The efficacy of Povidone-Iodine in reducing iatrogenic bacteraemia when used as a pre-treatment mouth rinse.

by

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CANDIDATE'S CERTIFICATE

This is to certify that the work presented in this thesis was carried out by the candidate in the Faculty of Dentistry, University of Sydney, Westmead Hospital Centre for Oral Health and Westmead Hospital Institute of Clinical Pathology and Medical Research. It has only been submitted to the University of Sydney for a higher degree.

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ABSTRACT

Bacteraemia caused by scaling may be implicated in infective endocarditis in "at risk" patients. This study investigated the effect of two one-minute pre-treatment rinses with 0.4% povidone-iodine solution on bacteraemia caused by ultrasonic scaling. Sixty healthy volunteers with plaque-induced gingivitis were recruited from the waiting list of Westmead Hospital Centre for Oral Health and randomly allocated to each of two groups. Thirty patients rinsed with a total of 20 mL of 0.9% sterile saline for two minutes prior to ultrasonic scaling and 30 patients rinsed for two minutes with a total of 20 mL of povidone-iodine solution. Venous blood samples were taken before scaling (baseline) and after 30 seconds and two minutes of ultrasonic scaling and were cultured aerobically and anaerobically using a semi-quantitative culture technique (lysocentrifugation). The micro-organisms recovered were identified to species level. The saline and povidone-iodine group patients were homogeneous for age, gender, smoking status, plating time, bleeding on instrumentation, PlI, mPMAI, probing depths and recession, with no statistically significant differences between the two groups found. The incidence of bacteraemia was 33% in the saline group and 10% in the povidone-iodine group. Regression analysis showed that povidone-iodine was approximately 80% effective in reducing the incidence of bacteraemia, with a statistically significant odds ratio of 0.189 (95% C.I. O.R.= 0.043 - 0.827). Viridans Group Streptococci comprised 11 of the 24 isolates recovered from the saline group. However, there were no Viridans Group Streptococci recovered from the three povidone-iodine group patients positive for bacteraemia. There was a trend towards a lower magnitude of bacteraemia, in terms of isolates recovered per patient, in the povidone-iodine group. The clinical and social parameters of the patients were investigated to find potential predictive factors for bacteraemia. The only statistically significant predictive factor was age, with an odds ratio of 1.4 for every 10-year increase in age (95% C.I. O.R.= 1.00 - 1.97). This study found that rinsing with povidone-iodine resulted in a lower incidence of bacteraemia, less Viridans Group Streptococci in the positive blood cultures and a lesser magnitude of bacteraemia as compared with rinsing with saline. Pre-scaling rinsing with povidone-iodine may therefore be indicated for patients with plaque-induced gingivitis who are at risk of infective endocarditis, as an adjunct to antibiotic prophylaxis.

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1. INTRODUCTION

It is well established that transient bacteraemia can result from periodontal treatments such as scaling (reviewed by Lockhart 2000). The bacteria that gain access to the bloodstream during such treatment are generally rapidly removed by the reticulo-endothelial system and in healthy individuals do not cause disease. However, bacteraemia from dental treatment has long been implicated in the development of infective endocarditis in susceptible patients (Pallasch & Slots 1996).

For those patients with underlying medical conditions that are considered to create a predisposition to the development of infective endocarditis, a great variety of prophylactic measures have been recommended. Pre-treatment administration of antibiotics has been advocated to inactivate the bacteria that gain access to the bloodstream during treatment. However, antibiotic cover does not appear to prevent infective endocarditis reliably following dental treatment (Durack et al. 1983). It has been proposed that the efficacy of the existing antibiotic prophylactic regimes could be enhanced by using a pre-treatment antimicrobial mouth rinse to reduce the numbers of viable plaque bacteria entering the bloodstream following manipulation of the oral tissues (Bender & Barkan 1989). The most recently published recommendations of the American Heart Association suggest using topical antimicrobials as an adjunct to systemic antibiotic cover, to enhance the efficiency of current prophylactic measures (Dajani et al. 1997).

The American Heart Association has recommended the use of chlorhexidine or povidone-iodine given as a pre-treatment mouth rinse, prior to periodontal treatments

such as scaling (Dajani et al. 1997). Mouth rinsing is considered preferable to subgingival irrigation since the latter has been shown to induce bacteraemia (Lofthus et al. 1991) and for this reason is not currently recommended by the American Heart Association.

There is a dearth of up to date studies examining the efficacy of mouth rinsing with antiseptic solutions as a means of reducing bacteraemia due to periodontal treatment. One antiseptic, povidone-iodine, is known to reduce the numbers of viable micro-organisms at the gingival sulcus (Randall & Brenman 1974). However, the use of povidone-iodine as a mouth rinse prior to scaling in patients with periodontitis reportedly does not reduce the incidence of bacteraemia (Witzenberger et al. 1982). Witzenberger and co-workers (1982) reported that rinsing and irrigation with povidone-iodine did not cause a reduction in bacteraemia. However, they did not use a control solution for rinsing and irrigation, nor did they identify the bacteria in positive blood cultures to a genus or species level. The study by Witzenberger and co-workers (1982) is cited by Greenstein (1999) as a reason for not recommending povidone-iodine as a pre-treatment mouth rinse to reduce bacteraemia.

Because of the shortcomings of the Witzenberger et al. (1982) study, the role of povidone-iodine in reducing bacteraemia following scaling requires clarification. There is a need, therefore, to investigate the efficacy of povidone-iodine as compared with a negative control solution in reducing bacteraemia due to scaling, when povidone-iodine is used as a pre-treatment mouth rinse. Once this effect has been investigated and its efficacy established, povidone-iodine could then be used as a

positive control when testing the effect on bacteraemia of other antiseptic mouth rinses, such as chlorhexidine.

2. LITERATURE REVIEW

2.1 INFECTIVE ENDOCARDITIS

2.1.1 Definition and Diagnosis

Infective endocarditis is characterized by colonization followed by invasion of the heart valves or the mural endocardium by a microbiologic agent from the circulation, leading to the local formation of thrombotic masses laden with micro-organisms; so called "infective vegetations" (Kumar et al. 1992). Infective endocarditis is generally divided into three categories: native valve endocarditis, endocarditis in intravenous drug abusers and prosthetic valve endocarditis. Infective endocarditis may also be classified as acute or subacute. Prosthetic valve endocarditis may be further divided into early and late prosthetic valve endocarditis.

The first precise clinical description of infective endocarditis is attributed to Lazare Riviere in 1723, which has been reproduced in an extensive account of the history of infective endocarditis by Weinstein and Brusch (1996a). The patient studied by Riviere complained of palpitations and the clinical examination revealed "small, irregular pulse with every variety of irregularity". Finally, dyspnoea, oedema of the legs and haemoptysis developed and the patient died. The autopsy revealed: "In the left ventricle of the heart, round caruncles were found like the substance of the lungs, the larger of which resembled a cluster of hazelnuts that filled up the opening of the

aorta." These "caruncles", as described by Riviere in 1723 may be the first description of infective endocarditis vegetations.

The diagnosis of infective endocarditis can be very difficult and may sometimes only be discovered at autopsy. The diagnostician needs to take into account the signs and symptoms of the disease that may be present as well as assessing the likelihood of the patient contracting the condition based on the cardiac history, such as the presence of known cardiac predisposing conditions.

Lists of criteria have been developed to aid in establishing the diagnosis of infective endocarditis. The two most recent and widely used criteria include the von Reyn (von Reyn et al. 1981) and the Duke criteria (Durack et al. 1994). The Duke Criteria for diagnosis of infective endocarditis appears in Appendix 1 (adapted from Bansal 1995). The more recent Duke criteria have been updated to include diagnostic information from echocardiography to take into account more features of endocarditis related to intravenous drug abuse (Bansal 1995). A persistent bacteraemia involving organisms commonly isolated from infective endocarditis lesions and an oscillating valvular mass, identified by echocardiography, is pathognomonic for infective endocarditis (Bansal 1995). This is reflected by the criteria shown in the appendix.

2.1.2 Incidence

The incidence of infective endocarditis is difficult to assess and studies quoting figures for the incidence are sparse. In a retrospective review of infective endocarditis

cases in Denmark from 1984-1993 (Benn et al. 1997), the overall incidence of infective endocarditis was 27 episodes per million people per year. The incidence was found to increase over the period of observation from 17.4 at the beginning to 36.5 per million per year towards the end of the period. The authors attributed the increase in incidence to factors such as: increased patient visits to doctors, increased use of blood cultures, introduction of echocardiography during the observation period and increased availability of cardiology services.

A meta-analysis of the literature reporting the incidence of infective endocarditis between 1940-1992 determined that the incidence of infective endocarditis varied from 0.68 to 6.5 cases per 100,000 person years (Drangsholt 1998). A drawback of this meta-analysis was that it was limited to studies using different criteria to decide whether the cases of infective endocarditis were related to dental treatment.

2.1.3 Age and Gender

Infective endocarditis affects subjects with a broad range of ages. In a study of 135 cases between 1985 and 1993, the age range was 17 to 87 years (Sandre & Shafran 1996). The mean age for endocarditis in intravenous drug users was 35 years, native valve endocarditis was 49 years and prosthetic valve endocarditis was 60 years. There was a male preponderance of 65%, which was reduced to 53% in the intravenous drug user group. In a review of 210 infective endocarditis patients over a ten-year period, ages ranged from newborn to 91 years with the median age being 60-70 years, with 55% of the patients being male (Watanakunakorn & Burkert 1993).

2.1.4 Mortality

The mortality rate for infective endocarditis has been reviewed by Sandre and Shafran (1996). The overall mortality rate for all microbes and all classes of infective endocarditis was 19%. Poly-microbial infections had the highest mortality rate (43%) in this study. Streptococcal infective endocarditis was associated with a mortality rate of 16%. The major cause of death from infective endocarditis was found to be congestive heart failure with sepsis and embolic events the next most common causes.

2.1.5 Pathogenesis

Infective endocarditis results from a complex interaction between valvular prostheses or damaged endocardium, local haemodynamic abnormalities, circulating bacteria and local and systemic host defences (Bansal 1995). The initiating event in the pathogenesis of infective endocarditis is the invasion of the bloodstream with micro-organisms (bacteraemia), thus affording the infecting organisms the opportunity to colonise susceptible cardiac tissue or prostheses (Livornese & Korzeniowski 1992). The most common pathogens in infective endocarditis are endogenous organisms that gain access to the bloodstream, resulting from the loss of epithelial integrity (Sipes et al. 1977).

There are a number of predisposing conditions associated with the development of infective endocarditis. The American Heart Association has categorised cardiac conditions according to their association with infective endocarditis (Dajani et al.

1997). Those conditions considered to pose a high risk for development of infective endocarditis include: prosthetic heart valves, previous infective endocarditis, complex cyanotic congenital heart disease and surgically constructed pulmonary shunts or conduits. Moderate risk conditions include: acquired valvular dysfunction (e.g. rheumatic heart disease), hypertrophic cardiomyopathy and mitral valve prolapse with valvular regurgitation and/or thickened leaflets. Negligible risk conditions include rheumatic heart disease without valvular dysfunction, cardiac pacemakers and implanted defibrillators and innocent murmurs.

2.1.6 Microbiology

The microbiology of infective endocarditis, in terms of the types of organisms detected in blood cultures from affected patients, is dependent on the demographics of the area from which the hospital receives patients (Steckelberg et al. 1990). Referral bias may also be a factor in the types of organisms detected (Steckelberg et al. 1990). Patients with infective endocarditis referred to a tertiary referral hospital may be more gravely ill with a more virulent infecting organism, than a patient with infective endocarditis being treated successfully in a smaller hospital. These factors must be kept in mind when interpreting studies reporting the incidence of micro-organisms recovered from infective endocarditis patients.

In a review of infective endocarditis cases seen at Westmead Hospital (a tertiary referral centre) between 1979 and 1992, 43% were found to be due to *Staphylococcus aureus* and 29% were due to Viridans Group Streptococci (Dwyer et al. 1994). The

authors attributed the high proportion of staphylococcal isolates to a large contribution of cases from patients with nosocomial infections or from inter-hospital transfers (43%). Further analysis of the study population revealed that the prevalence of Viridans Group Streptococci was 37% and *S. aureus* was 25% in community acquired infective endocarditis cases. Furthermore, it was found that most of the unusual fungi and Gram-negative organisms were recovered from the nosocomially acquired or transferred cases, which demonstrates the effect of study patient selection bias on the micro-organisms recovered from patients with infective endocarditis.

