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LA THÈSE A ÉTÉ  
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STUDIES ON ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS TO INERT  
PLASTIC MATERIALS

A Thesis submitted to the  
School of Graduate Studies  
University of Ottawa

In Partial Fulfillment of  
the Requirements for the Degree of  
Master of Science,  
Department of Microbiology and Immunology,  
School of Medicine.

by

Reina Laura Rivera-Calderon

January, 1985



UNIVERSITÉ D'OTTAWA  
UNIVERSITY OF OTTAWA

Dedicated to my Father and Mother  
whose love made this possible.

To Aunt Maria F. Rivera  
for her great support

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## SUMMARY

Implantation of prostheses and other foreign bodies is sometimes associated with infections that may interfere with the function of the implant.

Many factors are involved in the process of infection, and adherence of microorganisms to the surface of the biomaterials is a particularly significant factor in this respect.

A method for the precise measurement of adherence and viability is described in which a nutrient agar overlay is applied to a surface previously exposed to a suspension of bacteria. The surface is then incubated and colonies develop under the agar.

The adherence of Staphylococcus epidermidis to inert plastic materials was studied with this method. The surfaces included Polyvinylchloride (PVC), Silastic (SIL), and Polytetrafluoroethylene (PTFE) which are used in the manufacture of prostheses. Polystyrene (POS) petri dishes were used as a standard reference surface for the measurement of adherence.

Adherence was not affected by pH over the range 6.0 to 8.0, but was inhibited by low temperature (4 C). Adherence was lower to PTFE than to POS, PVC and SIL.

The prevention of adherence is a possible way of preventing prosthetic infections, and this procedure developed in this work would be applicable to the study of this problem.

## LIST OF ABBREVIATIONS

A B	ALCIAN BLUE.
A T P	ADENOSINE TRIPHOSPHATE.
B S A	BOVINE SERUM ALBUMIN.
C A	CELLULOSE ACETATE.
C S F	CEREBROSPINAL FLUID.
C V	CRYSTAL VIOLET.
D L V O	DERJAGUIN-LANDAU-VERWEY-OVERBEEK.
E T E C	ENTEROTOXIGENIC ESCHERICHIA COLI.
E M	ELECTRON MICROSCOPY.
F E P	POLY(TETRAFLUOROETHYLENE-CO-HEXAFLUOROPROPYLENE).
I A	INDEX OF ADHERENCE.
§ I A	§ INDEX OF ADHERENCE.
i v	INTRAVENOUS.
L T A	LIPOTEICHOIC ACID
N.A.	NON ADHERENT.
N.D.	NOT DONE.
N R	NEUTRAL RED.
O D	OPTICAL DENSITY.
P B S	PHOSPHATE BUFFER SALINE.
P O S	POLYSTYRENE.
P T F E	POLYTETRAFLUOROETHYLENE.
P V C	POLYVINYLCHLORIDE.
R.	CORRELATION COEFICIENT
R R	RUTHENIUM RED.
S E M	SCANNING ELECTRON MICROSCOPY.

S.I.L. SILASTIC.

T.E.M. TRANSMISSION ELECTRON MICROSCOPY.

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## INTRODUCTION

Shunt insertion procedures for the alleviation of hydrocephalus have been used for many years, and it is recognised that bacterial colonisation of the valve and tubing is a common and serious complication (Holt 1969; Bayston et al., 1974; Venes 1976; Garvey 1980). A frequent accompaniment of shunt infections is a persistent bacteraemia and the microorganisms responsible can cause a fulminating septicaemia (Ivan et al., 1980; Garvey 1980). The great majority of these types of infection occur in the early period after the placement of the device (Garvey 1980).

Experience has shown that it is very difficult if not impossible to eradicate an established prosthetic infection as long as the colonised shunt remains within the body. The bacteraemia may be controlled by antibiotics, however it has been found that antibiotics fail to sterilize the valve.

Adhesion of bacteria to cell surfaces has been recognised as the initial step in the production of infectious processes (Ofek et al., 1980) and similarly the infections associated with implants and devices in man are preceded by adherence of microorganisms on the surface of the biomaterials.

"In vitro" studies on bacterial adherence in which segments of catheters were briefly immersed in suspensions of coagulase-negative staphylococci, Escherichia coli or Staphylococcus aureus have demonstrated that bacteria, which adhered to the surface of the catheter, could multiply and form colonies (Sheth et al., 1983 a).

Shunt infections are mainly caused by Staphylococcus epidermidis (Holt, 1969; Bayston et al., 1974; Garvey 1980; Noble 1984) which is an inhabitant of the normal skin, and a very important opportunistic pathogen. It is part of the Genus STAPHYLOCOCCUS which is presented in detail as follows.

## STAPHYLOCOCCI

The genus STAPHYLOCOCCUS is part of the family MICROCOCCACEAE. The definition of staphylococci is as follows: Non-motile, non spore forming, gram positive cocci, catalase positive, facultative anaerobes, and ferment glucose anaerobically. Individual cells have a diameter of 0.7 to 1.2 micro meters and are characteristically grouped in irregular aggregates that resemble clusters of grapes. Differentiation of human staphylococcus species is based on biochemical characteristics. The most important is the coagulase test, which divides the staphylococci in two groups, the coagulase-positive staphylococci in which the only member is the Staphylococcus aureus, and the coagulase-negative staphylococci in which are included the other members, Staphylococcus epidermidis, S. saprophyticus, S. simulans, S. hominis, and S. haemolyticus.

Most shunt infections caused by coagulase-negative staphylococci are attributable to Staphylococcus epidermidis (Kuhn 1984). Staphylococcus aureus is responsible for most cases of staphylococcal disease in humans, but is rare as a cause of shunt infection.

## BACTERIAL ADHERENCE

The property of bacteria to adhere to and colonise almost any surface is a very important phenomenon, not only in the area of medicine but in other areas such as veterinary sciences, sanitary engineering, and in marine microbiology. The importance of adherence of microorganisms in the production of disease, is illustrated with the following examples;

Enterotoxigenic Escherichia coli are an important group of bacteria that cause acute diarrhoea in human and domestic animals. These bacteria have specific mechanisms, that allow them to attach to the mucosal surface of the small intestine and therefore produce disease (Smith et al., 1977 Knutton et al., 1984 a,b; Zielberberg et al., 1984). Neisseria gonorrhoeae has the ability to attach to mucosal surfaces and to multiply despite the flow of urine and mucus secretions, their adherence is of crucial importance in the pathogenesis of gonorrhoeae (Pearce et al., 1978; Watt and Ward 1980). Adherence of Bordetella pertussis to ciliated cells may be a means of evasion of clearance by the mucociliary transport system and this could be significant in the establishment of the infection in the human host (Tuomanen et al., 1983; Sato et al., 1980). Intimate association of Vibrio cholerae with mucosal surfaces may involve adherence to the microvilli of the intestinal epithelial cells (Jones et al., 1976).

## THE MICROBIAL CELL SURFACE AS RELATED TO ADHERENCE

Adhesion of the microbial cell to a surface of any sort, requires intimate contact. Both microbial cell surface and the substratum are, usually negatively charged. (Corpe 1980; Beachey et al., 1980). The substratum can be an epithelial cell which may be regarded as biological surface or can be a plastic or other inert surface that is referred to as a non biological surface. Since there is mutual repulsion between two surfaces of like charge it is necessary to explain the ability of bacteria to adhere. The most widely accepted hypothesis to explain the adhesion is the DVLO theory, (Derjaguin-Landau-VerWey-Qverbeek) (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948). In summary this hypothesis states that two surfaces of like charge approaching one another are subjected to several attractive and repulsive forces. These different forces, attractive and repulsive, vary with the distance between the two surfaces (Curtis 1973). There is a certain distance at which the repulsive force balances the attractive force and the surfaces are held close together but not touching. If energy can be supplied to overcome this repulsion barrier, then as the surfaces become approximated to each other against the electrostatic barrier, a second attractive force becomes operative and may, at very short distances, overcome electrostatic repulsion to permit attachment. The exact Physico chemical details involved in this process are not precisely defined and

probably vary according to different combinations of surface and bacterium. There is however general agreement on the fact that bacteria are initially unable to make direct physical contact with cell or inanimate surfaces, and that any hypothesis explaining adherence must include a mechanism to bridge the gap between the organism and the surface to which it is attaching.

Studies on surface charge and its influence on the attachment of bacteria to human epithelial cells have been carried out with Neisseria gonorrhoeae. In these experiments, it was shown that pili or fimbriae (see below), facilitated the attachment of N. gonorrhoeae by overcoming the initial electrostatic repulsive barrier which exists between the bacterial cell surface and the host cell. Modification of the charges of bacterial cell surface changed its capacity for attachment.

Blocking the negatively charged free carboxyl groups on the bacterial cell surface on non fimbriated gonococci, increased their capacity for attachment. One possibility is that the negative charge on fimbriae is less than that on the bacterial surface and hence they are better able to overcome the electrostatic barrier. Another possibility is that the initial contact is made by the pilus tip, either because it has different chemical composition or because it has smaller surface area that renders it less sensitive to the electrostatic barrier (Heckels et al, 1976).

## STRUCTURAL COMPONENTS INVOLVED IN BACTERIAL ADHERENCE TO BIOLOGICAL SURFACES

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The adherence of microorganisms to biological surfaces is often specific since there exist receptors on the cells that correspond to the adhesins on the surface of the microorganisms, (Beachey 1980), on the other hand, the attachment of microorganisms to inanimate surfaces is a non specific process since the inert surface does not have specific biological receptors.

Different structural components have been proposed to explain the attachment of microorganisms to biological surfaces. These are: fimbriae or pili, mostly in gram negative bacteria, lipoteichoic acid (LTA) in gram positive bacteria, extracellular polymers and glycocalyx. Motility is suggested as a factor that facilitates adherence but the flagella are not usually considered as adhesins.

FIMBRIAE are hairy projections consisting of protein, their size is approximately 4 to 10 nm in width and 0.5 to 4 micro meters long and are almost exclusively found in gram negative bacteria they are shorter and not as rigid as flagella (Duguid and Old, 1959; Duguid and Anderson, 1967). A distinction can be made between fimbriae and Pili which mediate the conjugative transfer of genetic information (Ofek et al., 1980). In the case of Escherichia coli,

studies with the electron microscope have revealed that pili or fimbriae are the structures responsible for the specific attachment of E. coli to human intestinal epithelial cells (Knutton et al., 1984, a,b,). Studies with E. coli K88, K99 and 987P strains which are diarrheagenic for animals showed that attachment mediated by pili was necessary for the production of disease. The adherence study was done with pig intestinal cells. In the study of K99 and 987P, non piliated mutants of these strains failed to adhere and did not produce disease (Middelpore et al., 1981; Isaacson et al.; 1978; Smith and Linggood 1971). Experiments with Neisseria gonorrhoeae have shown that their attachment is pili or fimbriae mediated, when they adhere "in vitro" to human tissues (Pearce et al., 1978). The attachment of Bordetella pertussis demonstrated that adherence is specific since these bacteria only adhered to the tufts of human trachea ciliated cells and did not adhere to the non. ciliated cells. Studies of these bacteria using electron microscopy (EM) showed a material surrounding the bacterial cells. Other studies demonstrated that some fibers exist on the surface of these bacteria and suggested that their attachment was mediated by pili or fimbriae (Tuomanen et al., 1983; Sato et al., 1980). More recently, the relationship between fimbriation and serotype of Bordetella pertussis has been investigated. It was found that agglutinogen 2 which may be associated with pathogenicity, appears to be fimbrial antigen (Carter and Preston 1984).

