non-supportive batch of Mueller-Hinton media.

The literature notes several possibilities for variations in results. Results could be influenced by the temperature of manipulations and incubations (Fletcher, 1980). Temperature can influence the quantity of extracellular polymer produced by reducing amounts at lower temperatures. Culture age has also been proposed as a factor which decreases attachment. Cooling of specimens, as in the methods utilized in this thesis, can reduce this aging. Finally, the phase of growth of the bacterial inoculum (stationary, logarithmic) can influence results (Sabath and Mokhbat, 1983). Experiments on cultures should be performed when the cultures are in the logarithmic growth phase. Bacterial specimens used in this thesis were assumed to be in the logarithmic phase of growth as staphylococcal cultures were newly incubated each night. In future experiments, these factors should be documented to ensure reproducible results.

#### 4.5. Summary and Conclusions

Results presented in this thesis demonstrate that various additives and antibiotics statistically, if not always biologically, reduce the adherence of several strains of *Staphylococcus epidermidis* to CAPD catheters. Reducing bacterial adherence to catheters in CAPD patients would be an important step in the application of the *in vitro* results presented in the present report.

Much study and experimentation remains to be performed on the attachment of *S. epidermidis* to CAPD catheters. The glycocalyx, charge

differences, pits in the catheter surface, and certain additives and antibiotics may play a role in bacterial adherence to the CAPD catheter, and these may be responsible for different stages in the colonization process. Many of the additives and antibiotics may inhibit attachment to the catheter due to their being large molecular weight charged molecules, since it is presumably not due to antibacterial effects of the treatments within a ten minute period. However, the exact mechanism for adherence has not been confirmed, but slime, a postulated virulence factor, may not be the major factor in the initial stages. More data on the mechanisms of adherence may help avoid serious complications with peritonitis. Future considerations should include the use of modalities that alter organism adherence and halt the initial steps of infection pathogenesis. For example, as both heat inactivated serum and serum heated for 1 hour (60° C) decrease control adherence indices, factors other than complement should be investigated which decrease adherence. Also, other antibiotics than the ones tested in this thesis may produce interesting results as to adherence indices. Antibiotic combinations may also provide useful results.

*In vivo* studies involving CAPD patients are required to evaluate whether results presented in this thesis *in vitro*, have relevance to the clinical situation. Only with more experimentation and experience with bacteria infecting and adhering to CAPD catheters can greater understanding of *S. epidermidis* peritonitis be obtained.

## Chapter 5

### Literature Cited

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