Viridans Group Streptococci are regarded as the most prevalent species contributing to community acquired infective endocarditis cases (Dwyer et al. 1994; Peat & Lang 1989; Venezio et al. 1984), with the prevalence of infection due to Viridans Group Streptococci for these studies being 37%, 53% and 35% respectively. Since this group of *Streptococci* spp. is commonly found in the oral cavity, the role of oral health and dental treatment in the production of streptococcal bacteraemia is considered of importance in the prevention of infective endocarditis (Barco 1991; Daly 1995; Cowper 1996; Pallasch & Slots 1996; Dajani et al. 1997)

2.1.7 Role of Oral Micro-organisms

Some members of the Viridans Streptococci group found commonly in the oral cavity (*Streptococcus mutans, Streptococcus mitior, Streptococcus sanguis*) have a greater propensity for involvement in infective endocarditis lesions than others of the same genus (Parker & Ball 1976). This is thought to be due to the ability of these

organisms to produce dextran extracellular polysaccharide (EPS). EPS forms the matrix for dental plaque and is an important part of the biofilm structure of plaque on tooth surfaces. Dextran EPS also forms the matrix for biofilm on heart valves when the infecting organism is a Viridans Group Streptococcus, increasing the size of the vegetations and improving survival of the infecting micro-organism in the face of antimicrobial therapy administered in the medical management of infective endocarditis (Dall & Herndon 1989).

It has been shown that some strains of *S. sanguis* have the ability to adhere to platelets. Two antigens that *S. sanguis* expresses on the cell surface facilitate adherence. One of these antigens is an adhesin (class I antigen), which facilitates the adhesion of the organism to platelets (Herzberg et al. 1983) and the other is a platelet aggregation associated protein (class II antigen), which mimics the part of collagen that binds platelets (Erickson & Herzberg 1987). These virulence factors are considered to lead to larger vegetations and a more severe clinical course for experimentally induced lesions of infective endocarditis (Herzberg et al. 1992).

2.1.8 Role of Dental Treatment

It is difficult to define accurately the temporal relationships between the occurrence of bacteraemia due to dental treatment and the development of infective endocarditis (Lockhart 2000). This difficulty is well illustrated by the statement: "Infective endocarditis may be the result of a single episode of failed host defence during thousands of repeat bacteraemia episodes over a lifetime" (Pallasch 1989). However,

given that bacteraemia is considered a normal day-to-day event, it is difficult to know which bacteraemia episode was responsible for a given case of infective endocarditis. Furthermore, it is not known whether dental treatment is able to produce a bacteraemia of sufficient magnitude to produce infective endocarditis, given that large numbers of organisms (10^4 to 10^9 colony forming units per mL) are required to produce experimental infective endocarditis in rabbits (Glauser & Francioli 1987).

There are no accurate figures for the incidence of infective endocarditis in New South Wales as infective endocarditis is not a notifiable disease and incidence studies tend to relate to individual hospitals (Dwyer et al. 1994). However, using the national incidence figures (36.5 cases per million people, per year) of infective endocarditis in Denmark (Benn et al. 1997), there would be approximately 140 cases of infective endocarditis per year in Sydney, having a population of approximately four million. Furthermore, of these 140 infective endocarditis cases, given that the infecting organism is likely to be a Viridans Group Streptococcus in 30% of infective endocarditis cases (Dwyer et al. 1994), only about 45 of these cases would have had a significant likelihood of originating from the oral cavity.

A significant number of these patients may not have undergone dental procedures in the weeks preceding their development of infective endocarditis, further reducing the numbers of cases attributable to dental treatment. In a review of 254 cases of infective endocarditis, it was found that only 7.9% of the patients had received dental treatment in the previous month and only 13% had received dental treatment in the previous three months (Bayliss et al. 1983). The incubation period for infective endocarditis is about two weeks (Starkebaum et al. 1977). Therefore, it is likely that

fewer than 13% of these patients would have received dental treatment in the two weeks preceding the development of infective endocarditis. Furthermore, it would only be possible to conclude that a case of infective endocarditis was attributable to a bacteraemia due to dental treatment if it could be shown that the patient had not been exposed to any spontaneous oral bacteraemia or bacteraemia due to home care procedures during the incubation period (Bayliss et al. 1983).

Guntheroth (1984) questioned the veracity of attributing an episode of infective endocarditis to dental treatment simply because the individual had undergone dental treatment within weeks or months prior to onset of the episode. Guntheroth (1984) calculated, for a one month period, for a patient with dental disease leading to an extraction, the relative exposure of the patient to random bacteraemia and bacteraemia from mastication and tooth brushing. This exposure time was compared to the time the patient was exposed to the bacteraemia from the extraction. The figure for the cumulative monthly exposure time to bacteraemia was 5376 minutes, only six of which were attributable to the extraction procedure. It is not clear, however, how these figures were calculated. Guntheroth (1984) concluded that the probability a given endocardial infection was seeded by an extraction procedure was about 1: 1000 and that if infective endocarditis was to develop after an extraction, the evidence linking the two events would be circumstantial unless a negative blood culture had been obtained before the extraction.

Recently, a well controlled epidemiological study failed to find a definite correlation between dental treatment and the development of infective endocarditis (Strom et al. 1998). In that study, 273 patients with community acquired infective endocarditis

(not due to intravenous drug abuse) were matched to "healthy" community residents by age, sex and community of residence. Information relating to the frequency of dental visits in both case and control groups was obtained by telephone interviews. It was found that in the two months before the study date, 16.8% of infective endocarditis patients and 14.3% of healthy controls had received dental treatment. In the three months before the study date, 23% of both infective endocarditis patients and healthy controls had sought dental treatment. Strom and co-workers (1998) concluded that dental treatment did not appear to be a risk factor for infective endocarditis given that the frequency of dental visits amongst their case and control patients was the same. Strom and co-workers (1998) found that more patients with infective endocarditis had underlying heart defects. The patient-recruiting process used in this study would be expected to select for infective endocarditis (case) patients with underlying conditions considered to put the patient at greater risk for developing infective endocarditis. The converse applies for the healthy control group.

From an epidemiological perspective, given the paucity of available data and the number of assumptions that must be made in deciding whether to attribute a case of infective endocarditis to a dental procedure at the patient level, it is currently not possible to arrive at a figure for the incidence of infective endocarditis due to dental treatment.

A more expansive view of the time course of the pathogenesis of infective endocarditis has been proposed by Drangsholt (1998), who suggests that chronic low-grade bacteraemia may cause endothelial cell damage and damage to the endocardium which could help to promote the formation of sterile vegetations on valve leaflets.

Transient bacteraemia, such as that resulting from dental treatment may then provide organisms that are able to seed the vegetations and cause endocarditis. Most dental procedures are thought to produce bacteraemia of similar magnitude to home care procedures (Pallasch & Slots 1996; Lockhart 2000). However, dental procedures that are considered to be more invasive, such as periodontal treatment and dental extractions are thought to be more likely to result in infective endocarditis (Pallasch & Slots 1996; Dajani et al. 1997; Lockhart 2000). It is thought that these more invasive dental procedures are more likely to result in infective endocarditis because they result in bacteraemia of greater magnitude. However, the magnitude of bacteraemia from home care procedures and of most dental procedures has not been measured to date.

The reason why the magnitude of the bacteraemia is thought to be important in the pathogenesis of infective endocarditis is that the inoculum needs to be large enough to colonise an affected native valve or a prosthetic valve. It has been speculated that low-grade bacteraemia may play a role in forming non-bacterial thrombotic endocarditis lesions on native valves (Drangsholt 1998). It has also been shown that relatively large numbers of bacteria are required to induce infective endocarditis in rabbits in which the heart valves have been experimentally damaged beforehand (Durack et al. 1973). According to the theory proposed by Drangsholt (1998), non-bacterial thrombotic endocarditis lesions produced by oral bacteraemia occurring spontaneously or due to home care procedures prime the heart valve for colonisation by a threshold inoculum of bacteria. However, there is no evidence in the form of data from prospective trials to support this theory.

2.2 BACTERAEMIA

2.2.1 Definition and Origins

A bacteraemia is defined as the presence of viable bacteria in the blood, as evidenced by positive blood cultures. The sources of infecting micro-organisms in regard to infective endocarditis are ostensibly commensals from the skin, mouth, genito-urinary system and the gut (Barco 1991). Thus, invasive procedures involving these areas may produce a bacteraemia involving micro-organisms that are clinically significant in terms of the frequency with which they are identified as the causative micro-organism in lesions of infective endocarditis.

2.2.2 Mechanism of Bacteraemia

A vital barrier preventing microbial access to human tissues is the integumentary system, which consists of epithelial and connective tissue components, namely skin and mucosa. In the gingival sulcus, polymorphonuclear leucocytes (PMN cells) form the primary defence around the teeth whilst the epithelial barrier is considered the second line of defence (Page 1986). Micro-organisms that are able to invade and disseminate within the body via the gingival sulcus first have to evade the PMN defences and then cross the physical barrier of the epithelium. Alternatively, the PMN and epithelial barriers may be breached because of trauma, such as de-epithelialisation of the sulcus or pocket lining during the use of dental instruments, allowing the entry of micro-organisms into the tissues.

Few papers discuss how micro-organisms that enter the oral soft tissues as a result of iatrogenic dental trauma may reach the bloodstream. Many authors (Cobe 1954; Diener et al. 1964; Romans & App 1971) have assumed that bacteria enter the vascular compartment directly. However, it has been proposed that the organisms involved in bacteraemia are more likely to gain access to the bloodstream via the lymphatic system (Guntheroth 1984). Following a traumatic injury to the oral mucosa, Guntheroth (1984) has questioned the ability of bacteria to gain direct entry to the bloodstream. A severed blood vessel such as an arteriole or capillary would have blood flowing from the proximal end, which would prevent bacterial ingress. Blood flow in the distal end of the severed vessel would cease due to a change in the hydrostatic pressure gradient that maintains flow in blood vessels, which would also prevent bacterial ingress (Guntheroth 1984).

Guntheroth (1984) contrasted the difference in patency of the blood and lymphatic vasculature in wounds, citing early studies which have demonstrated that following trauma, lymphatic vessels maintain their patency for more than 48 hours (McMaster & Hudack 1934). During this period, lymphatic vessels are able to take up Pontamine Blue dye, a high molecular weight compound, indicating that lymph drainage is still occurring (McMaster 1937). It has been shown using light microscopy, that lymphatic vessels are linked to the connective tissue framework by a network of reticular fibres (Pullinger & Florey 1935). Linking of the lymphatic vessels to connective tissue components is thought to be the mechanism by which lymphatic vessels dilate when tissues become oedematous (Pullinger & Florey 1935) and probably accounts for how the patency of lymph vessels is maintained when severed by traumatic injury.

Early *in vivo* investigations of bacteraemia have demonstrated that bacteria entering a wound gain access to the vascular compartment indirectly via the lymphatic system and not by direct entry into blood vessels (Barnes & Trueta 1941). In that study, the blood vessels and lymphatic drainage of the hind legs of rabbits were ligated separately. Following ligation of only the lymphatic drainage, leaving the blood supply to the limbs intact, freshly made deep calf wounds were deliberately contaminated with *Pseudomonas pyocyaneus.* The control rabbits, their lymphatic drainage intact, had similar wounds inoculated with the same concentration of the same bacterium. Blood cultures and spleen cultures from the test rabbits were negative, whilst those from the control animals (lymphatic drainage intact) were positive for the same bacterium. The micro-organisms that gained access to the sub-epithelial tissues entered the lymphatic vessels and were transported to local lymph nodes. Some of the bacteria were able to pass through the lymph nodes and enter the vascular compartment, where they were subsequently recovered by the blood sampling procedure.

The same authors (Barnes & Trueta 1941) demonstrated the effect of inflammation and oedema on lymph flow. Dogs' paws were scalded with hot water to produce inflammation and oedema and the lymphatic vessels of the corresponding limb were cannulated. The flow of lymph from the limb increased as it became oedematous. Pontamine Blue dye and *P. pyocyaneus* were introduced into the oedematous tissues and were subsequently recovered in less than one minute from the cannulae in the test animals, whereas they did not appear for three minutes in the control animals. The increased flow of lymph resulting from the inflammation caused a change in the time

course of the bacteraemia, with a more rapid progress of the micro-organisms from the lymphatic compartment into the bloodstream in the inflamed tissues.