LIPOTEICHOIC ACID (LTA). Teichoic acids are important components of the gram positive organisms. They appear to be situated mainly on the outer surface of the cell wall, but they may also pass through the peptidoglycan layer to contact the cytoplasmic membrane.

Studies on Streptococcus pyogenes adherence, showed that LTA plays a central role in the attachment of this bacterium to epithelial cells (Beachey et al., 1983). With the transmission electron microscopy (TEM), it has been shown that fibrillar structures containing the LTA adhesin, mediate the attachment of the organisms to epithelial cells. Antibodies directed against LTA, inhibit adherence.

Glycocalyx. - An acidic polymer has been observed in Pseudomonas, the glycocalyx, that is secreted when this bacterium attached to polystyrene petri dishes (Fletcher 1973). The glycocalyx is defined as a structure of bacterial origin, containing polysaccharides lying outside the integral elements of the outer membrane of gram negative cells and the peptidoglycan of gram positive cells. It has been proposed that glycocalyx, which has been observed in many bacteria in natural environments (Costerton et al., 1978; 1981) may be important in mediating adherence. The production of Pseudomonas aeruginosa to inanimate surfaces (Fletcher 1973; Costerton et al., 1978). The attachment of Streptococcus mutans to dental enamel, which plays a central role in the production of dental caries is probably mediated by polymers

synthesised from glucose (Mukasa et al., 1973; Gibbons and vanHoute 1980). The secretion of slime by Staphylococcus epidermidis has been associated with foreign body infections due to this organism and may be a virulence factor in this context (Bayston et al., 1972; Christensen et al., 1982). Although there is little doubt of the role of pili and LTA in specific adherence to biological surfaces the role of glycocalyx is less well defined. It may be the function of the glycocalyx is to permit the formation of microcolonies and produce a matrix for the growth of bacteria rather than to mediate primary attachment.

MOTILITY. - Motility is mediated by flagella which are helical structures composed of subunits of a single protein called flagellin (Dawes et al., 1976). This protein differs between species in aminoacid composition and serological properties. Bacterial flagella range from 3 to 70 micro meters long and 0.01 micro meters diameter (McCracken et al., 1983). After removing flagella, the microorganisms lose their motility. This property is recovered when flagella are resynthesized (Dawes et al., 1976; Stanley 1983). Flagella have been suggested to increase the chances of the bacterial cell to come in close contact with the surface of attachment. Experiments with vibrios have shown that nonmotile vibrio mutants had less capacity of adherence to rabbit intestinal brush border membranes than those vibrios that are motile. It was suggested that flagella may have a role in adherence (Jones et al., 1976; Attridge et al., 1983 b).

ADHERENCE OF BACTERIA TO INERT SURFACES  
(NON BIOLOGICAL SURFACES)

METHODS

Inert plastic surfaces clearly do not have specific biological receptors, and the adherence of microbes to these surfaces is probably mediated by mechanisms other than the specific "lock and key" mechanism described as the means of attachment to specific biological surfaces. A variety of methods have been used in previous studies for the measurement of adherence of bacteria to inert surfaces, but all follow the same principle. The surface is exposed to an inoculum for stated period of time, washed and the adherent bacteria are then counted. Methods used to measure the residual adherent microorganisms include light microscopy (Fletcher et al., 1979), epifluorescence microscopy (Paul, 1982, bioluminescence (Harber et al., 1983), counting of previously radiolabelled organisms (Feldner et al., 1979), rolling catheters on agar plates (Maki et al., 1977; Franson et al., 1984) scanning and transmission electron microscopy, (SEM and TEM) (Marrie et al., 1983; Fletcher et al., 1973). These experiments are reviewed below in greater detail.

## ADHERENCE OF ORGANISMS TO DIFFERENT PLASTICS

The adherence and growth of coagulase negative staphylococci on intravenous i.v. catheters has been studied (Peters et al., 1982). Two strains of Staphylococcus epidermidis were used, for these "in vitro" studies, and they were obtained from colonised catheters. In this study, the catheters were immersed in the bacterial suspension and incubated. After different intervals of time, the catheters were removed and prepared for SEM (Peters et al., 1982). The results showed that colonisation of the catheters increased with the increase of the exposure intervals. Surfaces, irregularities appeared to be the preferential site of attachment of the organisms. These experiments were done with thoroughly washed organisms in the presence of saline only. There was apparent corrosion of the catheter surface and replication of the organisms, implying that they were able to use the catheter material as a substrate. This needs further investigation. Another observation in these experiments was the presence of a slimy material covering the bacterial cells, which appeared to enhance the attachment to the plastic surface. This slimy material was also found by other investigators (Marrie et al., 1983; Christensen et al., 1982), when they studied the attachment of Staphylococcus aureus and S. epidermidis to plastic surfaces. This slime might prevent the recovery of bacteria from blood cultures and also act as a protective substance against the antimicrobial drugs (Peters et al., 1982).

Another morphological study used catheters removed from patients and examined directly. The microorganisms involved in this study were S. epidermidis, S. aureus, Enterobacter sp., Achromobacter xylosoxidans, Serratia sp., Klebsiella sp. and Candida albicans. The catheters were made of polytetrafluoroethylene (PTFE). These were removed from the patient and immediately cut in segments that were rolled over the surface of an agar plate and the resultant colonies were then identified. Other segments of catheter were prepared for TEM and still others were stained with Ruthenium Red (RR) for examination with SEM. The results showed that some of these microorganisms were associated with extensive slime production. The production of this slime was correlated with clinical pathogenicity (Marrie et al., 1983). "In vitro" studies have shown that the synthesis of the slime was enhanced by presence of glucose in the medium. When S. epidermidis was incubated in media with no glucose the slime was not present (Christensen et al., 1982). Slime producing strains of Staphylococcus epidermidis were investigated in relation to attachment to smooth surfaces. The strains were isolated from intravenous (i.v.) catheters and the test surfaces were plastic or glass tubes and i.v. catheters. The slime was observed attached to the walls of the tube after growing the bacteria in it. After incubation of the segments of catheters with the bacterial suspension the specimens were stained with Alcian Blue (AB), SEM showed that bacteria were immersed in an extracellular material which stained with AB suggesting its polysaccharide

nature (Christensen et al., 1982).

In studies done with Candida albicans, it was shown that the presence of sucrose and galactose enhances the attachment of these yeast to acrylic plastic or buccal epithelial cells (MacCourtie et al., 1984). In experiments with Streptococcus mutans and their attachment to glass surfaces it was shown that adherence was possible in the presence of sucrose because this bacterium synthesises a polymer from this sugar (Mukasa et al., 1973. Mycoplasmas were studied adhering to glass and also the presence of glucose increased their attachment (Feldner et al., 1981). These sugars seems to be important in the environment to enhance the attachment of some microorganisms to inert surfaces.

Experiments with coagulase-negative staphylococci have been done comparing their adherence between i.v. catheters made of different materials. The different i.v. catheters were made of polyvinylchloride (PVC) and PTFE and the segments were immersed in the bacterial suspension. After the incubation time the segments were removed, rinsed and then rolled on agar plates. The resulting colonies were counted. It was found by this semiquantitative method, that these strains of coagulase-negative staphylococci, had greater adherence to PVC than to PTFE. These experiments suggest that the catheter material may be an important factor in the determination of adherence of Staphylococcus

epidermidis. In this study it was also observed that D-mannosamine could inhibit between either the PVC catheter surface or the bacterial cell surface occurred with the amino sugar (Franson et al., 1984). More experiments are needed to understand why this amino sugar could inhibit attachment of this strain to plastics. Another semiquantitative method has been employed to compare adherence between different plastics. S. aureus coagulase negative staphylococci and Escherichia coli were compared in their ability to adhere to i.v. catheters made of PVC and PTFE (Sheth et al., 1983 a, b.). Segments of the catheter were placed in bacterial suspensions for 2 minutes, removed and rinsed. After rinsing, the catheters were rolled directly onto blood agar plates (Maki et al., 1977) and then cultures in tryptic soy broth (TSB). After 18 hours of incubation time they were rinsed and stained with crystal violet (C.V.) for examination under the microscope, and some were prepared for SEM.

When the segments of catheters were incubated in TSB, many bacteria would be expected to grow in the broth and multiply and the number of bacteria at the end of the incubation period would not closely reflect the number of original adherent organisms. This method is therefore only semiquantitative. Subject to this criticism, greater bacterial adherence to PVC than to PTFE catheters was shown. The coagulase-negative staphylococci adherence was greater on PVC than it was for an E. coli strain (Sheth et al., 1983 b).

The adherence characteristics of S. epidermidis and S. saprophyticus were investigated using poly (tetrafluoroethylene co-hexafluorpropylene) (FEP), and cellulose acetate (CA). The method consisted in immersing the segments of catheters in the bacterial suspension, after a certain period of incubation, the samples were rinsed and the adherent microroganisms were counted under the microscope. The results obtained showed that S. epidermidis was much less on to CA than to FEP (Hogt et al., 1983).

Botta et al., (1984), investigated the effect of washing of various organisms adherent to PTFE and polyethylene. The organisms were, Escherichia coli, Pseudomonas aerugonosa, Enterococci, Staphylococcus aureus and Candida sp. In this study adherence per se was not investigated. The results indicated that washing removed less organisms from the polyethylene rather than from the PTFE.

The adherence of Candida sp. to PTFE and to PVC has been studied. Yeast cells were labelled with C-14-Glucose, then the pieces of the catheters were immersed in the yeast suspensions. After an incubation time, the samples were washed and the organisms that remained attached to the surface of the materials were counted. The results obtained with this method showed more adherence to PVC than to PTFE (Rotrosen et al., 1983).

Another method used to study bacterial adherence is bioluminescence. E. coli adherence was investigated with this method, for quantifying adherence to polystyrene. The bacterial suspension was incubated in polystyrene tubes, after this the tubes were rinsed. The ATP of the adherent bacterial cells were extracted and measured by bioluminescence (Harber et al., 1983).

The adherence of Streptococcus mutans to glass surfaces was investigated. Bacterial cell suspensions were incubated in glass tubes, after a certain period of time the tubes were rinsed, and the cells that remained in the tube were resuspended in buffer, and the optical density (OD) was measured (Mukasa et al., 1973). Studies with this method showed that S. mutans synthesised a polymer in the presence of sucrose and it was required for adherence of this bacterium.

#### STRUCTURES INVOLVED IN BACTERIAL ADHERENCE TO INERT SURFACES

Data from several studies have given some information concerning possible structures involved in the mechanisms of adherence of bacteria to inanimate surfaces.

GLYCOCALYX, has been observed in many bacteria, adhering to different inert materials. Studies on adherence of a marine bacterium to millipore filters, demonstrated that an

extracellular acidic polysaccharide is involved in adherence (Fletcher and Floodgate 1973). Experiments on Thiobacillus albertis showed by glycocalyx is an important factor for attachment when tested on elemental sulfur tablets (Bryant et al., 1983).

A slimy material has been observed in some strains of Staphylococcus epidermidis. This slime has not been studied in great detail, but it commonly surrounds the bacterial cells when they adhered to glass or plastic. It was suggested to be involved in adherence of bacteria to inert surfaces or to be a substance that bind bacterial cells together (Christensen et al., 1982; Peters et al., 1982).

S. epidermidis adherence to FEP has also been stated to be related to protein containing structures from the bacterial cell surface (Hogt et al., 1983).

All the experiments reviewed above have limitations which are further considered in detail in the discussion. In summary the adherence is only measured semiquantitatively, and the viability of the adherent organisms was not considered, and these are the main issues addressed in the present study.

## SECTION 1

### M A T E R I A L S   A N D   M E T H O D S

#### ORGANISMS

Nine strains were used in these experiments. Three strains of Staphylococcus epidermidis and one strain of Staphylococcus capitis, were isolated from healthy volunteers, and five strains of Staphylococcus epidermidis were isolated from the CSF of patients with shunt infections. All the strains were identified by coagulase test and the API Staph-ident System (Analytab Products, N.Y).

#### MEDIA AND SOLUTIONS

BLOOD AGAR, was made with Columbia base agar (DIFCO) adding 5% Sheep blood.

OVERLAY AGAR, for adherence experiments consisted of Tryptone Soy Agar (OXOID), supplemented with Glucose 1% and Neutral Red (NR), (0.015%).

BROTH, used as growth medium for the adherence experiments was Tryptone Soy Broth (OXOID).

PHOSPHATE BUFFER SALINE (PBS). pH 7.2

Na Cl	0.8 g/l
K <sub>2</sub> HPO <sub>4</sub>	1.21 "
KH <sub>2</sub> PO <sub>4</sub>	0.34 "

ARTIFICIAL CEREBROSPINAL FLUID (C.S.F.), pH 7.2 (Mann et al., 1978).

This was made up according to:

NaCl .....	8.57 g/l
NA <sub>2</sub> HPO <sub>4</sub> .....	0.057 "
NaH <sub>2</sub> PO <sub>4</sub> -H <sub>2</sub> O .....	0.014 "
KCl .....	0.268 "
MgSO <sub>4</sub> .....	0.045 "
or-MgSO <sub>4</sub> -7H <sub>2</sub> O ...	0.0297 "
CaCl <sub>2</sub> .....	0.128 "
Glucose .....	1.8 ( "

BUFFER SOLUTIONS, for the experiment on effect of pH were made as follows:

Solution A, Na H<sub>2</sub> PO<sub>4</sub>.2H<sub>2</sub>O .... 31.2 g/l (0.2 M)

Solution B, Na<sub>2</sub> H PO<sub>4</sub> ..... 28.39 g/l (0.2 M)

Final buffer solutions were prepared as follows:

<u>ml</u> <u>A</u>	<u>ml</u> <u>B</u>	<u>ml</u> <u>H<sub>2</sub>O</u>	<u>pH</u>
87.7	12.3	100	6.0
74.5	26.5	100	6.4
51.0	49.0	100	6.8
28.0	72.0	100	7.2
13.0	87.0	100	7.6
5.3	94.7	100	8.0

## SURFACES USED IN ADHERENCE EXPERIMENTS

Surfaces used were:

Polystyrene dishes, Falcon #1007 manufactured from polystyrene with approximately 1% stearate as a plasticiser, with no other surface additives. The dishes were washed twice with distilled water and twice with alcohol. It was found that distribution of adherent colonies was more even on washed than on unwashed dishes.

Polytetrafluoroethylene (PTFE) tape, was purchased from a hardware store. It was washed twice with alcohol before use.

Catheters, Polyvinylchloride (PVC) Catheters (Deseret), medical grade Silastic (SIL) tubing (Dowing Corning Corporation) and Polytetrafluoroethylene (PTFE) Catheters (Jelco) were used as supplied.

PTFE tape and segments of catheters were attached to the bottom of petri dishes with a drop of paraffin wax at each end (Figure 1).

Microscope glass coverslips were used in the experiment which compared hydroxypolystyrene, glass and polystyrene. The coverslips were coated with hydroxypolystyrene and polystyrene.

## BACTERIAL SUSPENSIONS

The bacteria were cultured in broth (TSB) for 18 h at 37°C. After this the bacterial cells were washed three times with PBS. The suspensions of the bacteria were prepared in PBS or in CSF. The final concentration was  $10^5$ /ml adjusted spectrophotometrically and serial dilutions were prepared containing  $10^3$ ,  $10^2$ , and  $10^4$  c.f.u. per ml.

## ADHERENCE STUDIES

Ten ml of bacterial suspension in various suspending fluids (see results), were poured into the polystyrene petri dishes, five replica dishes were used per dilution. Five hours were usually allowed for attachment. The surfaces were then washed carefully using as far as possible a standard form of agitation. An overlay of molten TSA at 45°C with NR was poured, allowed to set, and incubated 48h at 37°C. The colonies on the plastic surfaces under the agar could be observed after this time, they were fixed with 5 ml of formaldehyde for 18 to 24 h. Then, the agar was removed and the colonies were stained with Crystal Violet (CV) and counted (figures 2, 3, 4, 5 and 6).

## CALCULATION OF INDEX OF ADHERENCE

This is defined as follows:

$$\% \text{ Index of adherence (I.A.)} = \frac{\text{N}^\circ \text{ of c.f.u./cm}^2 \text{ surface}}{\text{N}^\circ \text{ of c.f.u./ml inoculum}} \times 100$$

In the case of the polystyrene dishes and the PTFE tape the surface areas were calculated from direct measurement. The surface area of the Catheter segments were calculated for the upper half of the outside surface, because the washing under the lower part was incomplete and not reproducible.

### Example:

$$\begin{aligned} \text{Density of adherent colonies} &= 50 \text{ cfu/cm}^2 \\ \text{Inoculum} &= 10^5 / \text{ml} \\ \% \text{ IA} &= (50/10^5) 100 = 0.05 \end{aligned}$$

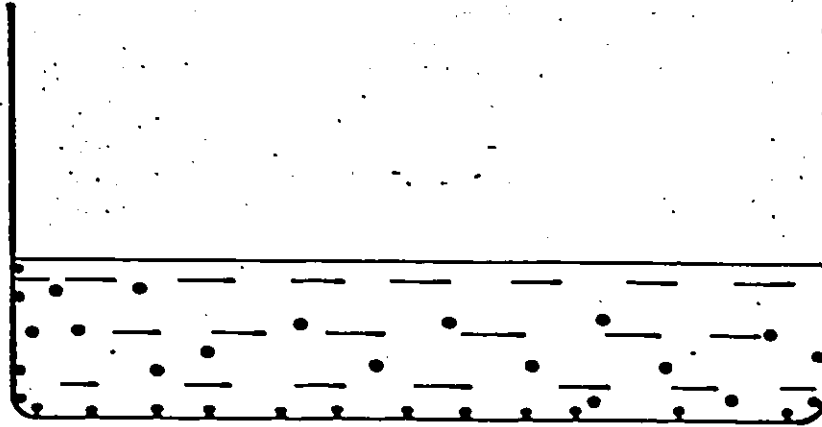
## EVALUATION

Three or five replicate dishes were used in each experiment at each dilution. The statistical tests used in each experiment are discussed in the results.

The % IA values of the median values for each set of replicates and the figures in parentheses are the mean deviations. Comparisons between groups were evaluated for significance by the Willcoxon non parametric rank test (Hollander and Wolfe, 1973).

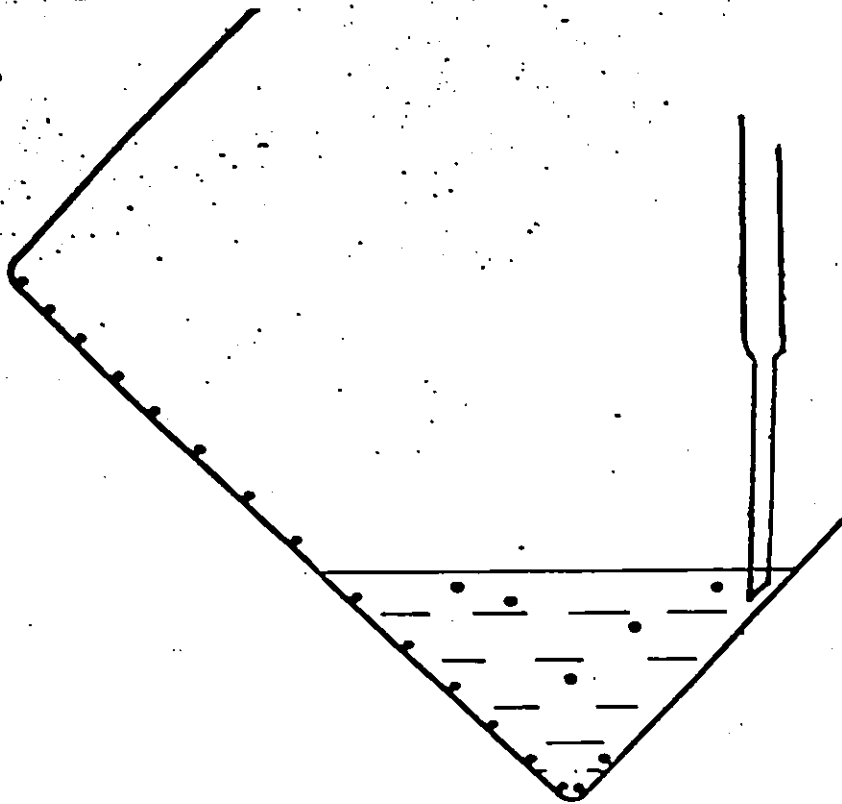


FIGURE 1.- PVC, SIL, AND PTFE CATHETERS IN POLYSTYRENE (POS) PETRI DISH. The materials were fixed at the bottom of the petri dish with molten paraffin at the ends.



Adhesion (5 hours)

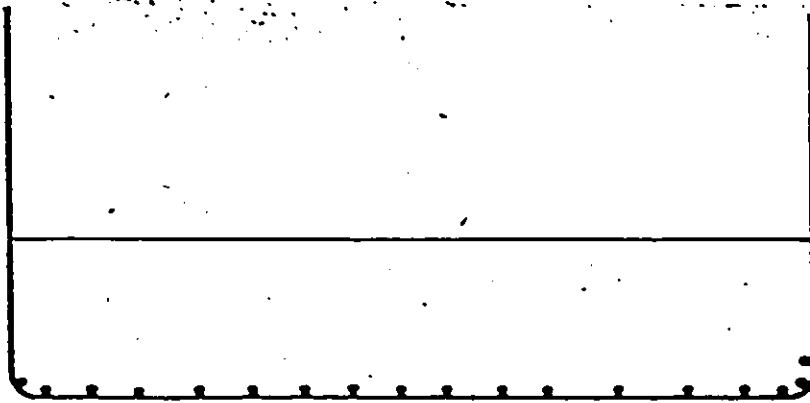
FIGURE 2.- ADHESION FOR FIVE HOURS. Ten ml of the bacterial suspension were poured into the polystyrene (POS) petri dishes which contains the different materials used as surfaces of attachment. The time allowed to attach was 5 hours.