The lymph nodes that drain the periodontal structures include the submandibular, submental, retropharyngeal and deep cervical nodes. These nodes then drain inferiorly to the deep cervical nodes, which ultimately receive all the lymph from the head and neck region. Their efferent vessels form the jugular trunk, which drains lymph to the right lymphatic duct or to the thoracic duct on the left, to be returned to the circulatory system (Hiatt & Gartner 1987). Bacteria arising from the oral region that enter the lymphatic system and which are not trapped by the local lymph nodes spill into the vascular compartment via the major head and neck efferent lymph vessels. To date, no studies have been reported that clarify the relative roles of the lymphatic and vascular systems in bacteraemia arising from trauma to the dento-gingival area. Since it is possible to isolate and cannulate the lymphatic vessels of the head in sheep (Au et al. 1996), it may be possible to utilize a sheep model to investigate the factors affecting the development of dentally induced bacteraemia.

2.2.3 Elimination of Bacteraemia

Macrophages in the lungs, liver and spleen neutralise bacteria that gain access to the vascular compartment. Gram-negative organisms are generally opsonised and destroyed by antibody and complement mediated lysis, whilst Gram-positive organisms are opsonised by antibody and complement and ingested and destroyed by phagocytic cells (Seymour et al. 1995). Bacteria may gain access to the bloodstream

in small clumps, which would rapidly be broken down in the small blood vessels of the lungs (Roberts et al. 1992).

Studies examining the time course of the recovery of micro-organisms involved in bacteraemia have given an indication of the clearance rate of micro-organisms from the bloodstream. A study of bacteraemia in dogs revealed that following introduction of bacteria into the venous system, peak recovery of isolates from the resulting bacteraemia occurred within 30 to 60 seconds (Silver et al. 1975). Recovery rates dropped sharply from 90 seconds to four minutes, depending on the organism introduced. Clearance of bacteria from the blood was usually complete within ten minutes and always complete within 20 minutes (Silver et al. 1975). Silver and co-workers (1975) explained the difference in clearance rates of the bacterial species from the bloodstream as being due to differences in the virulence of the micro-organisms and the presence of specific antibody.

2.2.4 Bacteraemia of Oral Origin

It has long been established that routine dental procedures are capable of precipitating bacteraemia (reviewed by Lockhart 2000). Any dental procedure that causes gingival bleeding is generally considered as having the potential to induce a bacteraemia (Dajani et al. 1997). Transient bacteraemia is well documented following treatments for periodontal disease such as gingivectomy (83% of patients; Bender et al. 1984), supra and subgingival scaling (25-61% of patients; Baltch et al. 1988), and

subgingival irrigation (30% of patients; Lofthus et al. 1991). Periodontal probing has also been shown to induce a bacteraemia (Daly et al. 1997).

Home care procedures may induce bacteraemia. For example, it has been shown that the incidence of bacteraemia following manual brushing in subjects with apparently minimal periodontal disease ranged from 0 to 26% (Rise et al. 1969, Berger et al. 1974). In subjects with various degrees of periodontal disease, the incidence was 24.2% (Cobe 1954). In patients with "infected gums", a ten-minute gingival massage resulted in a bacteraemia incidence of 17.6% (Richards 1932). Five out of 30 patients were shown to develop a bacteraemia following the use of an electric toothbrush (Sconyers et al. 1973). Madsen (1974) reported on the incidence of bacteraemia due to tooth brushing and toothpick use. Nineteen percent of gingivitis patients and 54% of patients with periodontitis were positive for bacteraemia. However, the patient groups were small, having 13 and 16 subjects respectively.

More recently, Schlein and co-workers (1991) reported on the incidence of bacteraemia during tooth brushing in patients undergoing orthodontic treatment with full fixed appliances. The incidence of bacteraemia due to tooth brushing in this group of 20 patients was 25%, in blood samples taken five minutes after the completion of tooth brushing. The blood samples were cultured using aerobic and anaerobic culture techniques. None of the patients had an underlying bacteraemia, which would have been indicated by a positive pre-brushing blood sample.

The incidence of bacteraemia relating to various dental procedures undertaken on 735 children undergoing general anaesthesia has been evaluated (Roberts et al. 1997). Roberts and co-workers (1997) found that the procedures associated with the highest incidence of bacteraemia were intra-ligamental injection (96.6%), multiple extractions (50.9%), scaling teeth (40%), muco-periosteal flap (39.2%), single extraction (38.7%), tooth brushing (38.5%), matrix band placement (32.1%), rubber dam placement (29.4%) and polishing teeth (24.5%). All treatment groups contained at least 30 randomly allocated patients and one 8 mL blood sample was taken from each subject 30 seconds after each of the procedures. Although this study investigated children only, it was a comprehensive study in its attempt to compare an array of dental procedures within the one investigation.

2.2.5 Microbial Translocation

The hypothesis that microbes may be able to cause damage at sites distant to the area of infection is not new and has been reviewed recently by O'Reilly and Claffey (2000). William Hunter (1910) delivered an address at the opening of McGill University in Montreal about the dangers of sepsis, in which he launched an attack on conservative dentistry, or "septic dentistry" as he called it:

"No-one has probably had more reason than I have had to admire the sheer ingenuity and mechanical skill constantly displayed by the dental surgeon. And no-one has had more reason to appreciate the ghastly tragedies of oral sepsis which misplaced ingenuity so often carries in its train. Gold fillings, gold caps, gold bridges, fixed

dentures, built in, on, and around diseased teeth which form a veritable mausoleum of gold over a mass of sepsis to which there is no parallel in the whole realm of medicine."

Hunter's views had a profound effect on the dental profession, to the extent that endodontic treatment was virtually abandoned as a treatment option for teeth with necrotic pulps, in favour of extraction (Belizzi & Cruse 1980). During the following decades, advances in endodontic therapy improved the outcomes of treatment significantly and the Focal Infection Theory all but sank into obscurity (Grossman 1974).

In 1915, Billings (cited in an extensive review of the history of oral sepsis by O'Reilly and Claffey 2000) defined the term Focal Infection (which replaced the term Oral Sepsis) as a "circumscribed area of tissue infected with pathogenic organisms". Billings (1916) stated that the term Focal Infection implied 1) that such a focus or lesion of infection existed, 2) that the infection was bacterial in nature and 3) that as such it was capable of dissemination, resulting in systemic infection of other contiguous or non-contiguous parts. The concept of Focal Infection and its implications has since been revisited and reinterpreted by many authors.

Bacterial translocation is an example of the focal infection theory at work. It is apparent that bacteria are able to disseminate in some instances or produce direct and indirect effects at distant sites. It has been reported that under certain conditions, bacteria within the lumen of the gut are able to cross the mucosal barrier and enter the mesenteric lymphatic system from where they spread systemically (Berg &

Garlington 1979). These authors described this phenomenon and coined the term "bacterial translocation". The gut lumen under certain conditions can act as a focus of infection and dissemination of bacteria may subsequently occur.

Bacterial translocation has been defined as the passage of viable bacteria from the lumen of the gastro-intestinal tract into other organ systems via the mesenteric lymphatic system (Berg & Garlington 1979). It has been shown that endotoxin administered intramuscularly can induce bacterial translocation across the wall of the gut (Deitch et al. 1987). The mechanism is probably due to alteration in the tight junctions between gut epithelial cells (Walker & Porvaznik 1978) and this effect has been observed irrespective of a genetic tolerance to endotoxin (Deitch et al. 1987), meaning that all subjects are susceptible to bacterial translocation from the effects of endotoxin (LPS) activity.

Factors involved in translocation in the gut involve bacterial overgrowth (local effect of LPS), impaired host immunity, physical disruption of the gut mucosal barrier (Deitch et al. 1987) and a reduction in blood flow to the gut (Saydjari et al. 1991). Glucocorticoids such as dexamethasone have been shown to promote bacterial translocation by reducing levels of secretory IgA (Alverdy & Aoys 1991). A decreased amount of secretory IgA reduces mucosal resistance to bacteria by allowing increased adhesion of bacteria to cells, which is the critical first step in the translocation process.

The extent of gut inflammation has been purported to have an important effect on the mode of bacterial translocation (Mainous et al. 1991). These investigators postulated

that when only mild inflammation was present, micro-organisms gained access to the bloodstream via the lymphatic system. However, with more severe inflammation bacteria could enter the bloodstream directly, resulting in large numbers of bacterial isolates being recovered from the portal blood. The mechanism by which bacteria gain direct access to the efferent bloodstream of the gut during translocation has not been elucidated to date.

An early and important event in the development of gingivitis is ulceration of the sulcus lining (Page 1986). If the integrity of the epithelial layer is compromised, bacteria are able to enter the underlying connective tissue (Page 1986). It is not known whether the pathogenesis of gingivitis mirrors that of LPS mediated bacterial translocation. If bacterial translocation were found to occur in the periodontium, this would add support to the view that spontaneous bacteraemia are more prevalent and of greater magnitude in people with poor oral hygiene and increased levels of gingival inflammation (Okell & Elliott 1935; Everett & Hirschmann 1977; Silver et al. 1975; Bender et al. 1984; Guntheroth 1984). It would be consistent with the work of Barnes and Trueta (1941), which showed an increased likelihood of bacteraemia in inflamed tissues.

2.3 ORAL MICRO-ORGANISMS

2.3.1 Biofilm Concept

Oral micro-organisms *in vivo* may exist in either a planktonic state in saliva or in a biofilm structure adherent to dental and mucosal surfaces. Dental plaque is currently

thought of as a biofilm. Costerton's model of aquatic biofilm, which is defined as matrix enclosed bacterial populations adherent to each other and/or to surfaces or interfaces, has been used to study dental plaque and has added to our understanding of plaque biology (Costerton et al. 1995).

The first substance deposited on newly cleaned enamel surfaces is dental pellicle, sometimes known as acquired dental pellicle (Genco et al. 1990). Pellicle constituents, such as glycoproteins, contain sugars which provide attachment sites for the bacterial species that initially colonize tooth surfaces (Darveau et al. 1997). Pellicle provides specific receptors for bacterial adhesion. For example, *Streptococcus gordonii* and *Actinomyces naeslundii* bind different regions of proline rich proteins found in pellicle. Initial colonization of enamel by bacteria occurs in three stages (Liljemark et al. 1997). Bacteria from saliva (initially *S. sanguis*), binds until saturation of pellicular binding sites occurs, whereupon further accumulation occurs as the bacteria spread across the tooth surface and further bacteria are deposited, until a critical density is reached. The third phase of growth occurs once a critical density is reached and is therefore termed the density–dependent growth phase (Bloomquist et al. 1996). During the density dependent growth phase, plaque mass increases very rapidly from bacterial cell division, such that 90% of the plaque biomass at 24 hours could be attributed to cell division (Liljemark et al. 1997). The earliest colonizers on a clean tooth surface are mostly *Streptococci* spp. which account for 47-85% of the cultivable cells found during the first four hours following professional tooth cleaning (Nyvad & Kilian 1987).

Once the initial colonizers have adhered to the pellicle, specific genes are activated which enable the bacteria to begin synthesizing EPS (Burne et al.1997), which forms the biofilm matrix. The main gene that controls cell metabolism in relation to EPS production has been referred to as the sigma factor gene, which codes for a subunit of the enzyme Ribose Nucleic Acid (RNA) polymerase (Carlsson 1997). The product of the sigma factor gene present in *Streptococci* spp. is a glucose-specific IIA protein (IIA^{glc}), a controlling enzyme in the cell's glucose metabolism pathway responsible for EPS production. A similar sigma factor gene, known as the Alg-C gene, has been identified as the gene responsible for the production of alginate matrix by *P. aeruginosa* (Deretic et al. 1990).

Diversification of species in the adherent plaque develops as genetically distinct species recognize specific binding sites on their respective surfaces and adhere to each other in a process called co-aggregation (Kolenbrander et al. 1993). Secondary colonisers may synthesize protein adhesins that recognize receptors on primary colonizers such as *Streptococcus* spp. and *Actinomyces* spp., further allowing for diversification of the dental plaque (London 1991). *Fusobacterium* spp. bind statherin in the pellicle and have been shown to co-aggregate with all other plaque species (Whitaker et al. 1996). *Fusobacterium nucleatum* is regarded as an important species in the maturation of plaque biofilm as it is capable of linking the more complex pathogenic plaque organisms to the early colonizers of the tooth surface (Kolenbrander & London 1993). Gilbert and co-workers (1997) described how these factors could contribute to the complexity of mature plaque biofilm, leading to vast arrays of vertical and horizontal stratifications inside the biofilm, making possible the existence of obligate anaerobes in what may appear to be an aerobic environment.

Plaque has recently been studied with the aid of Confocal Scanning Laser Microscopy (Lawrence et al. 1991), which allows the study of "live" biofilm. The arrangement of the bacteria within the biofilm is highly significant in terms of the behaviour of the bacterial community. The viable microbes are arranged in micro-colonies of cells within a dense extra-cellular polysaccharide matrix (Costerton et al. 1995).

There is an extensive network of channels surrounding the micro-colonies that reach to the deepest layers of the biofilm (Costerton & Lewandowski 1997). These channels contain fluid, which exhibits bulk flow characteristics. Fluid is able to enter the channels and move by convective flow throughout the considerable extent of the channel system (DeBeer et al. 1994a, b). The same authors have used micro-probes to show that bacteria within the micro-colonies may live anaerobically and that those near the edge of the fluid channels, only 100 microns away, live in an aerobic environment. The biofilm arrangement allows cells with very different requirements to live in close approximation (Wolfaardt et al. 1994).

The EPS biofilm matrix has been thought of as a potential barrier to the penetration of antimicrobials, thus explaining the lack of biofilm sensitivity to these substances (Slack & Nichols 1981). Three mechanisms have been proposed for this effect (Govan et al. 1979). Firstly, the EPS may provide a static layer through which the antimicrobial must diffuse. Secondly, the negatively charged biofilm matrix may hinder the penetration of positively charged antimicrobials, which would have to saturate all the negatively charged biofilm sites before exerting an effect. Thirdly, there may be a reduction in the rate of diffusion of a charged antimicrobial in an oppositely charged biofilm matrix. Some inhibition of diffusion of cationic

aminoglycosides has been observed in the mucoid EPS of *P. aeruginosa*, whereas the diffusion of neutral or negatively charged β-lactam antibiotics was not inhibited (Slack & Nichols 1981).

It has been proposed that increased rates of bacterial growth results in increased efficiency of antimicrobial agents (Eng et al. 1991). However, it has been found that a spatial distribution of cells of different metabolic rates exists within biofilm (Costerton et al. 1995), such that cells of varying growth rates may exist in close proximity. None of the antimicrobial agents tested by Eng and co-workers (1991) resulted in more than three orders of magnitude of killing against slowly growing *S. aureus*, and only quinolones were effective against slowly growing or non-growing gram-negative bacteria. In contrast, rapidly growing micro-organisms experienced complete killing. It has been proposed, therefore, that the window of opportunity for antimicrobials to act against plaque biofilm is at the time of maximal cell growth (Liljemark et al.1997). This would most likely occur early in the plaque formation process.

2.3.2 Biofilm and Antibiotics

The biofilm arrangement is known to increase the resistance of micro-organisms to antibiotics (Nickel et al. 1985; Wilson 1995; Gilbert et al. 1997). The first group to determine this effect showed that *P. aeruginosa* in a biofilm arrangement had a Minimum Inhibitory Concentration (MIC) 500 times greater than that of the same species in planktonic solution, when exposed to the antibiotic tobramycin (Nickel et

al. 1985). A similar effect has been demonstrated with *Porphyromonas gingivalis* biofilm (Wright 1997) and *S. sanguis* biofilm (Larsen & Fiehn 1996).

The effect of amoxycillin and doxycycline on *S. sanguis* biofilm was determined using a flow cell model (Larsen & Fiehn 1996). A flow cell is a tube containing biofilm through which a nutrient solution, in this case brain heart infusion broth (BHI), was pumped at a constant rate. The antimicrobials were added to the BHI in varying concentrations, after which the biofilm was sampled and cultured to enumerate the remaining viable cells. Amoxycillin and doxycycline had little effect on the biofilm when added to the BHI for 48 hours in a concentration equal to the MIC that was determined (in planktonic solution) for the same micro-organism. Growth of the biofilm was inhibited only after exposure to the antibiotics at concentrations 500 times greater than the MIC.

P. aeruginosa was seeded onto a latex substrate and grown in a flow cell in the presence of urine, in a study designed to show the effect of tobramycin on urinary catheter infections (Nickel et al. 1985). Once the biofilm was established, sterile urine containing 1000 μg/ml tobramycin was pumped through the flow cell. Following 12 hours of exposure to this high concentration of tobramycin, a significant proportion of the micro-organisms within the biofilm were still viable. The MIC for the micro- organisms used to seed the flow cells and for the viable cells recovered from the biofilm, following exposure to tobramycin, was 0.4 μg/ml. The biofilm growth pattern clearly conferred a significant resistance to the antibiotic tobramycin.

The biofilm growth pattern confers similar antibiotic resistance to metronidazole (Wright et al. 1997). Wright and co-workers grew *P. gingivalis* in broth cultures (planktonic growth), on agar plates and as a biofilm on hydroxyapatite slabs suspended in broth cultures of *P. gingivalis*. Metronidazole was added in varying concentrations, which enabled determination of the MIC for metronidazole on *P. gingivalis* under the varying growth conditions. The MIC for *P. gingivalis* grown in broth culture was 0.125 μg/mL, in agar culture 0.375 μg/mL and as a biofilm, 40-100 μg/mL. Thus, the MIC values for *P. gingivalis* grown as a biofilm were up to 320 times that of *P. gingivalis* grown in planktonic culture. Furthermore, the MIC was determined for the micro-organisms grown as a biofilm following re-suspension in broth as a planktonic culture. The re-suspended micro-organisms had an MIC equal to that of the micro-organisms grown only as a planktonic culture.

These studies confirm that micro-organisms in a biofilm structure are resistant to antibiotic concentrations which would normally inhibit those in a planktonic growth situation. The implication of these findings for the prevention of infective endocarditis is that the administration of systemic antibiotics prior to invasive dental procedures (e.g. scaling) would not be expected to be bactericidal for micro-organisms present in dental plaque. This would help to explain the finding that systemic antibiotics do not prevent bacteraemia when administered prior to invasive dental procedures (Hall et al. 1993). Antibiotics exert their effects on bacteria that have already gained access to the bloodstream.

2.3.3 Biofilm and Antiseptics

Biofilm is similarly resistant to the effects of commonly used antiseptics. Chlorhexidine was able to effectively neutralise the *S. sanguis* biofilm grown for 24 hours in a flow cell at ten and 50 times the MIC for planktonic cultures of *S. sanguis* (Larsen & Fiehn 1996). Chlorhexidine at 100 times the MIC, (corresponding to 0.16% chlorhexidine), was required to kill the biofilm bacteria after four hours. Chlorhexidine (0.16%) was able to eliminate *S. sanguis* biofilm within 24 hours at ten and 50 times the MIC for the planktonic culture. It would be impossible to maintain this concentration of chlorhexidine for a similar length of time in the mouth. Furthermore, as this study relates to a single species biofilm, it may not be possible to draw conclusions regarding the likely effects of chlorhexidine against native plaque.

Similar differences in susceptibility of *S. sanguis* to chlorhexidine and cetylpyridinium chloride have been demonstrated, when these micro-organisms are grown in planktonic culture and as a biofilm (Wilson et al. 1996). With the aid of a constant depth film fermenter, *S. sanguis* biofilm was exposed to 0.2% chlorhexidine and 0.05% cetylpyridinium chloride. The constant depth film fermenter produces biofilm of a uniform depth, controlling for parameters related to penetration, onto which antimicrobial substances can be applied. No viable micro-organisms remained after five minutes' exposure to these antimicrobials in planktonic culture. However, when grown as a biofilm, viable micro-organisms were recoverable after four hours exposure to the antimicrobial solutions tested (Wilson et al. 1996).

Povidone-iodine has been shown to be effective against supragingival plaque bacteria (Randall & Brenman 1974). In their study, periodontal patients with plaque deposits of unspecified age rinsed with povidone-iodine or a placebo following initial plaque sampling. After rinsing with povidone-iodine, it was found that the colony counts fell by approximately 33%, whereas they fell by only 8% following rinsing with the placebo. It is possible that the observed anti-microbial effect of povidone-iodine may have been due to the capacity of povidone-iodine to diffuse into the bacterial micro-colonies of the plaque biofilm. Such diffusion may be facilitated by the relatively small size of the iodine (I_2) molecule, which has a molecular weight of 106.

There has been a series of recent *in vivo* studies examining the effects of Listerine® on dental plaque. The effect of Listerine® on interproximal and supragingival plaque has been determined using sterile water as a control in a single blind crossover trial (Fine et al. 2000). Twenty nine subjects rinsed with 20 mL of solution for 12 days, after which plaque samples were taken and cultured (one hour after rinsing with the given solution) to determine the numbers of viable plaque bacteria using serial dilution and plating techniques. Fine and co-workers (2000) found that Listerine® had a differential effect on the plaque micro-organisms, with an average reduction in the numbers of recoverable *Streptococci* spp. of 69.9%, relative to the saline mouth rinse. This study is limited in that the culture technique was selective for *Streptococci* spp. The effect of Listerine® on biofilm as a whole, therefore, could not be demonstrated.

Development of vital staining techniques has enabled the study of the survival of micro-organisms in plaque biofilm following rinsing with antimicrobial mouth rinses, without the need for culturing. The effect of Listerine® on one day old plaque *in vivo*

has been determined in a crossover trial, with the aid of a vital staining technique (Pan et al. 2000). Baseline plaque samples were taken before each of the 17 subjects rinsed with 20 ml of Listerine® or sterile saline for 30 seconds. Plaque was then sampled from contralateral teeth and stained using a vital stain technique. The stain fluoresced live and dead bacteria green and red respectively. Using this staining technique, Pan and co-workers (2000) determined that the proportion of non-viable micro-organisms in plaque following rinsing with saline and Listerine® was 27.9% and 78.7% respectively. Further study is required to assess the effect of anti-microbial mouth rinses on more mature biofilm using vital staining techniques. It would be useful to study the effect of povidone-iodine mouth rinse and other antiseptics on plaque biofilm using this technique in order to determine the efficacy in reducing numbers of viable micro-organisms, relative to Listerine®, which could be used as a positive control.

Little is known about how biofilm reacts when only part of the bacterial population is rendered non-viable by antimicrobial mouth rinses. For example, it is not known what effect a partial kill would have on the growth rates of the micro-organisms within the biofilm and whether some or all species revert to planktonic growth rates to reconstitute the biofilm.

2.4 PREVENTING ENDOCARDITIS CAUSED BY DENTAL TREATMENT

2.4.1 Current Practice

The administration of systemic antibiotics prior to invasive dental treatment, particularly periodontal treatment, has been and continues to be standard practice (Lockhart 2000). However, the differing antibiotic prophylactic regimes proposed by the American Heart Association (Dajani et al. 1997), British Society for Antimicrobial Chemotherapy (Simmons et al. 1992), The Australian Dental Association (Woods et al. 1994), the Victorian Drug Usage Advisory Committee (1995) and the National Heart Foundation of New Zealand (Ellis-Pegler et al. 1999), to name but a few authorities, serve to exemplify the fact that there is no single antibiotic prophylaxis regime which is universally recommended. Further, the limitations of antibiotic prophylaxis are well documented.

2.4.2 Limitations of Antibiotics

There are several problems associated with antibiotic prophylaxis regimes. Firstly, antibiotics do not decrease the magnitude of bacteraemia (Hall et al. 1993). Secondly, there are no human placebo-controlled trials to demonstrate the efficacy of any of the recommended antibiotic prophylaxis regimes for the prevention of infective endocarditis. Clinical practice should be evidence-based to ensure that the treatment delivered is effective and of a high standard. Evidence to support the efficacy of prophylactic antibiotic regimes in the form of controlled, prospective, randomised

human clinical trials is not likely to be forthcoming as there are seemingly insurmountable barriers in the performance of such studies in relation to infective endocarditis. For example, it would not be possible from an ethical standpoint to run a placebo-controlled study. Due to the low incidence of infective endocarditis, a prohibitively large number of subjects would need to be enrolled in such a study. It is estimated that at least 6000 at-risk patients would be required for an appropriate double-blind study (Durack 1990). Lack of evidence to support the use of prophylactic antibiotic regimes is likely to remain a significant problem. Despite this lack of evidence, most advisory bodies, including the National Heart Association of New Zealand (Ellis-Pegler et al. 1999), consider that a major change in antibiotic prophylaxis guidelines is not warranted at present.

There are thought to be approximately four hundred bacterial species that are able to exist in the oral cavity (Socransky & Haffajee 1992). Antibiotics, by nature, are selective in their effect against bacteria and bacteria are showing increased resistance to antibiotics (Maskell et al. 1990). Not one of the eight antibiotics that have been recommended for prophylaxis can inhibit all of the oral bacteria implicated in infective endocarditis (Walker et al. 1985). It is conceivable and indeed probable therefore, that all bacteria that gain access to the bloodstream during dental treatment may not be susceptible to the particular prophylactic antibiotic administered. It is also possible that antibiotic regimes involving a combination of antibiotics may not neutralise all micro-organisms that may gain access to the bloodstream during dental treatment (Pallasch & Slots 1996).