WASH

FIGURE 3.- WASHING WITH PBS.-

The non adherent bacterial cells were removed by washing the surfaces 3 times with PBS.



### Agar Overlay

FIGURE 4.- AGAR OVERLAY.- After washing the surfaces, an agar containing NR is poured into the polystyrene petri dishes. This covered all the materials investigated. The colonies will be present on the materials surfaces, after 48 hours of incubation at 37'C.

COLOURED PICTURES  
Images en couleur

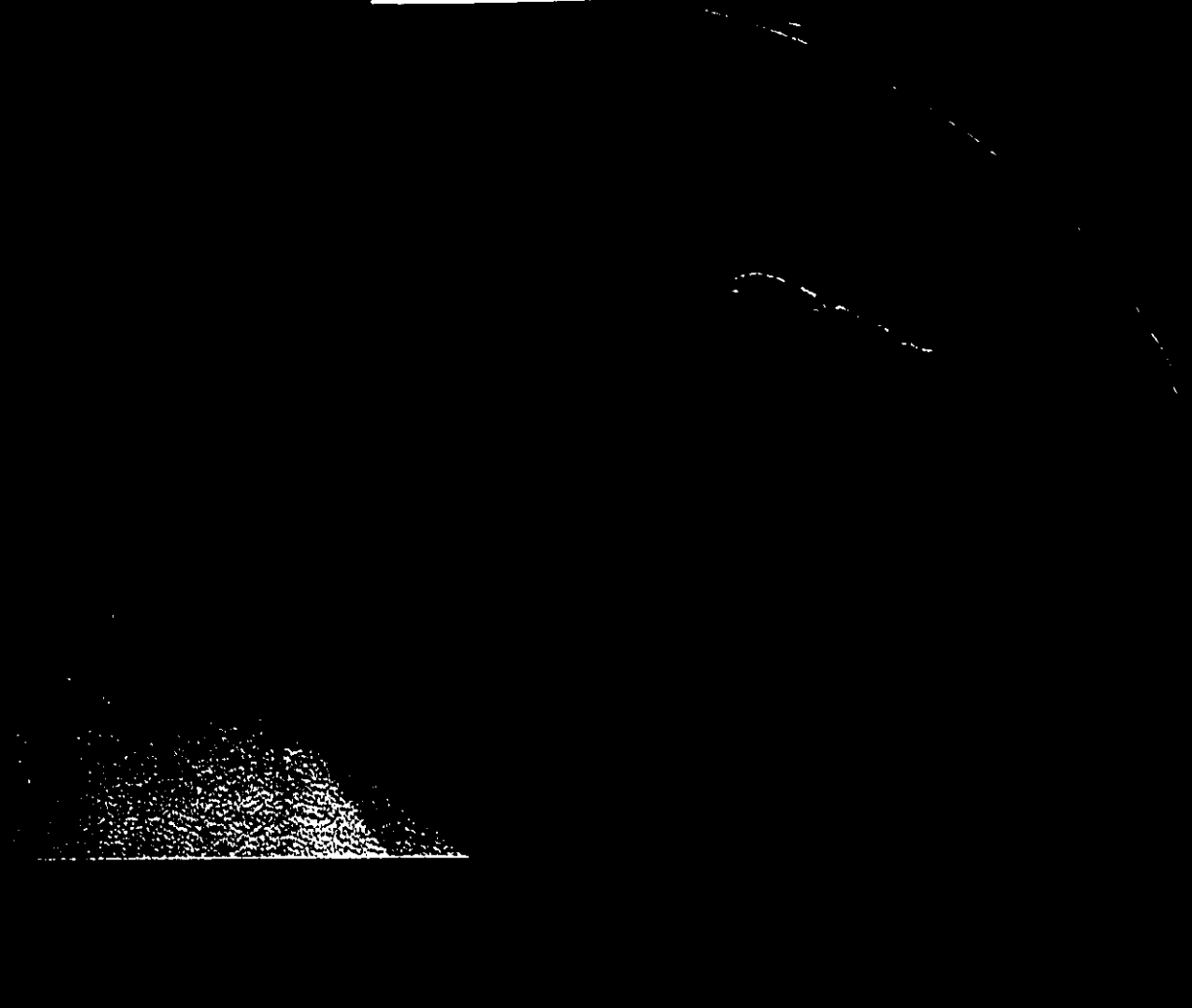


FIGURE 5.- REMOVING THE AGAR.- There are no colonies within the agar, indicating complete removal of non adherent organisms in the washing procedure.

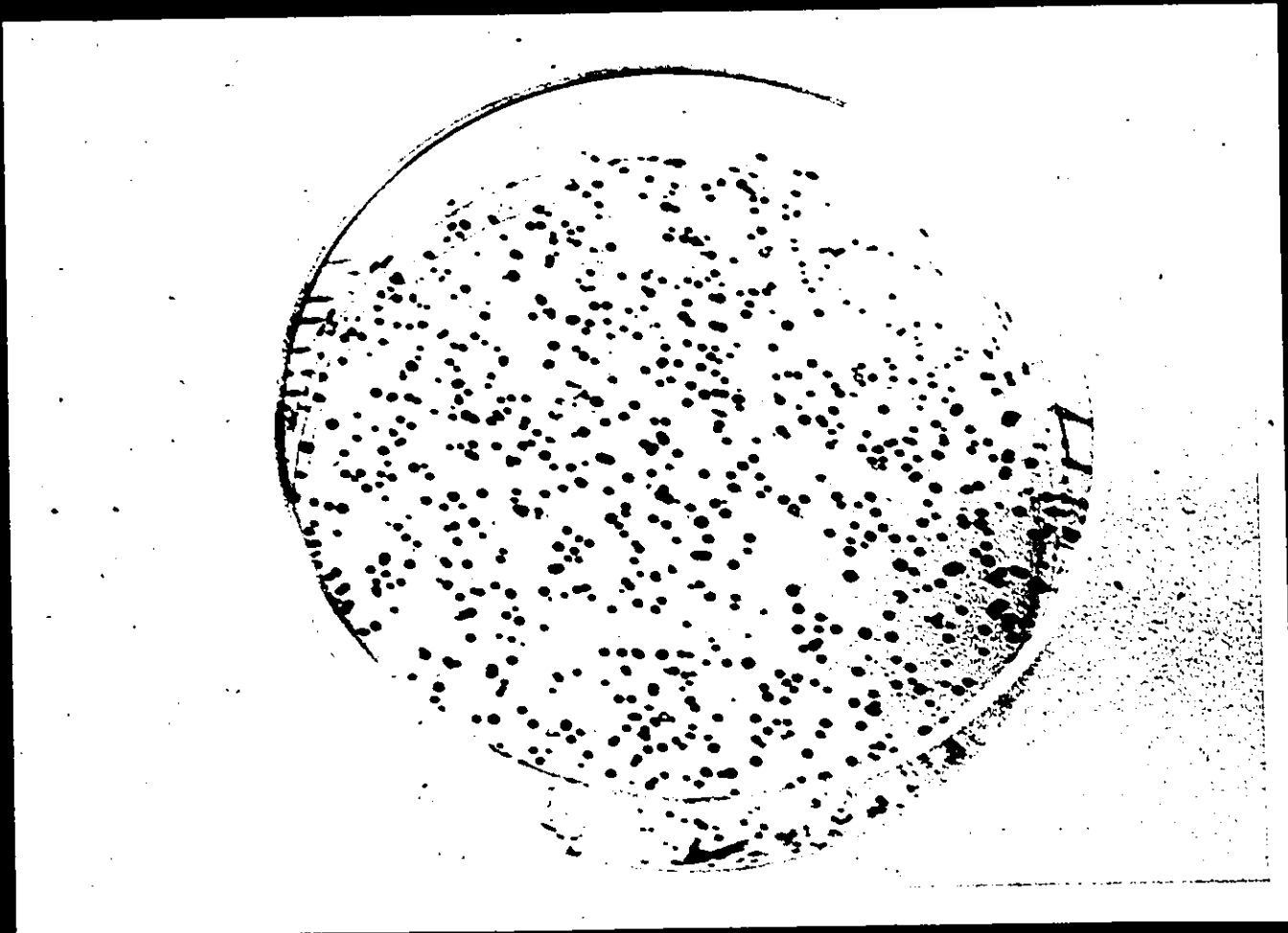


FIGURE 6.- STAINING WITH CV AND COUNTING.- After fixing, the adherent colonies were stained with crystal violet (CV), and then counted.

## SECTION 2

### RESULTS

Table 1 shows the results of an experiment to determine the reproducibility within a day. Three experiments were done under identical conditions with the same suspension of organism. Dilutions were prepared separately for each experiment. There is no significant difference between any of the three results.

Figure 7 and table 1 show the results of thirteen experiments comparing the difference between adherence of Staphylococcus epidermidis strain #3 to polystyrene and to PTFE-tape. A sample plate from these experiments is illustrated in figure 8. The day to day variation in the adherence of this organism to both polystyrene and PTFE is greater than the variation within the same day as is shown in table 2. There is a good correlation ( $R=0.72$ ,  $P 0.01$ ) between the adherence to polystyrene and to PTFE.

Tables 3 and 4 and figure 9 illustrate the relationship between time allowed for adherence and the % adherence index for polystyrene and PTFE tape. Three experiments were done. In the first two experiments (Table 3) adherence to polystyrene and to PTFE tape was measured at 1 hour and 5 hours, using Staphylococcus epidermidis strain #3. In this

experiment it was noted that adherence to polystyrene did not differ significantly between 1 hr. and 5 hr., but adherence to PTFE tape was increased at 5 hr. as compared with 1 hr. In the third experiment adherence was measured every 15 minutes from zero time to 1 hr. and at 5 hr., the result is shown in figure 9. Adherence to polystyrene was almost complete within 15 minutes, but adherence to PTFE tape was much slower and still very low at one hour compared with 5 hour. Those three experiments confirm that the time relationship of adherence of this strain differ between the two plastics. All subsequent experiments, and those illustrated in Tables 2, 3 and 4 and figure 9 were done within 5 hours adherence periods.

Experiments to define the role of temperature indicated that adherence at room temperature did not differ significantly from adherence at 37°C but that at 4°C adherence was reduced. This applied to polystyrene. However for PTFE-tape adherence was low at 4 C and at 37 C (Table 5).

Serum has a marked effect on adherence of Staphylococcus epidermidis strain #3 to polystyrene on which the adherence was significantly decreased, and to PTFE tape, on which the adherence was increased (figure 10). This effect was demonstrated to occur when serum was used in the suspending fluid (Table 7); and also when the surface was pretreated with serum and washed with PBS (Tables 8 and 9).