There have been a number of instances where infective endocarditis has not been prevented following dental treatment, despite the recommended antibiotic prophylaxis regime having been followed (Durack et al. 1983). Furthermore, the bacteria cultured from these lesions have been shown to be sensitive to the prophylactic antibiotic administered. The value of antibiotic prophylaxis has been questioned by a number of workers on the basis of the community prevalence of penicillin allergy. From a review of the literature, Pallasch (1989) concluded that the number of lives saved by the prevention of infective endocarditis from the prophylactic administration of penicillin was probably outweighed by the death rate from anaphylactic reactions to penicillin, for patients at lower risk for infective endocarditis.

2.4.3 Role of antiseptic agents as an adjunct to antibiotics

As antibiotics have been shown to be ineffective in some situations (Durack 1983), it has been suggested that prevention of infective endocarditis must not depend on antibiotic agents alone and that the practitioner should consider adjunctive methods, such as mouth rinses, to reduce the risk of complications from transient bacteraemia (Tzukert et al. 1986). One such adjunct that has been proposed is the use of topically applied antiseptic agents at the gingival margin to reduce the numbers of viable micro-organisms entering the bloodstream during dental treatment (Bender & Barkan 1989; Randall & Brenman 1974; Dajani et al. 1990, 1997). This could result in fewer viable bacteria entering the bloodstream and could enhance attempts to protect susceptible patients from bacteraemia by reducing the size of the bacteraemia. It could be postulated that if fewer micro-organisms were to gain access to the

bloodstream, then the chance of such micro-organisms evading the host's immune mechanism and the prophylactic antibiotics deployed against them would be decreased.

The American Heart Association recommends the use of a pre-treatment antimicrobial mouth rinse as an adjunct to antibiotic prophylaxis, to reduce the incidence of iatrogenic bacteraemia (Dajani et al. 1990, 1997). Chlorhexidine digluconate has been recommended as an agent to be used. However, the authors have not cited evidence to show that a pre-treatment chlorhexidine mouth rinse is of benefit in reducing the occurrence of iatrogenic bacteraemia.

The American Heart Association bases its recommendation for the use of chlorhexidine on that of a previous review paper (Pallasch & Slots 1996), which states that chlorhexidine is the antimicrobial agent of choice, applied by "gentle oral rinsing for one to two minutes prior to dental treatment". Pallasch and Slots (1996) support this by citing a regime proposed by Dajani and co-authors some years earlier (Dajani et al. 1990), where it was suggested that chlorhexidine be painted on isolated dried gingiva for three to five minutes before dental treatment. However, no reference to a clinical study was given by Dajani and co-authors (1990) to support their assertion that this practice would decrease the incidence of iatrogenic bacteraemia.

As the recommendation for the use of a pre-procedural oral rinsing with chlorhexidine (Dajani et al. 1997) does not appear to be based on cited clinical studies, other antiseptics, such as povidone-iodine, should be evaluated. Antiseptic solutions do not enter the gingival crevice or periodontal pocket when administered as a mouth rinse

(Pitcher et al. 1980), although using an irrigating syringe and needle has been shown to enhance the penetration of such solutions into pockets (Pitcher et al. 1980). However, subgingival irrigation with chlorhexidine and with sterile water has been shown to cause a bacteraemia (Lofthus et al. 1991). For this reason, subgingival irrigation with antiseptic solutions is not recommended as a prophylactic measure in patients at risk of infective endocarditis (Pallasch & Slots 1996; Dajani et al. 1997).

2.5 POVIDONE-IODINE

2.5.1 History

Elemental iodine was discovered in 1811 by Bernard Courtois, a French chemist. It derives its name from the Greek word *ioeides*, meaning violet, due to the intense violet colour of its vapours. Iodine has been used as a wound antiseptic for over 150 years (Fleischer & Reimer 1997). The early iodine preparations such as iodoform and ethylic iodide tincture were lacking in stability and were highly aggressive towards skin and mucosa. Binding it to various macromolecules further detoxified iodine. More tissue-friendly preparations came in the form of iodophores such as povidone-iodine, which emerged towards the end of the 1960s (Fleischer & Reimer 1997).

2.5.2 Chemical Nature

Povidone-iodine is classed as an iodophore. Iodine is linked to povidone (polyvinylpyrrolidone), a dextran-like molecule, via hydrogen bonds. An equilibrium

reaction occurs in aqueous environments where approximately 10% of the bound iodine is released as free iodine to exert an antiseptic effect. This equilibrium reaction allows further free iodine to dissociate from the povidone-iodine molecule as it is used up, which helps to maintain the anti-microbial effect of povidone-iodine (Fleischer & Reimer 1997).

2.5.3 Actions

Free iodine has a strongly oxidative effect. The povidone molecule, by virtue of its affinity for cell membranes, delivers diatomic free iodine (I_2) directly to the bacterial cell surface (Zamora 1986). It is thought to exert its effect on amino acids and unsaturated fatty acids, which results in the destruction of cell membranes and enzymes (Schreier et al. 1997). Electron microscopic studies have revealed that povidone-iodine interacts with cell walls to form permanent or transient pores or solid liquid interfaces, which results in the loss of cytosolic components and the denaturation of bacterial enzymes within the cytosol (Schreier et al. 1997). When exposed to povidone-iodine, sulfhydryl compounds, peptides, proteins, enzymes, vitamin C, lipids and cytosine are iodinated and oxidised by free iodine, resulting in inactivation of molecules that are essential for the biologic activity of the micro-organism (Zamora 1986). Povidone-iodine appears to be active against all micro-organisms, including Gram-positive and Gram-negative bacteria, spores, cysts, mycobacteria, fungi, viruses and protozoa (LeVeen et al. 1993).

2.5.4 Local Toxicity

The safety of povidone-iodine has been evaluated in terms of its effect on wound healing. Povidone-iodine as a dry powder preparation, sprayed onto the abdominal wounds of rats, has been shown to have no adverse effects on wound healing (Strokon 1977). Povidone-iodine has been shown to be toxic to phagocytic cells *in vitro* in concentrations greater than 0.05%. Unbound iodine was toxic at 10% of this concentration, which stands to reason as povidone-iodine only releases 10% of its iodine content as free iodine (van den Broek et al. 1982). Van den Broek and co-workers (1982) suggested that it was the iodine exerting the toxic effects and not the povidone molecule. However, for granulocytes (basophils, eosinophils and neutrophils), the toxic concentrations of povidone-iodine and free iodine were the same (van den Broek et al. 1982).

Hypersensitivity reactions to povidone-iodine seem to be related to the povidone molecule and less often to the free iodine released (van Ketel & van den Berg 1990). van Ketel and van den Berg (1990) conducted skin patch tests on eight patients exhibiting contact eczema, three from skin and five from wound exposure to povidone-iodine. All patients showed varying degrees of sensitivity when patch tested with various povidone-iodine preparations. Five of the eight patients were patch tested with potassium iodide in petrolatum, of which none displayed sensitivity. Five of the eight patients were tested with povidone-iodine in petrolatum, four of these experiencing sensitivity reactions. These results seem to suggest that the povidone molecule is responsible for the sensitivity reactions. However, the results

are equivocal because not all the patients were tested with the potassium iodide and iodine tincture control preparations.

2.5.5 Systemic Toxicity

Long-term use of povidone-iodine has been implicated as a cause of thyroid dysfunction due to systemic absorption of iodine (Nobukuni et al. 1997). The 27 subjects in this study were all chronically ill and had daily applications of povidone-iodine to cannula or tracheostomy sites. The 11 patients who developed problems related to iodine toxicity had only mild thyroid dysfunction, which only manifested in six patients after at least 40 weeks of administration. There was only one case of mild hyperthyroidism, the other patients experiencing subclinical hyperthyroidism or hypothyroidism.

There are few studies of the toxic effects of povidone-iodine in preparations designed for oral use. A prospective study of six months' duration has shown that daily rinsing with Perimed® (5% povidone-iodine, 1.5% H_2O_2) or 5% povidone-iodine and water mixture results in the systemic absorption of iodine (Ader et al. 1988). Systemic iodine absorption was evidenced by elevated levels of serum total iodine and inorganic iodide concentrations and increased urinary iodine excretion. However, serum triiodothyronine and thyroxine concentrations and the free thyroxine index did not change. Serum Thyroid Stimulating Hormone levels were raised but within the normal range, which reflected a normal physiologic response to an increased intake of

iodine. There was no evidence of thyroid dysfunction during the course of the study (Ader et al. 1988).

2.5.6 Antimicrobial Effects

The optimum concentration for povidone-iodine when used in the oral cavity has not been determined. There is evidence to suggest that dilute povidone-iodine is more effective than stock solutions (Berkelman et al. 1982). Dilutions of 10% povidone-iodine solutions of up to 1:100 have demonstrated more rapid killing of *S. aureus* and *Mycobacterium chelonei* than the undiluted solution (Berkelman et al. 1982). The mechanism for this effect of increased antibacterial activity at lower concentrations is thought to be due to weakening of the bonds between the povidone molecule and the iodine, leading to a higher level of free iodine (Trueman 1971).

2.5.7 Povidone-Iodine: Oral Formulations

Povidone-iodine is available in Australia as a Schedule 2 medication for oral use in the following formulations:

(i) Minidine® Sore Throat Gargle,

(Sigma Pharmaceuticals Pty Ltd, Croydon, VIC);

2.5% v/v povidone-iodine to be diluted 1:20

(ii) Betadine® Sore Throat Gargle,

(Faulding Pharmaceuticals, Mulgrave North, VIC);

7.5% w/v povidone-iodine to be diluted 1:20

(iii) Nyal Medithroat® Gargle,

(SmithKline Beecham International, Ermington, NSW);

75 mg/mL povidone-iodine to be diluted 1:20

2.5.8 Studies of Povidone-Iodine in Oral Bacteraemia

Investigations of the effect of povidone-iodine mouth rinses and/or irrigation on bacteraemia of oral origin have involved mainly extraction studies with only a few examining periodontal treatment.

2.5.8.1 Extraction Studies

Most of the early studies involved rinsing or irrigation with iodine since povidone-iodine was not introduced until the late 1960s. One of the first such studies (Keosian et al. 1956) examined the effect of 170ppm aqueous diatomic iodine (I_2) mouth rinse on the incidence of post-extraction bacteraemia. The I_2 group (101 patients) rinsed with five aliquots of 20 mL of solution for 20 seconds each. The control group (100 patients) rinsed with saline mouthwash. Blood samples were obtained before and two and 12 minutes after extraction of the teeth and were cultured using aerobic and anaerobic techniques. The incidence of bacteraemia in the I_2 group was 20% and 27% for the control group. However, the extraction technique was poorly defined and the study population was not homogeneous with respect to periodontal or pulpal and periapical pathology. The authors addressed this problem in part, noting that for the bacteraemia positive patients in the I_2 group, all but one had "local infection" which included presence of a dento-alveolar abscess, chronic pulpitis or periodontal involvement. No analysis was done for the patients who rinsed with saline. Keosian and co-workers (1956) concluded that rinsing with aqueous diatomic iodine was effective in reducing bacteraemia uncomplicated by the presence of dental infection, but had little or no effect in those complicated by such infection.

Scopp & Orvieto (1971) examined the effect of 0.5% povidone-iodine solution as compared with a flavoured, coloured placebo, in a controlled double-blind study consisting of 64 male patients in their third to seventh decades of life. The povidone-iodine and placebo groups had 31 and 28 subjects respectively. The mouth rinse solutions were applied as two 30 second rinses using 10-20 mL of solution. This was

followed by irrigation of the sulcus and marginal gingiva for one minute using a blunt syringe and a further 10-20 mL of solution. Two blood cultures were taken, the first at baseline and the second, at three minutes after the extraction procedure.

It was found that the povidone-iodine resulted in a statistically significant reduction in the incidence of bacteraemia resulting from dental extractions (Scopp & Orvieto 1971). The incidence in the povidone-iodine group was 28% and that in the placebo group was 56%. However, plaque samples were obtained from the gingival sulcus following rinsing and this procedure could have contributed to the incidence of bacteraemia in both groups. Further, the number of teeth extracted was not specified. Since the number of teeth extracted has been shown to affect the occurrence of bacteraemia (Okabe et al. 1995), this could have influenced the results of the study.

Macfarlane and co-workers (1984) evaluated the effect of 1% v/v povidone-iodine and 1% v/v chlorhexidine solutions relative to a normal saline control group, on the incidence of bacteraemia following the extraction of a single mandibular posterior tooth (excluding third molars). There were 20 age and sex-matched patients in each of the three study groups. A blunt ended needle was used to irrigate the gingival sulcus of the tooth to be extracted, using 10 mL of the solution to be tested and the patients held the solution in their mouths for two minutes before expectorating. Exclusion criteria included gross caries, presence of conditions such as advanced periodontal disease, periapical lesions or swelling and antibiotic treatment within the previous three months.

Blood samples were taken immediately before and 30 seconds after tooth extraction and were subsequently cultured under anaerobic and aerobic conditions. The bacteraemia incidence for the saline group was 80%, chlorhexidine 25% and povidone-iodine 40%. However, the difference between the povidone-iodine and chlorhexidine groups, with respect to the incidence of bacteraemia, was not statistically significant. Statistical significance may have been reached if more patients had been enrolled in the study. The largest group of bacterial isolates recovered was composed of anaerobic streptococci, followed by isolates of *S. sanguis*, *S. mutans* and *Streptococcus mitior*.

Yamalik and co-workers (1992) examined the effect of sulcus irrigation using 1% povidone-iodine delivered as a subgingival lavage on the incidence of bacteraemia following extraction of a molar tooth. Four groups, each of 20 patients, age and sex matched where possible, were used to compare the effects of 0.02% chlorhexidine, 1% povidone-iodine, 3% hydrogen peroxide and no irrigation. Blood samples were taken before and after the extraction procedure, which took less than three minutes to complete. The bacteraemia incidence observed for the control group (no irrigation) was 70%, whereas for the povidone-iodine group it was 35%, the hydrogen peroxide group 50% and the chlorhexidine group was 40%. A statistically significant reduction in bacteraemia was observed only for the povidone-iodine group. The results need to be interpreted with caution, as there was no irrigation performed with a negative control solution such as saline. There was no verification of homogeneity between the study groups in the form of a statistical analysis of clinical parameters and patient numbers in the study groups were low. Further, the timing of the post-operative blood samples was not specified. Since bacteria are cleared rapidly from the bloodstream

following dental manipulations (Pallasch & Slots 1996), the timing of blood sampling needs to be clearly specified to permit useful comparison with other studies. The authors note that steps were taken to exclude patients with periapical and periodontal infections. However, the screening procedure for exclusion of these patients was not described.

Rahn and co-workers (1995) devised a single-blind trial to determine the effect of sulcus irrigation on bacteraemia, using 0.2% chlorhexidine or 10% povidone-iodine solutions. Control group patients were irrigated with saline. There were 120 patients in total; half underwent a single molar tooth extraction and the other half had an intra-ligamentous injection. Forty patients were randomly allocated to each of the groups but the randomisation procedure was not described. The authors reported that the three treatment groups were homogeneous with respect to the clinical parameters recorded but no values for these clinical parameters were presented.

Blood samples were obtained prior to sulcus irrigation and at two, four and six minutes after treatment. The blood samples were then cultured and subsequently subcultured for identification if growth was detected. There were no positive baseline blood cultures reported. The incidence of bacteraemia was found to be least in the povidone-iodine group (27.5%), followed by the chlorhexidine group (45%) and the saline group (52.5%). The incidence of recovery of Viridans Group Streptococci was 10% in the povidone-iodine group, 35% in the chlorhexidine group and 32.5% in the saline group. The authors make a seemingly contradictory statement when attributing statistical significance to the Viridans Group Streptococci bacteraemia results, in that they inadvertently imply that there was a statistically significant difference in

bacteraemia incidence between the chlorhexidine and saline groups. For an unknown reason, Rahn and co-workers (1995) grouped the povidone-iodine and chlorhexidine patients together but still obtained statistical significance with respect to the saline controls. Based on their results, chlorhexidine had no effect on reducing the incidence of Viridans Group Streptococci bacteraemia.

2.5.8.2 Periodontal Treatment

Winslow and Millstone (1965) were amongst the first to determine the effect of an iodine solution on the incidence of bacteraemia resulting from dental scaling, root planing and curettage of gingival crevicular epithelium. The iodine solution tested was made of one part of 15% phenol, two parts tincture of aconite, three parts of 3.5% tincture of iodine, and four parts glycerine. The iodine solution was formulated to reduce the tissue damage that results from the use of elemental iodine on mucous membranes. Thirty- two patients with periodontitis were included in the study, as the authors considered that patients with gingivitis were not prone to bacteraemia resulting from routine dental treatment. The iodine solution was syringed into the periodontal pockets before the commencement of scaling. A baseline blood sample was taken, followed by a second sample after two minutes of scaling and the blood samples were cultured aerobically and anaerobically. No bacterial growth was observed in any of the blood samples. It is difficult to draw any conclusions from these results as the study was not controlled. Winslow and Millstone (1965) compared their results to a similar study in which no antimicrobial irrigation was used (Winslow & Kobernick 1960), which resulted in a bacteraemia incidence ranging

from 9.5% for patients with gingivitis, to 42% for those with periodontitis. *Staphylococcus* spp. accounted for half the isolates recovered from the post-scaling blood samples. This suggests a high rate of contamination with non-oral species during the sampling or culturing procedures. The sample population was small and the identity of bacterial isolates was not reported in the later study (Winslow & Millstone 1965), which does not allow a reasonable basis to compare the two studies in terms of bacteraemia incidence in relation to scaling procedures and the effect of the iodine solution.

Brenman and Randall (1974) showed that two 30 second rinses with 20 mL of 0.5% povidone-iodine, followed by a one minute lavage of the gingival sulcus and attached gingiva using the same volume of solution, resulted in a reduction of bacteraemia incidence in the order of 35% following gingivectomy as compared with a negative control group who were irrigated with an unspecified placebo solution. The reduction in bacteraemia incidence was statistically significant. The incidence of bacteraemia in the povidone-iodine group was 23%.

The most recent study examining the efficacy of povidone-iodine in reducing bacteraemia due to scaling and root planing concluded that a pre-treatment povidone-iodine mouth rinse and sulcus irrigation had no effect on the incidence of bacteraemia (Witzenberger et al. 1982). This study involved 20 patients undergoing a split mouth trial involving two quadrants with periodontally involved teeth. Each quadrant had three teeth with at least one molar, with probing depths of at least 4 mm and with at least one surface with subgingival calculus deposits. The study was conducted over three appointments, the first involving a complete periodontal examination. At the

second appointment ten days later, a cannula was inserted into a vein and a 5 mL baseline blood sample was taken. At this visit, patients rinsed with 15 mL of Betadine® gargle for one minute, although the concentration of povidone-iodine was not stated. The teeth in the test quadrant were then isolated and the gingival sulci were irrigated with 10 mL of 10% povidone-iodine solution with a blunt tipped irrigating syringe, after which the solution was allowed to remain in the mouth for a further three minutes. The second blood sample was then obtained. The third blood sample was taken after two minutes of scaling and the fourth on completion of the scaling procedure. Patients returned within 14 days for treatment of the control quadrant. At this visit, no negative control mouth rinse nor irrigation was used before scaling of the three teeth. Blood samples were taken prior to and after two minutes of scaling and at the end of the scaling procedure. All blood samples were cultured using anaerobic culture techniques.

Witzenberger and co-workers (1982) found that the bacteraemia rate for both the control and test visits was 55%. The 11 patients who were positive for bacteraemia without prior rinsing or irrigation were the same patients who were bacteraemia positive following rinsing and sulcus irrigation with povidone-iodine solution. The authors concluded that povidone-iodine mouth rinsing and irrigation neither increased nor decreased the incidence of bacteraemia following scaling. However, there are significant deficiencies in the methodology that preclude reliable conclusions being drawn from the results.

Firstly, the study was uncontrolled as no negative control solution was used with which to compare the effect of povidone-iodine. Secondly, bacterial isolates were not

identified and consequently there is no way of verifying that the isolates were oral species resulting from the scaling procedure. It is possible that the bacterial isolates recovered may have been skin contaminants entering the bloodstream from the venepuncture site.

Witzenberger and co-workers (1982) attributed the lack of efficacy to the failure of povidone-iodine to penetrate into the periodontal pockets. However, this explanation cannot be verified because the bacterial isolates were not identified and therefore no conclusions can be made regarding the effect of povidone-iodine on the periodontal pocket flora or the bacteraemia isolates recovered. Witzenberger and co-workers (1982) conceded that as a non-quantitative culture technique was used, it was not possible to determine the effect of povidone-iodine on the magnitude of the bacteraemia resulting from scaling and root planing.

The authors used statistical analysis to determine whether there were clinical periodontal parameters which could have indicated the likelihood of patients developing bacteraemia. No relationship was found between bacteraemia recovery and presenting clinical parameters. However, this finding is equivocal as the periodontal parameters (except the plaque index) were only recorded at the first visit and not on the days of the treatment appointments. The methodology allowed for up to 24 days to elapse between the first and third visits. Consequently, a Hawthorne effect could have led to an improvement of the clinical parameters, particularly the amount of gingival inflammation, which was not quantified and which could have degraded the quality of the clinical periodontal data. Furthermore, the results for the control quadrants may not be independent from those of the povidone-iodine rinse

quadrants. It is not clear whether treatment was randomised or whether all patients received the povidone-iodine at the first treatment visit. The authors stated that up to two weeks elapsed between the appointments for treatment of the control and povidone-iodine patients. This may mean that for some patients only a few days may have elapsed between treatment appointments. If so, there may have been a "carry-over" effect with the povidone-iodine solution.

A major deficiency of this study was that no negative control was used for rinsing and irrigation. In order to ascertain whether povidone-iodine is of no value in reducing bacteraemia due to scaling, it is necessary to compare it with a negative control solution. Otherwise, it is possible to speculate that rinsing and irrigation with a control solution such as sterile saline could be superior to povidone-iodine. A further deficiency relates to the lack of identification of the bacterial isolates recovered. Without such identification, it can be speculated that the bacteraemia was not entirely of oral origin. In order to clarify the value of povidone-iodine in reducing bacteraemia due to scaling, it would be necessary to design a study overcoming the above deficiencies.

2.6 SUMMARY OF LITERATURE REVIEW

The entry of oral bacteria into the systemic circulation following invasive dental treatment is widely accepted as a risk factor for the development of infective endocarditis in those patients who are readily susceptible to that condition. The value of reducing the numbers of viable bacteria entering the bloodstream by utilisation of pre-treatment mouth rinses has been referred to by the American Heart Association. One such mouth rinse, povidone-iodine, has been shown to be effective in reducing bacteraemia when used in patients undergoing dental extractions and gingivectomy. However, its use in patients undergoing scaling has not been recommended (Greenstein 1999) due mainly to the findings of the Witzenberger et al (1982) study. Due to the study design problems of that investigation, it is necessary to design a well controlled study to determine whether a povidone-iodine mouth rinse, used prior to scaling, is effective in reducing the incidence of bacteraemia.

3. HYPOTHESIS

The hypothesis of this study was that the use of povidone-iodine mouth rinse can reduce the incidence of bacteraemia due to scaling, relative to that of sterile saline. For statistical purposes, the null hypothesis was that rinsing with povidone-iodine for two minutes before scaling has the same effect on the incidence of bacteraemia as rinsing with saline solution.

4. AIMS

The aims of this study were:

i) to assess the effect of povidone-iodine mouth rinse on the occurrence of bacteraemia due to scaling;

ii) to identify the micro-organisms recovered in the positive blood cultures following scaling;

iii) to determine the magnitude of bacteraemia;

iv) to determine whether the likelihood of bacteraemia occurring could be predicted on the basis of the subjects' age, gender, smoking status or clinical data.

5. MATERIALS AND METHODS

5.1 ETHICAL APPROVAL

The study was approved by the Scientific, Human Ethics and Drug Committees of the Western Sydney Area Health Service and the Human Ethics Committee of the University of Sydney.

5.2 SUBJECTS

5.2.1 Selection Criteria

The subjects were obtained from the waiting list of Westmead Hospital Centre for Oral Health. The waiting list consisted of patients who had been assessed for treatment and placed on a waiting list. Following the assessment procedure, all patients on the waiting list had a Community Periodontal Index of Treatment Needs (CPITN) score (Ainamo et al. 1982) and an OPG radiograph. A waiting list of 3500 patients was maintained by Westmead Hospital Centre for Oral Health, which provided the source of new patients for the dental hospital clinics. As at February 1st 2001, there were 2000 female patients and 1228 male patients on the waiting list.