The adherence of Staphylococcus epidermidis #3 to different materials was investigated in 3 experiments. Results showed lower adherence to PTFE-catheter (Table 10 and figure 11).

The adherence of 9 different strains of Staphylococcus epidermidis to different catheter materials is shown in Tables 11, 12 and figure 12. The origin of the strains is shown in the materials and methods section. There is a progressive diminution in the index of adherence (IA) of all 9 strains in the order POS, PVC, SIL, and PTFE catheters.

Table 13 shows the results from an experiment in which adherence of Strain # 3 was compared between polystyrene and hydroxypolystyrene.

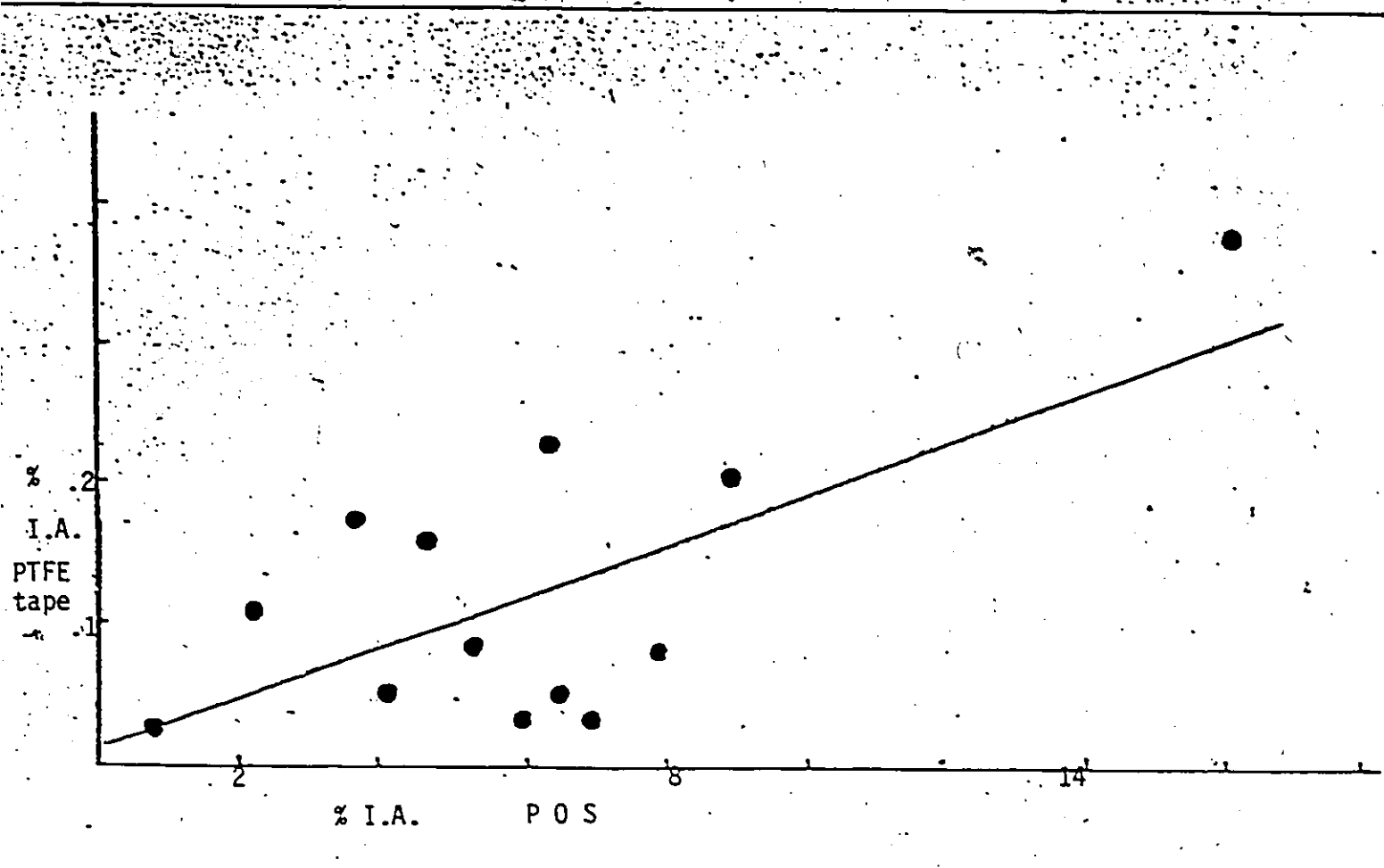


FIGURE 7.- ADHERENCE TO POLYSTYRENE AND PTFE-tape

Relationship between adherence to polystyrene and to PTFE tape in 13 experiments.

Staphylococcus epidermidis strain # 3.

Time of adherence 5 hours.

Five replicate plates per dilution.

Mean adherence to polystyrene 6.18

Mean adherence to PTFE tape 0.26

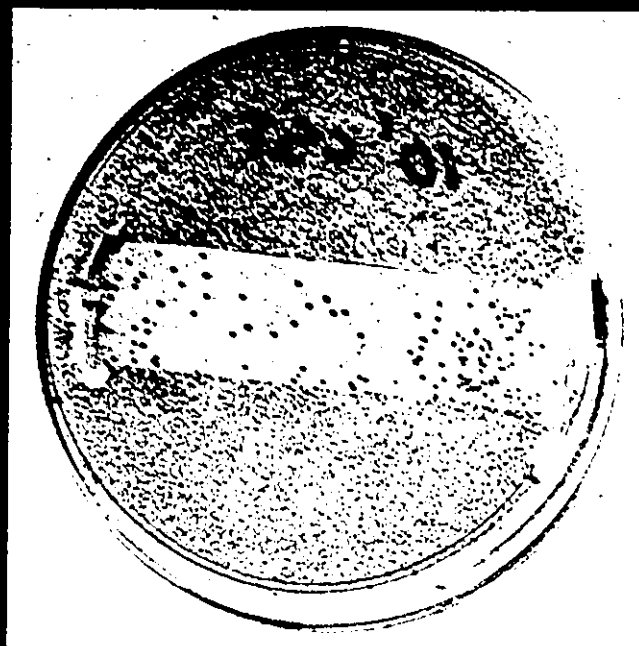


FIGURE 8.- ADHERENCE TO POS AND PTFE-tape. Adherence of Strain #  
3 is lower to PTFE.

TABLE 1

REPRODUCIBILITY BETWEEN DAYS

§ IA OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN #13,

OBTAINED IN 13 EXPERIMENTS IN DIFFERENT DAYS

ADHERENCE TO POLYSTYRENE AND PTFE-tape

POLYSTYRENE

POLYTETRAFLUOROETHYLENE-tape

16.7(±2.62)	0.78(±0.33)
8.84(±0.85)	0.43(±0.034)
7.83(±0.32)	0.18(±0.10)
7.0 (±1.14)	0.086(±0.015)
6.5 (±0.1)	0.12(±0.026)
6.36(±1.69)	0.47(±0.067)
6.0 (±0.80)	0.088(±0.01)
5.37(±0.57)	0.18(±0.069)
4.62(±0.59)	0.33(±0.052)
4.18(±0.36)	0.12(±0.081)
3.62(±0.34)	0.36(±0.005)
2.38(±0.54)	0.23(±0.055)
0.95(±0.17)	0.075(±0.035)

TABLE 2

REPRODUCIBILITY WITHIN A DAY

---

<u>EXPERIMENT #</u>	<u>MEAN OF § I A</u>
1	6.7 (+0.76)
2	5.8 (+0.6)
3	6.0 (+0.7)

---

Adherence of Staphylococcus epidermidis strain # 3 to polystyrene dishes. Three separate experiments were performed of 5 plates per dilution. Time of adherence 5 hours.

TABLE 3

TIME RELATIONSHIP OF ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS  
STRAIN #3 TO POLYSTYRENE AND PTFE-tape

---

<u>TIME</u>	<u>HOURS</u>	<u>POLYSTYRENE</u>		<u>P T F E -tape</u>	
		<u>EXP # 1</u>	<u>EXP # 2</u>	<u>EXP # 1</u>	<u>EXP # 2</u>
1		2.78(+0.59)	2.56(+0.03)	0.025(+0.01)	0.13(+0.017)
5		5.37(+0.57)	4.6(+0.59)	0.18(+0.04)	0.33(+0.05)
		P = 0.004	P = 0.004	P = 0.016	P = 0.004

---

Organism: Staphylococcus epidermidis strain # 3.

Time of adherence 1 and 5 hours

Five plates per dilution.

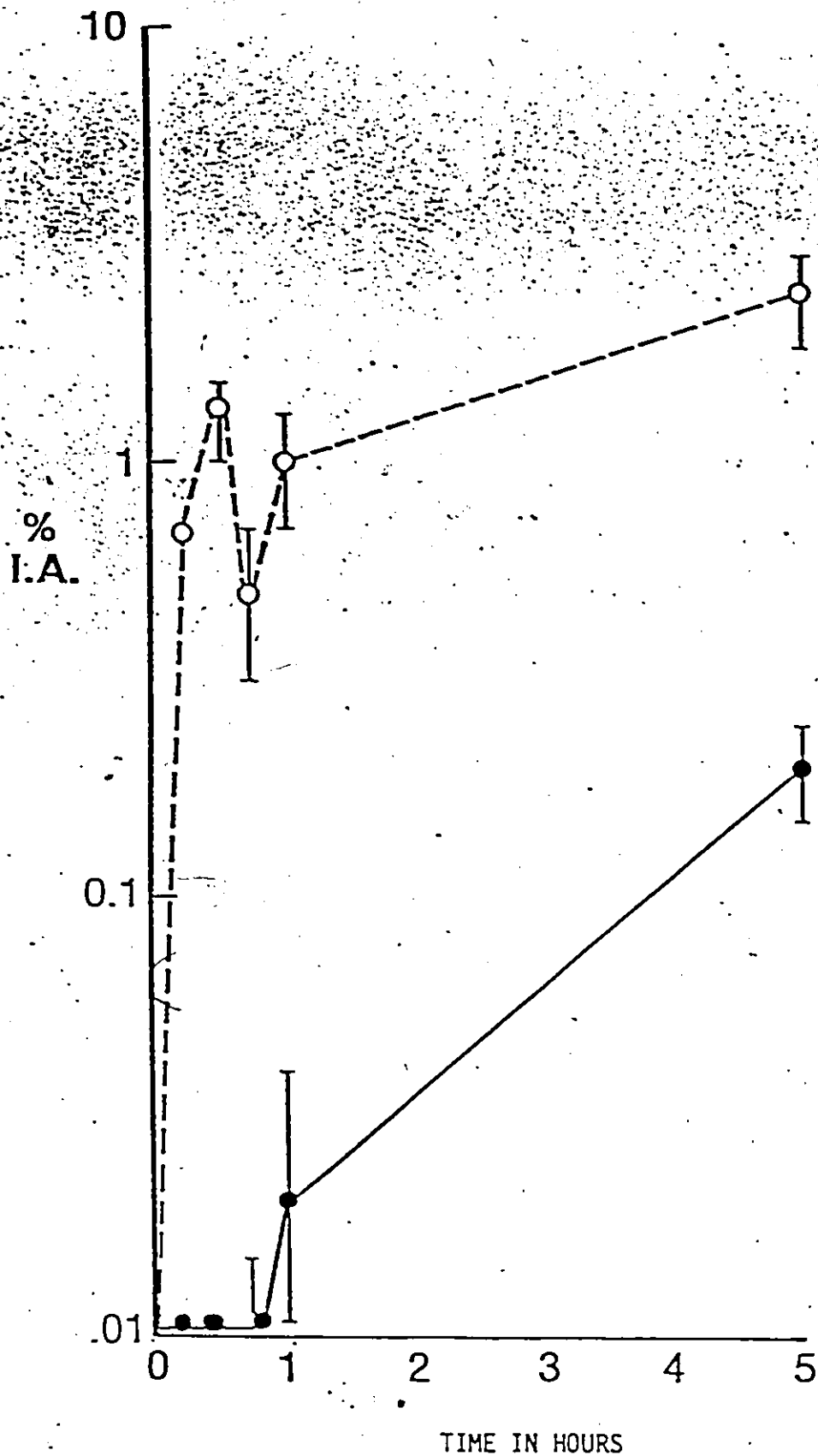


FIGURE 9.- TIME RELATIONSHIP OF ADHERENCE TO POS AND PTFE-tape. — Adherence of Strain # 3 measured in intervals of 15 minutes during 1 hour, and every hour during 5 hours. ● = PTFE ○ = POS