For selection, patients were required to have a palpable vein in an antecubital fossa and to be in good general health. They were also required to have plaque induced gingivitis, as defined by the American Academy of Periodontology (AAP) involving five adjacent teeth (Mariotti 1999), with visible plaque and bleeding on provocation. Pocket depths on these teeth had to be less than or equal to 4 mm.

Patients were excluded from involvement in the study if they were known to have:

a) significant medical problems e.g. diabetes, systemic lupus erythematosus, rheumatoid arthritis or infection with the human immunodeficiency virus; b) If they were known to be allergic to iodine; c) If they had taken antibiotics in the last three months or were taking corticosteroid or immunosuppressive medications; d) If they had a history of cardiac defects or other conditions requiring the administration of prophylactic antibiotic cover; e) If they were pregnant; f) If they had probing depths of 4mm or greater; g) or if they had received periodontal treatment or scaling/cleaning within the previous six months.

Sixty subjects were sought. Subjects with CPITN scores of one or two (Ainamo et al. 1982) were selected from the Westmead Hospital Centre for Oral Health treatment waiting list. A printed list of patients at least 16 years of age and with appropriate CPITN scores was obtained. Patients were telephoned consecutively. The experimental procedure and purposes of the study were explained briefly. A screen of their medical history was undertaken to confirm that they were able to be included in the study. An appointment was then arranged for the clinical procedure. All patients fulfilling the

screening criteria were included in the study. Patients were instructed not to brush for at least 30 minutes before the appointment to avoid the possibility of any tooth brushing-induced bacteraemia (Sconyers et al. 1973). The patient's hospital file was available during the appointment in order for the OPG radiograph to be visualized.

A detailed information sheet was provided for the volunteers as they arrived for the clinical component of the study. Each patient then signed a witnessed consent form approved by the Western Sydney Area Health Service. An interpreter was provided when necessary. The patient information and consent forms that were used are presented in Appendix 2.

5.2.2 Patient Data

Smoking status, gender and age were recorded. Patients were asked if they had brushed their teeth in the 30 minutes prior to the appointment.

5.2.3 Clinical Data

Five adjacent teeth that satisfied the parameters for the diagnosis of plaque induced gingivitis (Mariotti 1999) were identified. These five teeth were the "study teeth" and usually consisted of teeth in quadrant three, namely 35 to 31 inclusive. Teeth in quadrant four, namely 41 to 45 were used if the teeth of interest were teeth missing in quadrant three. The recordings of clinical indices made for the teeth to be scaled were the Papilla,

Margin, Attached Gingiva Index (mPMAI), modified for the purposes of the study (after Schour & Massler 1947) and the Plaque Index (PlI), (Silness & Löe 1964). These indices were recorded at the buccal and lingual surfaces for each study tooth. The non-invasive clinical parameters or those that were not likely to cause a bacteraemia were recorded before the scaling procedure (PlI, mPMAI). Those that may have caused a bacteraemia were recorded following collection of the final blood sample (probing depths and recession).

The PMA index was modified for the purposes of the study in two ways. Firstly, the values were recorded in a way that facilitated statistical analysis and a category was added for the absence of inflammation. Secondly, the index was applied to posterior teeth that were included in the study. The PMA index (Schour & Massler 1947) was used to describe the gingival condition in anterior regions. Table 1 shows how the new values were allocated to the PMA score.

Table 1 Modification of the PMA Index values

Extent of Inflammation	**PMA score**	**Modified score**
No visible signs of inflammation	-	0
Papillae only	P	1
Papillae and Margins	M	2
Papillae, Margins & Attached Gingiva	A	3

Following recording of these clinical parameters (PlI, mPMAI), the volunteers were randomly allocated to either the experimental or the control group. The allocation was decided at the toss of a coin.

5.3 EXPERIMENTAL DESIGN

5.3.1 Experimental group treatment

Experimental patients rinsed with 10 ml of povidone-iodine 7.5% w/v solution (Betadine® Sore Throat Gargle), which had been diluted 1:20 with water according to the manufacturer's directions, using the measuring container provided with each bottle. The final concentration of the mouth rinse was therefore 0.375% povidone-iodine (w/v). Patients rinsed for one minute and were then asked to expectorate. Following an interval of 30 seconds, the patients repeated the one-minute rinse with a further 10 ml of the same solution.

Immediately following the Betadine® rinses, an ultrasonic scaler (Siemens Siroson®, Germany) was used to clean the test teeth for a total of two minutes. The scaler was used at maximum power and maximum water flow. Supragingival calculus was removed first, within the first ten seconds of scaling. The scaler tip was then placed at the base of the sulcus at the distal study tooth. The side of the scaler tip was used to clean the tooth

surface using horizontal strokes keeping the scaler tip at the base of the sulcus. The scaler tip was tilted to reach the interproximal areas and a vertical lifting motion was used to remove any interproximal deposits. All the lingual surfaces were cleaned before moving to the disto-buccal aspect of the most distal study tooth whereupon all the buccal surfaces were cleaned.

Bleeding from the gingival margin was recorded during and after scaling. If a line of blood filled the gingival sulcus, this was gently irrigated and washed away with the scaler until the source of the bleeding was identified. Bleeding was recorded at six sites per tooth. This clinical parameter was given the title "Bleeding on Instrumentation". Finally, the probing depths and recession for the study teeth were measured using a Williams probe with Michigan markings (Premier USA, Cat No. 03672) at six sites for each tooth (Mesio-Buccal, Mesio-Lingual, Mid-Buccal, Mid-Lingual, Disto-Buccal, Disto-Lingual). The mean probing depths and mean recession were then calculated for each subject.

5.3.2 Control group treatment

The control group patients were asked to rinse with 10 ml of sterile normal saline (0.9% NaCl, Astra Pharmaceuticals Pty. Ltd., North Ryde, NSW) for one minute and were then allowed to expectorate. A 30 second respite was allowed, as for the test patients, before the rinse was repeated for a further minute. Ultrasonic scaling and then measurement of the clinical parameters were carried out as for the experimental group.

5.4 BLOOD SAMPLES

5.4.1 Venepuncture technique

All blood samples were taken via an intravenous cannula inserted into a vein in the antecubital fossa with the most easily palpable vein, using an aseptic technique. All the cannulae were inserted by the same operator. The insertion of the cannula was undertaken following allocation to the test or control group, before rinsing with the mouthwash. Each patient was instructed to remove clothing (when feasible) that prevented easy access to an antecubital fossa. A tourniquet was then applied to the arm midway between the shoulder and the elbow in order to easily palpate and visualize the cephalic vein or the median cubital vein.

After donning examination gloves, the skin overlying the vein was swabbed with a sterile 70% isopropyl alcohol wipe (Kendall, WEBCOL® Alcohol Prep, 2-Ply Medium, Mansfield, MA) for a period of ten seconds. The skin was allowed to dry for one minute before the intravenous cannula (Terumo SURFLO® 22G x 25 mm) was sited in the chosen vein. The cannula was fitted with a one-way valve, which acted as a stopper and had a port for connection to a syringe for drawing the blood samples and for flushing the IV apparatus with heparinised saline (Braun SAFSITE® VALVE, Bethlehem, PA, USA). All care was taken to avoid contamination of the cannula site following cleansing with the alcohol wipe. Following insertion, a cannula dressing was placed to secure the cannula and to help prevent contamination of the wound (OPSITE IV3000™ 6 x 8.5 cm, Smith & Nephew, Hull, U.K.). The cannulation apparatus is shown in Figure 1.

5.4.2 Sampling times

Three blood samples were taken from each volunteer:

i) 10 ml of blood was sampled as a baseline measurement, to ensure the absence of a pre-existing bacteraemia. This was collected following rinsing with the mouthwash provided.

ii) 10 ml of blood was sampled 30 seconds after scaling was commenced.

iii) A further 10 ml of blood was sampled at the completion of two minutes of scaling.

Thus, a total of 30 ml of blood was collected from each patient.

The time of collection of the first blood sample was recorded. Each blood sample was drawn using a sterile 10 ml eccentric tip syringe (Terumo®, Elkton, MD, USA) after connecting it to the intravenous cannula. In order to prevent bacterial contamination of the cannula and valve, the intravenous cannula and SAFSITE® valve were flushed with heparinised saline solution between samples, (2 x 2.5 ml of 50 IU Heparin Sodium in 5ml 0.9% Saline, ASTRA Pharmaceuticals, NSW, Australia). The cannula flushing apparatus and heparinised saline vials are shown in Figure 2.

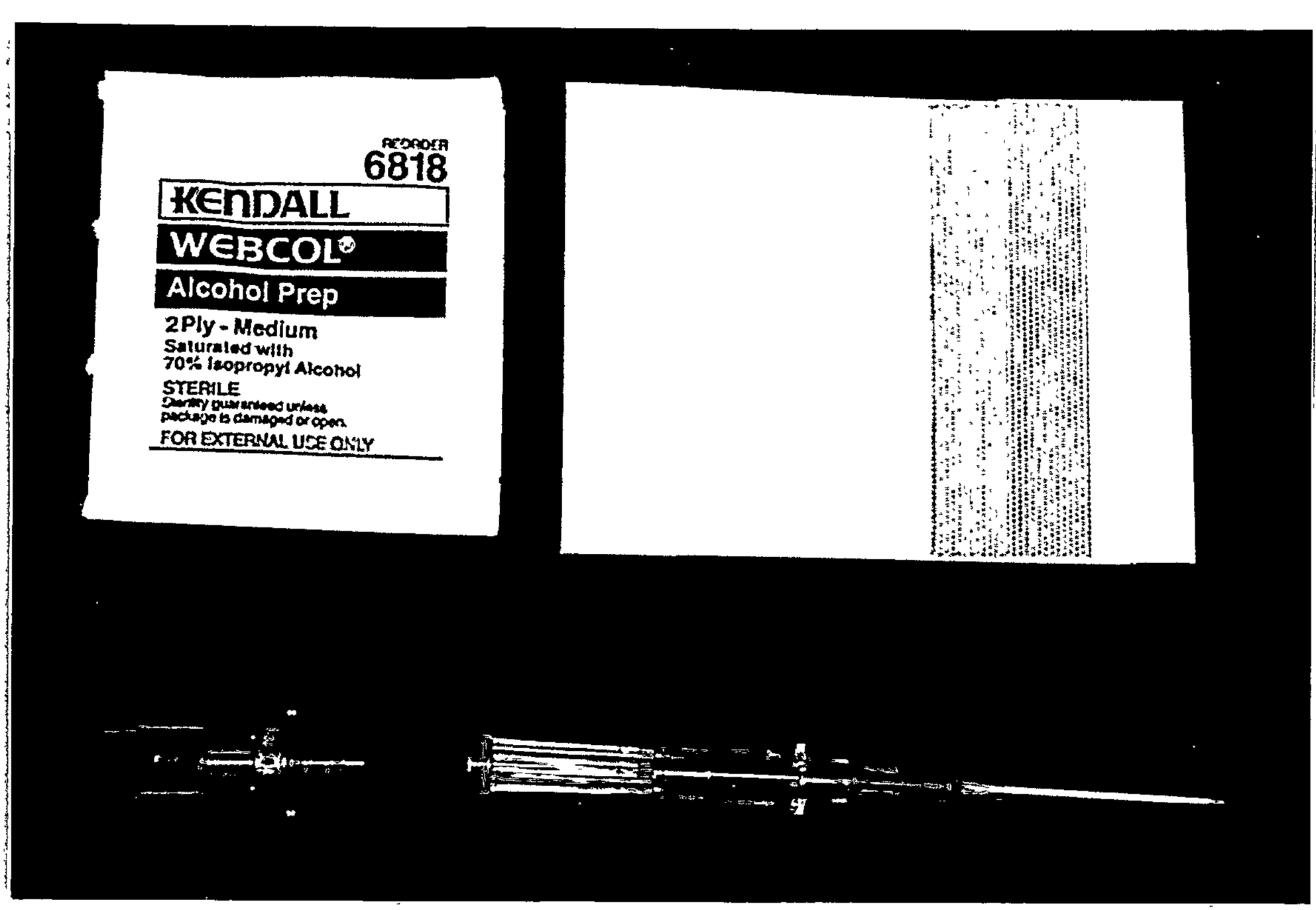

Figure 1: Cannulation apparatus (clockwise from top left): alcohol wipe, cannula dressing, cannula, SAFSITE® valve.

5.4.3 ISOLATOR™ tube inoculation

A lysocentrifugation tube (Oxoid Wampole ISOLATOR™ 10) was inoculated with each blood sample immediately following collection. The stoppers of the lysocentrifugation tubes were decontaminated with WEBCOL® alcohol wipes and allowed to air dry before being filled with blood. A sterile 19-gauge needle was fitted to each syringe containing the blood samples (TERUMO® 1.1mm x 38 mm, Elkton, MD, USA) to enable transfer to the respective labelled lysocentrifugation tube. The types of syringe and lysocentrifugation tube used for the blood sampling procedures are shown in Figure 3.