TABLE 4

TIME RELATIONSHIP OF ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS  
STRAIN #3 TO POLYSTYRENE AND PTFE-tape

---

<u>TIME HOURS</u>	<u>POLYSTYRENE</u>	<u>PTFE-tape</u>
1	2.8(+0.5)	0.10(+0.005)
2	5.0(+0.001)	0.17(+0.01)
3	4.3(+0.008)	N. D.
5	7.8(+0.3)	0.18(+0.10)
	P=0.05 (1-5H)	P=0.05 (1-5H)

---

Adherence of Staphylococcus epidermidis strain # 3.

Three replicate dishes per dilution.

Time of adherence 1,2,3 and 5 hours

TABLE 5

EFFECT OF TEMPERATURE ON ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN #3 TO POLYSTYRENE AND PTFE-tape

<u>TEMPERATURE</u>	<u>POLYSTYRENE</u>	<u>PTFE-tape</u>
room temp	6.5(+0.1)	0.12(+0.026)
4°C	0.1(+0.01)	0.023(+0.009)
	P < 0.05	P < 0.05
room temp	6.3(+0.16)	0.47(+0.067)
37°C	7.0(+3.8)	0.067(+0.038)
	P = 0.421	P = 0.004

Organism: Staphylococcus epidermidis strain # 3.

Five replicate dishes per dilution.

Time of adherence 5 hours, during which dishes were held at the temperature indicated.

TABLE 6

INVESTIGATION ON EFFECT OF pH ON ADHERENCE OF STAPHYLOCOCCUS  
EPIDERMIDIS STRAIN #3 TO POLYSTYRENE AND PTFE-tape

---

<u>pH VALUE</u>	<u>POLYSTYRENE</u>	<u>PTFE-tape</u>
6.0	6.3(+0.6)	0.17(+0.01)
6.4	6.6(+0.5)	0.14(+0.06)
6.8	7.2(+0.5)	0.11(+0.09)
7.2	6.0(+0.8)	0.068(+0.018)
7.6	6.2(+1.0)	0.27(+0.13)
8.0	6.8(+0.2)	0.20(+0.04)

---

Organism: Staphylococcus epidermidis strain # 3.

Suspending medium: Buffered Saline at pH values indicated.

Solutions made up as shown in Materials and Methods.

Time of adherence 5 hours.

Five replica plates per dilution.

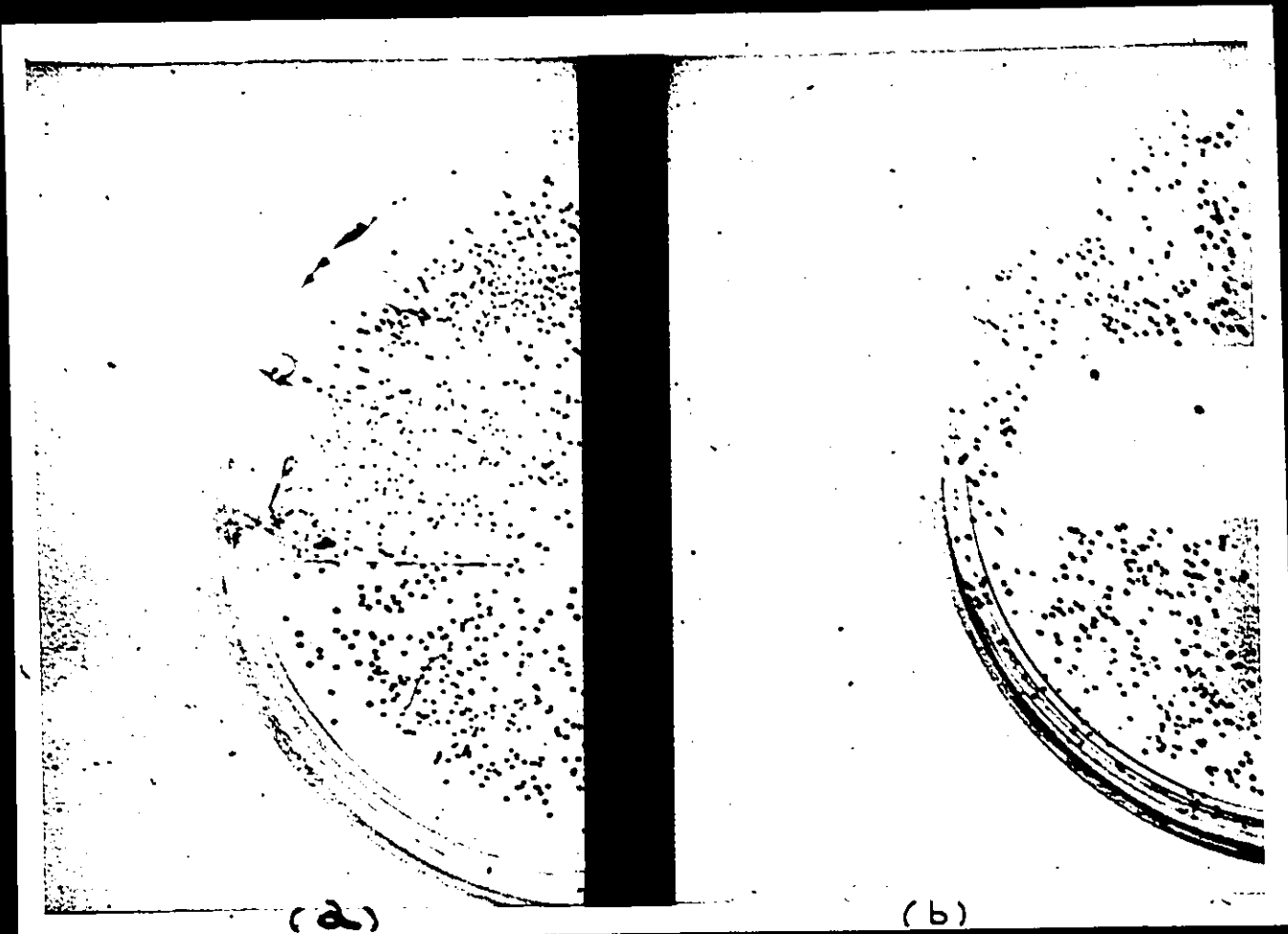


FIGURE 10.- EFFECT OF SERUM ON ADHERENCE TO POS AND PTFE-tape.-  
The presence of serum increased the adherence of Strain # 3 to PTFE-tape, and decreased adherence to POS (a). In the serum free control, adherence was higher to POS (b).

TABLE 7

EFFECT OF THE PRESENCE OF SERUM (10%) ON THE  
ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN #3 TO POLYSTYRENE  
AND PTFE-tape. SERUM ADDED TO THE BACTERIAL SUSPENSION

---

<u>MATERIAL</u>	<u>CSF SERUM FREE</u>	<u>CSF SERUM</u>	<u>P</u>
<u>POLYSTYRENE</u>			
EXP # 1	9.54(± 0.82)	2.42(± 0.16)	0.004
EXP # 2	7.0 (± 1.1)	1.73(± 0.24)	0.004
<u>PTFE-tape</u>			
EXP # 1	0.12(± 0.08)	2.37(± 0.24)	0.004
EXP # 2	0.086(± 0.015)	2.5(± 1.0)	0.004

---

Organism: Staphylococcus epidermidis strain # 3.

Time for adherence 5 hours.

Five replicate plates per dilution.

Serum was added to artificial CSF (10%).

TABLE 8

PRETREATMENT OF POLYSTYRENE AND PTFE-tape SURFACES WITH SERUM  
(108) IN THE STUDY OF ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS  
STRAIN #3

---

<u>MATERIAL</u>	<u>CSF SERUM FREE</u>	<u>SERUM PRETREATED</u>	<u>P</u>
POLYSTYRENE	16.7(+2.6)	5.15(+1.0)	0.004
PTFE-tape	0.78(+0.33)	4.75(+0.61)	0.004

---

Experimental details as in Table 6.

Surfaces were pretreated with serum for 5 minutes and then washed 3 times with PBS.

TABLE 9

PRETREATMENT OF CATHETERS WITH SERUM IN THE STUDY OF ADHERENCE  
OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN #3

---

<u>MATERIALS</u>	<u>CSF SERUM FREE</u>	<u>SERUM PRETREATED</u>	<u>P</u>
POLYSTYRENE	7.37(+ <u>1.0</u> )	2.3(+ <u>0.68</u> )	0.004
P V C	2.2(+ <u>0.44</u> )	1.9(+ <u>0.42</u> )	>0.05
SILASTIC	2.5(+ <u>1.0</u> )	2.5(+ <u>0.72</u> )	>0.05
P T F E cath.	0.14(+ <u>0.06</u> )	0.33(+ <u>0.07</u> )	0.028

---

Experimental details as in Table 6.

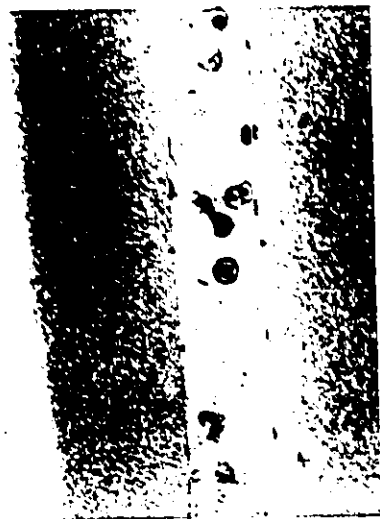
Surfaces were pretreated with serum (10%) for 5 minutes.



a



b



c

FIGURE 11.- ADHERENCE TO DIFFERENT CATHETERS.- The adherence was lower on PTFE-catheter (c), the other materials are PVC (a) and SIL (b).