Following inoculation of the lysocentrifugation tubes, the syringe and needle were disposed of in a sharps container and each tube was inverted at least five times to ensure mixing of the blood with the reagents within the tube. A list of the tube reagents can be found in Appendix 3.

The tubes were marked with two numbers, one to identify the patient (study reference number) and another to identify the sample sequence. The patient numbers did not show laboratory staff to which treatment group the patient belonged. Following collection of the blood samples, the cannula was removed and a gauze dressing was placed over the wound. The gauze dressing was replaced with a sticking plaster after allowing a few minutes for haemostasis at the cannula wound site. Each patient was instructed to apply firm finger pressure to the gauze dressing and to keep his or her arm straight in order to minimize bruising.

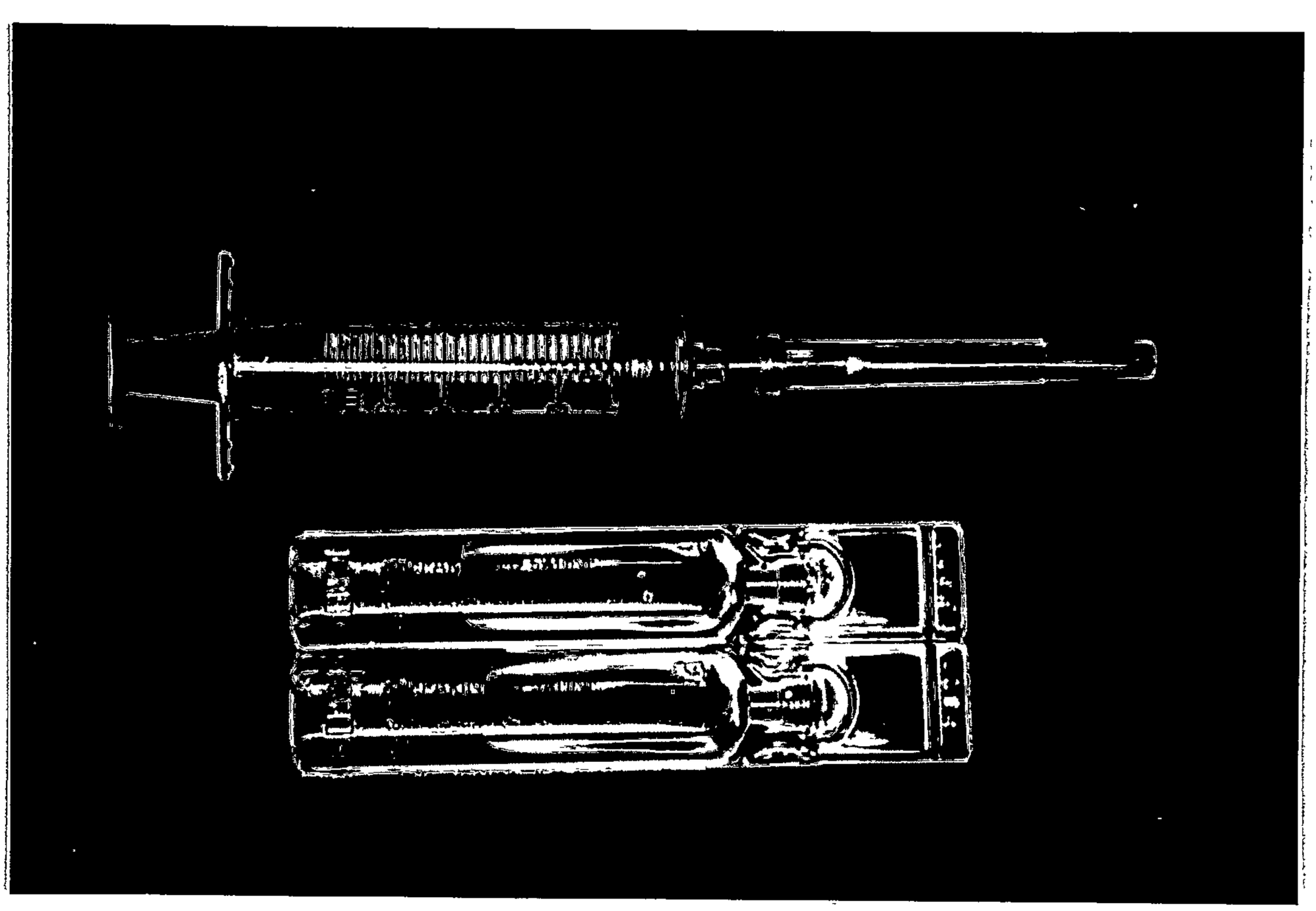

Figure 2 Cannula flush; heparinised saline and syringe.

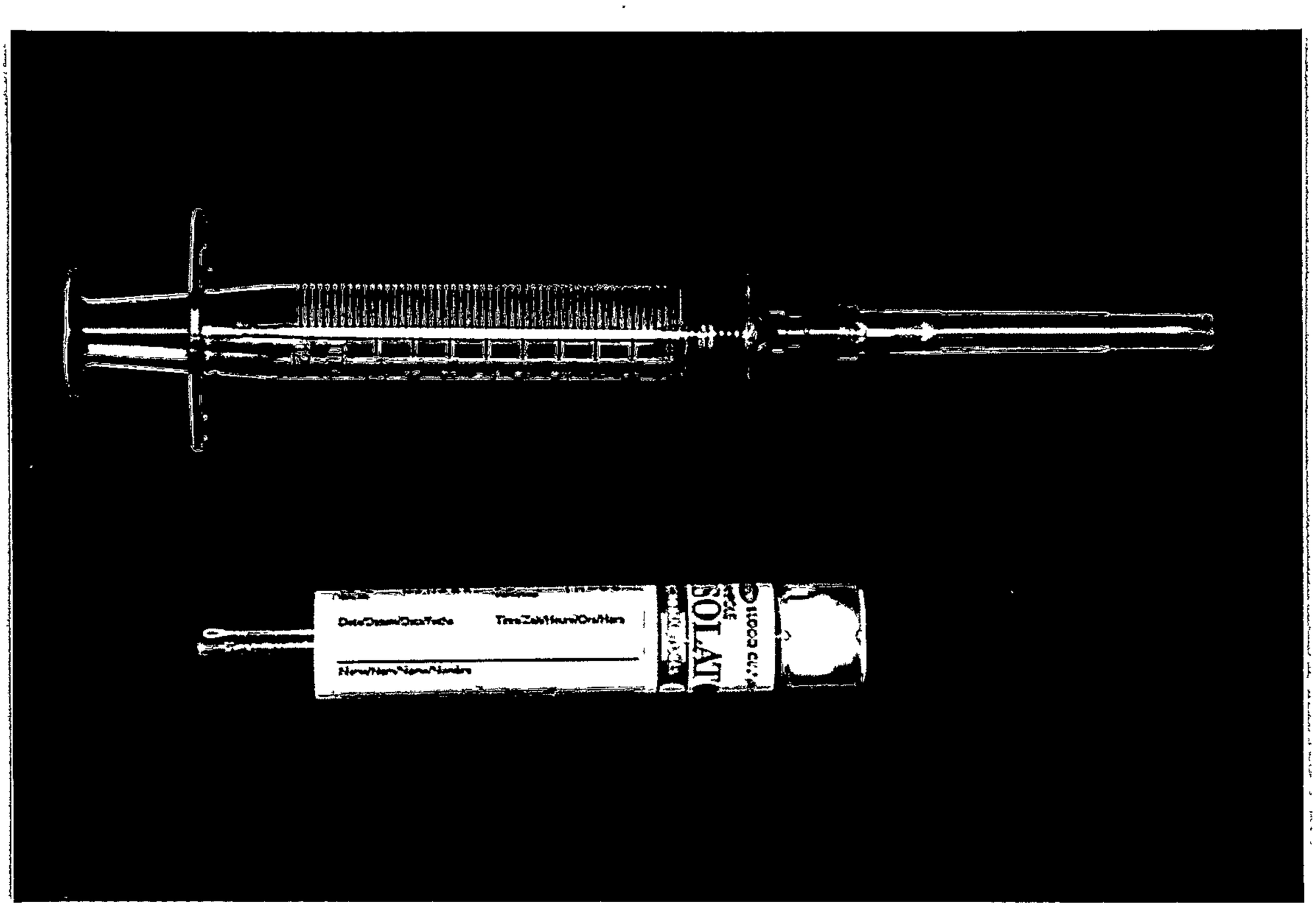

Figure 3 Blood sampling syringe and lysocentrifugation tube.

5.5 BLOOD CULTURING

5.5.1 Processing of blood samples

Following the collection of the blood samples, the lysocentrifugation tubes were transported to the laboratory for centrifugation in order to recover a pellet containing the bacteria within the sample. The pellets were subsequently cultured in order to enumerate and identify any bacterial species present. The protocol used was that described in the Oxoid Wampole ISOLATOR™ 10 instruction manual, which forms the basis of the processing procedure described below.

Specimens were processed as soon as possible after collection. The time of plating of the blood samples was recorded. The elapsed time between collection of the first blood sample and plating of the pellet following centrifugation was recorded and was referred to as the plating time. The patient appointment times were scheduled so that a maximum of eight hours could elapse from collection to the processing of the blood specimens.

5.5.2 Processing procedure

The lysocentrifugation tubes were centrifuged at room temperature for 10 minutes at 5000G (Heraeus Christ, Labofuge GL, Osterode, GmbH, 35° fixed angle rotor centrifuge). A blank ISOLATOR™ 10 tube was used as necessary to balance the rotor. The lysocentrifugation tubes were transported in a rack following centrifugation, to avoid

the potential of mixing of the tube contents due to heating of the glass wall from handling.

The centrifuged tubes were placed in an ISOSTAT (BC502C) rack and press (Oxoid Wampole, UK). The press is designed to push a cap down over the end of the tube to pierce the tube stopper, in order to gain access to the tube contents for removal. The tube stoppers were once again decontaminated with a WEBCOL® isopropyl alcohol swab and allowed to air dry. After drying, each tube was fitted with a sterile ISOSTAT cap. Care was taken not to contaminate the cap aperture or the internal spike. The press was activated for at least five seconds to ensure that the cap was fully seated. The cap aperture and the tubular internal spike allow for the insertion of pipettes, to enable aspiration of the contents of the tube.

Following the fitting of the cap, the tubes were transported to a Class II Hepa-filtered Laminar Flow Cabinet (Westinghouse AIRPURE, Model # 1687/2340/612.2) for recovery of the pellet and the plating procedure. The supernatant was subsequently removed using disposable supernatant pipettes (ISOSTAT). A supernatant pipette was removed from the heat-sealed pack and the pack was immediately re-sealed. Care was taken not to contaminate the pipette stem at any time. Before inserting into the tube, the bulb at the end of the pipette was squeezed to remove the air within. The air must be removed at this stage to avoid mixing of the tube contents, should any air bubbles be forced from the pipette whilst the tip is below the surface of the supernatant.

The supernatant pipette was placed into the tube so that the lower edge of the bulb was against the tube cap. The bulb was then released, aspirating the supernatant. It was apparent that all the supernatant had been removed when air was aspirated into the pipette stem. The pipettes were withdrawn from the tube without expelling any of the supernatant. The pipette containing the supernatant was subsequently disposed of in a contaminated waste receptacle. This procedure was repeated for all the tubes, using a new sterile pipette for each.

Following removal of the supernatant, the pellet containing the material to be cultured was re-suspended in the remaining liquid. This was accomplished with the aid of a vortex mixer (Chiltern MT-19, Auto Vortex Mixer). The tube contents were vortexed for 15 to 20 seconds at the maximum setting. A concentrate pipette (ISOSTAT™) was then removed from its heat-sealed pack. As with the supernatant pipette, care was taken not to contaminate the stem of the pipette at any stage. The bulb was squeezed to expel the air and the pipette was inserted into the tube through the aperture in the cap, without releasing pressure on the bulb. A concentrate pipette is longer then a supernatant pipette and is designed to reach to the bottom of the tube to remove all the remaining contents. Slowly releasing pressure on the bulb and rotating the tube to aspirate any remaining matter accomplishes this. The pipette was then used to transport the pellet material to the culture plates, where the contents were evenly divided between each plate. This procedure was repeated for all the tubes in turn, using a new concentrate pipette for each tube.