TABLE 10

ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN # 3 TO DIFFERENT MATERIALS

---

<u>MATERIALS</u>	<u>% I A</u>		
	EXP 1	EXP 2	EXP 3
POLYSTYRENE	0.7(+0.05)	7.37(+0.59)	9.94(+0.39)
P V C	0.12(+0.02)	2.2(+0.44)	5.0(+0.5)
SILASTIC	0.18(+0.02)	2.5(+1.0)	1.7(+0.074)
PIFE cath.	0.007(+0.008)	0.14(+0.06)	1.9(+0.5)

---

Adherence of Staphylococcus epidermidis strain # 3 to different catheters.

Three replicate plates per dilution.

Time of adherence 5 hours.

TABLE # 11

ADHERENCE OF DIFFERENT STRAINS TO POLYSTYRENE AND PTFE-tape

STRAINS	POLYSTYRENE		PTFE-tape	
	EXP 1	EXP 2	EXP 1	EXP 2
*C228	6.0 ( $\pm$ 0.38)	0.22( $\pm$ 0.04)	0.014( $\pm$ 0.001)	n. a.
1K	4.3 ( $\pm$ 0.45)	18.00( $\pm$ 4.77)	1.2 ( $\pm$ 0.24)	1.66( $\pm$ 0.16)
*C117	1.63( $\pm$ 0.3)	0.96( $\pm$ 0.41)	<0.0025	0.22( $\pm$ 0.09)
*S. 3	1.07( $\pm$ 0.3)	8.84( $\pm$ 0.85)	0.31 ( $\pm$ 0.1 )	0.43( $\pm$ 0.03)
*C1006	0.89( $\pm$ 0.39)	n. d.	<0.0025	n. d.
5N	0.71( $\pm$ 0.18)	0.54( $\pm$ 0.04)	0.45( $\pm$ 0.13 )	0.34( $\pm$ 0.16)
4D	n. a.	20.60( $\pm$ 0.93)	n. a.	0.21( $\pm$ 0.18)
3L	incountable	3.82( $\pm$ 0.19)	1.00( $\pm$ 0.44)	0.09( $\pm$ 0.04)

1K, 3L, 4D and 5N, are strains isolated from skin.

\* strains are from shunt infections.

Time for adherence 5 hours.

Three replicate plates per dilution.

10  
% I.A.

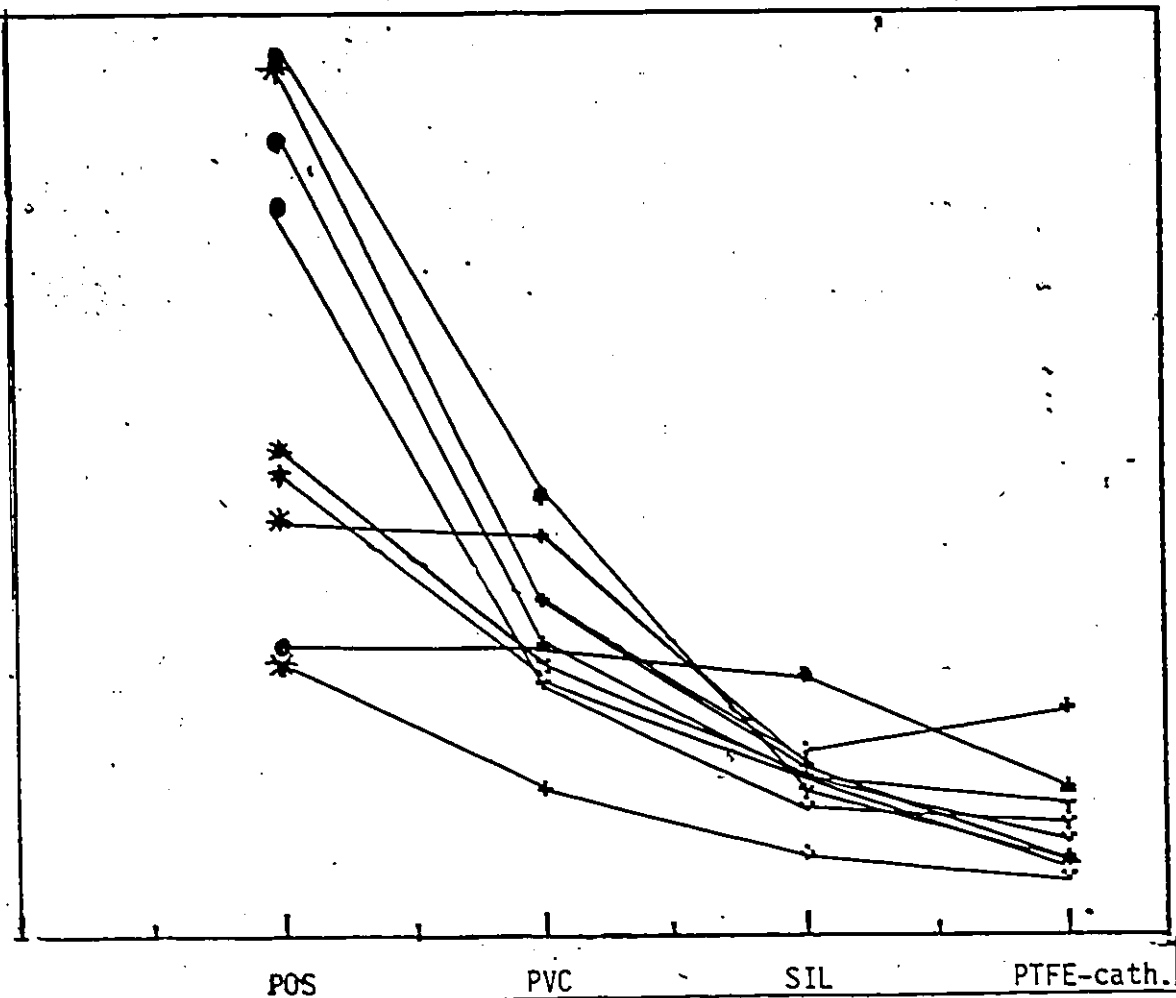


FIGURE 12.- ADHERENCE OF 9 STRAINS TO DIFFERENT MATERIALS.

Three plates per dilution.

Adherence time 5 hours.

The lines join experimental observations in the same strain.

The materials are POS, PVC, SIL and PTFE-catheters.

● = Skin isolated.

\* = Isolated from shunt infections.

TABLE 12

ADHERENCE OF DIFFERENT STRAINS OF COAGULASE-NEGATIVE STAPHYLOCOCCI TO DIFFERENT CATHETERS

<u>STRAINS</u>	<u>POS</u>	<u>FVC</u>	<u>SIL</u>	<u>PIFE -cath</u>
IK	18.6(+0.46)	7.8(+1.4)	2.92(+0.88)	3.87(+0.61)
*C1006	18.6(+0.1)	8.8(+0.96)	2.1(+0.02)	0.41(+0.03)
SN	16.8(+1.36)	5.38(+0.74)	4.68(+0.93)	2.0(+0.15)
3L	15.3(+0.95)	4.51(+0.55)	2.5(+0.41)	0.96(+0.14)
*S.3	9.94(+0.39)	5.0(+0.53)	1.72(+0.07)	1.93(+0.51)
*C117	9.33(+0.47)	4.77(+0.4)	2.55(+1.0)	1.25(+0.15)
*C228	8.3(+2.0)	6.37(+0.54)	2.5(+0.36)	0.25(+0.04)
4D	5.3(+0.4)	5.33(+0.29)	2.25(+0.66)	1.74(+0.27)
*C1455	4.84(+1.0)	2.0(+0.29)	0.56(+0.22)	0.12(+0.43)
	P = 0.005	P < 0.05		P = 0.016

Experimental details as in Table #10.

P values indicate significance of difference in adherence between different plastics for the whole population of strains.

TABLE 13

ADHERENCE OF STAPHYLOCOCCUS EPIDERMIDIS STRAIN #3  
TO POLYSTYRENE, GLASS AND HYDROXYPOLYSTYRENE

<u>POLYSTYRENE</u> <u>DISHES</u>	<u>GLASS</u>	<u>POS ON GLASS</u> <u>COVERSLIPS</u>	<u>HYDROXYPOLYSTYRENE</u> <u>ON GLASS COVERSLIPS</u>
0.60(± 0.26)	10.39(± 1.90)		
1.13(± 0.36)		2.10(± 0.5)	
1.61(± 0.76)			3.68(± 1.43)

Organism: Staphylococcus epidermidis strain # 3.

Three replicate plates per dilution.

Time for adherence 5 hours.

Glass coverslips were coated with polystyrene and hydroxypolystyrene.

## DISCUSSION

### THE METHOD

The new method described in this thesis represents an advance on previous methods for the measurement of Bacterial Adherence to inanimate surfaces.

The methods that have been used up to now all require a relatively high density of challenge inoculum ( $10^8$  to  $10^9$  cfu/ml) and also measure adherence only without regard to the viability of the adherent organisms. This method measures not only adherence but also viability.

The method is precise and reproducible as colony counts are performed for the enumeration of both the inoculum and the viable adherent microorganisms. The method has very high sensitivity as it would be possible to measure a small number of adherent organisms from a high inoculum density. In these experiments a relatively low inoculum density (dilution ranging from  $10^5$  to  $10^2$  cfu/ml) was used. It is likely that clinical infections are initiated from low inocula which increases the relevance of this model.

The reproducibility within a day is high (table 1) but between day reproducibility is rather less. This could be due to variations in the bacteria, in the suspending medium or in the surfaces.

The suspending medium was a simple electrolyte solution resembling CSF as far as possible but without the protein, which was omitted in order to retain the medium as a defined medium. High grade chemicals were used and it is unlikely that the day to day variation was the result of variations in the suspending medium. It is possible that there were variations in the surfaces to which adherence was being measured. It was not possible to do all the experiments on a single batch of petri dishes and therefore variation of adherence due to inconstancy in the manufacturing process can not be excluded, but a single product was used throughout for the polystyrene and it was confirmed with the manufacturer that no surface additives were present on the type of dish used in this study.

It has been shown that growth conditions and growth phase can affect adherence of organisms. Studies with marine *Pseudomonas* have been done to investigate the effect of culture concentration, age, time and temperature on adherence (Fletcher 1977). The number of attached bacterial cells increased with both the inoculum density and the time allowed for attachment, and the attachment of this marine bacterium was also dependent on growth phase, culture and temperature. Log-phase cultures adhered more readily to polystyrene followed by stationary and death-phase cultures. It is possible that synthesis of the adhesive substance may be influenced by the growth phase of the bacterial cells as a result of variations in the cell surface polymers during the growth cycle (Fletcher and Floodgate, 1973). Other investigators studied *Pseudomonas aeruginosa* cells from different

stages of growth cycle and examined their ability to adhere to stainless steel. Results showed that cells in early or late log-phase adhered better than those in stationary phase. This agrees with the results from Fletcher (1977). It was suggested that there was a change in the surface of stationary phase bacterial cells which may change their ability to adhere (Stanley 1983). In contrast to these results, Hogt et al (1983), stated that the growth phase did not affect the adherence of Staphylococcus epidermidis to FEP. Studies on adherence of bacteria to hydrocarbons have shown that Serratia marcescens adhered strongly to hexadecane, xylene and octane (hydrocarbons) when tested with stationary phase cells and very little adherence was observed with those in the exponential phase (Rosenberg and Rosenberg 1980).

All the experiments in this study were done on stationary phase organisms from overnight growth. This procedure was kept constant and the same batch of broth was used in all experiments.

Figure 7. demonstrates that there is a good correlation ( $R=0.72$ ) for strain # 3 between adherence to the PTFE-tape and adherence to polystyrene. The adherence to the PTFE is less than to polystyrene, and this difference is highly significant. This correlation establishes the validity of comparisons between different surfaces done in a single experiment. It also establishes the necessity to include in all experiments a standard surface and to relate indices of adherence to this standard. In this work the standard surface was polystyrene dishes (Falcon).

## EFFECT OF DIFFERENT ENVIRONMENTAL FACTORS ON ADHERENCE.

### TIME

The time dependance of the adherence process was investigated in three experiments (Tables 2, 3 and figure 9)

In all cases the adherence at 5 hours was greater than the adherence at one hour for both PTFE tape and polystyrene. Figure 9, illustrates the results of the experiments in which adherence was measured every 15 minutes for the first hour and then at 5 hours. Adherence to polystyrene was almost complete at 15 minutes as compared with 5 hours, whereas the adherence to PTFE was delayed and even at the end of 45 minutes was minimal.

Other investigators have found that adherence is a time dependent phenomenon (Marshall et al., 1971; Fletcher 1977, 1979; Feldner et al., 1979; Stanley, 1983; Peters et al., 1982). When Pseudomonas aeruginosa adherence was studied using stainless steel it was found that bacterial cells began to attach in less than one minute and the number adhering to the surface increased with time (Stanley 1983). In studies on colonisation of intravenous catheters by coagulase negative staphylococci the observation by SEM of the catheters demonstrated colonisation progressing with an increase in the exposure interval (Peters et al., 1982). At least two stages are recognised in the attachment of bacteria to inert surfaces: the reversible sorption or primary or temporary adhesion and the secondary sorption or irreversible

adhesion. One possible explanation for the results of these timing experiments is that two types of mechanisms are involved when bacteria adhere to surfaces.

Reversible adherence of bacterial cells is governed by brownian motion which acts to randomize the distribution of the cells and also increases the number of bacterial collisions with the surface of attachment increasing in this way the opportunity for attachment. Brownian motion may also enable the bacterial cells to overcome the electrostatic forces of repulsion that exist between the bacterial cell surfaces and the inert surface of attachment (Curtis 1973). At this stage the bacterial cells are easily removed by washing the surfaces, and after this stage, the secondary or irreversible attachment takes place in which the bacterial cells can not be removed from the surface (Marshall et al., 1971). Irreversible attachment may include the production or synthesis of some adhesin that does not permit removal of the bacterial cells after a period of time.

These results in the present study are compatible with this hypothesis. The primary or reversible attachment occurs when the staphylococci are settling down on the surface, and they are easily removed by rinsing the surface. This was shown in the time variation experiment in which bacterial cells adhered in low numbers to polystyrene during the first 15 minutes. At this stage, bacterial cells are removed when the surface is washed. After 5 hours, the number of adhering bacterial cells was high and they could not be easily washed off. This is the irreversi-

ble attachment (figure 9).

### THE EFFECT OF TEMPERATURE ON ADHERENCE

Temperature was another factor investigated in the attachment of Staphylococcus epidermidis strain # 3. It was found that lowering of the temperature decreased the number of bacterial cells that attached to polystyrene and PTFE-tape.

Adherence studies with psychrophilic marine pseudomonas have been done at low and room temperatures. The results demonstrate that low temperatures diminish adherence. However when bacterial cells were observed at low and room temperatures electron microscopic examinations did not show obvious structural differences (Fletcher 1977).

These results were obtained with a marine Pseudomonas in sea water, which contains 3% salt, and the relevance to our experimental situation is questionable.

Studies with mycoplasmas, resulted in very little or non adherence when the temperatures were low ( 4 °C or 0 °C) and the best adherence was obtained at 37 °C (Gorsky et al., 1977; (Feldner et al 1979).)

There are two alternative explanations for these results:

- 1- that the synthesis of an adhesin is diminished at 4°C,
- 2- that the adherence process it self does not proceed at the lower temperature even though preformed adhesin may be present.

### THE EFFECT OF pH ON ADHERENCE

The effect of pH on the adherence of strain # 3 was of interest. Over the range pH= 6.0 to pH= 8.0 there was no change in adherence of this organism to either polystyrene or PTFE-tape.

In studies with E. coli adherence to polystyrene pH= 6.8 was optimal for attachment of the strongly adherent strains, and for those classified as weakly adherent variation in pH did not affect the attachment of this microorganism (Harber et al., 1982). In the case of marine bacteria pH and electrolyte concentration significantly affected adherence (Fletcher 1973).

Hogt et al (1983) found that attachment of coagulase-negative staphylococci was not affected by changes in the pH from 5.0 to 9.4 when they tested adherence to FEP surfaces.

The results in the present study are similar. The pH stability of the adherence process indicates that the adhesin is both stable and able to function over the pH range tested.

### EFFECT OF SERUM

The effect of the addition to the suspending medium of 10% serum was measured. It was found that the presence of serum consistently caused a small reduction in the % IA for polystyrene and a substantial increase in the % IA for PTFE. This effect was demonstrated with the addition of serum to the suspending medium, or when the serum was preadsorbed to the surface.

The attachment of Mycoplasma pneumoniae to glass surfaces is reduced by the addition of BSA but subsequent addition of BSA after attachment does not cause organisms to become detached (Feldner et al., 1979). Attachment of marine pseudomonas strains to polystyrene is also reduced by BSA when added to the bacterial suspension or as a preadsorbed film (Fletcher, 1975).

One possible explanation for the effect of protein in our experiments is that the protein adsorbs to the surface to cover it completely, and that the adherence index being measured relates to the protein rather than to the underlying plastic surface. Steric exclusion has also been suggested as a mechanism by Maroudas (1975).

Whatever the mechanism this effect of protein is important in the interpretation of these results relative to the clinical situation in which serum proteins would normally be present in the "in vivo" situation. The demonstration that serum increases adherence to PTFE is therefore important, as it reduces the apparent advantage of PTFE in the clinical context.

#### ADHERENCE TO DIFFERENT MATERIALS

All 9 strains of Staphylococcus epidermidis in our study showed a progressive diminution in adherence to the different plastics studied in the order POS- PVC- SIL- PTFE. This was true both for the population as a whole and also in respect of each individual strain (figure 11)

Greater adherence to polystyrene as compared to PTFE has been previously noted in the case of a marine *Pseudomonas* (Fletcher 1979) *Staphylococcus epidermidis* (Hogt et al., 1983) and coliform organisms (Botta et al., 1984). Comparisons between PVC and PTFE have been done in the case of *Candida* (Rotrosen et al., 1984) and coliform organism (Botta et al., 1984). The results were similar showing that adherence to PTFE was less than to PVC.

Comparisons between Silastic and other plastics as described here have not been previously reported.

The reason for the difference in adherence of organisms to different plastics is not known. A relationship between adherence and the angle of contact of water (wettability) of the plastic has been reported. The higher the angle of contact the more water repellent the plastic is and the less readily do bacteria adhere (Fletcher 1975).

The results in this thesis would be consistent with this explanation.

Hydrophobicity has been related to adherence of bacteria. *Staphylococcus epidermidis* and *S. saprophyticus* have been studied in relation to adherence to biomaterials. *Staphylococcus epidermidis* showed more hydrophobic character and higher adhesion to FEP than the encapsulated *S. saprophyticus*. Decreased hydrophobicity resulted in lower adhesion. It was suggested that interactions between *S. epidermidis* and FEP are mainly caused by hydrophobic bonding (Hogt et al., 1983.)

Rosenberg and Rosenberg (1980) studied bacterial adherence

to hydrocarbons. They showed that Staphylococcus aureus and Serratia marcescens adhered to a variety of liquid hydrocarbons. Hydrophobicity increased in Serratia marcescens with increase in culture age and Escherichia coli rough mutants were observed to be more hydrophobic than non rough strains. In general, the adherence characteristic of these strains increased with the hydrophobicity.

## CONCLUSIONS

In infections with implants and devices, "in vivo", thrombus formation, fibrin deposition, attachment of platelets and the presence of an inflammatory reaction in the vicinity of the foreign body, as well as bacterial adherence, might also play a part in the establishment of the infection process.

Prosthetic infections can be classified into early and late infections according to the time between operative insertion and the manifestation of the symptoms. The majority of the prosthetic infections are early infections (Garvey, 1980) and are probably intraoperative contamination due to deposition of the bacteria onto the surface of the device.

Primary bacterial adherence itself to the prostheses is likely to be the most important factor in the initiation of these infections.

The study described here demonstrates the readiness with which Staphylococcus epidermidis adheres to plastic surfaces. There are significant differences in the degree of adherence to the various plastics examined in this study. It appears from the point of view of prevention of infection that PTFE would be the most satisfactory material for the manufacture of prostheses.

One approach to prevention of prosthetic infection might be inhibition of the initial process of adherence. This might be accomplished by the design of plastics to which the bacteria do

not adhere, or by physiological prevention of the process of adherence. The experiment with hydroxypolystyrene represents an attempt at the first of these two approaches. It was hoped that the hydroxypolystyrene might prove toxic to the bacteria by virtue of the incorporation of phenolic groups into the structure of the plastic, but both adherence and growth were as great on the hydroxypolystyrene as on the polystyrene. It is possible however that a more extensive investigation might yield a polymer with the required properties.

The idea of preventing adherence is more promising. The data in this thesis are consistent with, but do not prove, the role of lipoteichoic acid in adherence of Staphylococcus epidermidis to plastic surfaces. It has been shown by Beachey (1980), that adherence of Streptococcus pyogenes to erythrocytes is mediated by LTA and that this adherence is reduced by pretreatment of the bacteria with penicillin. It would be appropriate using the system described in this thesis, to investigate effects of antibiotics on adherence of Staphylococcus epidermidis to plastics. Cationic detergents and penicillin can be adsorbed to the surface of plastics and this might be used to inhibit adherence or growth after adherence. These are two examples of the way in which the experimental method developed in this thesis might be used to investigate the possibility of preventing prosthetic infections by preventing adherence or growth of adherent organisms.

## CONTRIBUTION TO KNOWLEDGE

This thesis represents an important step in the search for a method to study the Bacterial Adherence. The new method developed here, might be useful to study adherence of organisms to inert materials and the possible inhibition of it.

- This is the most precise and quantitative comparison between different plastic materials in the study of adherence of Staphylococcus epidermidis. It is the first method that measures accurately the viability of the adherent organisms.

- It is the first work that include SILASTIC as surface of adherence.

- It is also the first report on effect of Temperature and presence of Serum on adherence of Staphylococcus epidermidis to inert plastic materials.

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