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**Ksiezycki-Ostoya, Beata Katarzyna**

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# INDUCTION OF PERIODONTITIS IN THE CHACMA BABOON

BEATA KATARZYNA KSIEZYCKI-OSTOYA

A dissertation submitted to the Faculty of Health Sciences of the University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry by research and coursework.

Johannesburg, 1999.

DECLARATION

I hereby declare that this research report is my own work and has not been submitted or incorporated in another dissertation or thesis for any other degree.

Beata Katarzyna Ksiezycki-Ostoya

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I would like to thank my supervisor, Prof U Ripamonti, Bone Research Unit, MRC/ University of the Witwatersrand, Johannesburg, for his guidance and assistance throughout this study, and for the honour of working with an international authority in the field of bone research.

My sincere appreciation to Mrs Arvinda Sooka in the Anaerobic Unit of the Department of Medical Microbiology, SAIMR, for her tireless work and sound advice on the microbiological component of this study.

My gratitude to Mrs Marianne Hendrikse, Marina Hengelbrecht and Raymond Cherry, of the Central Animal Service, University of the Witwatersrand, for their assistance during the collection of the data.

A special thanks to Mrs Aviva Petri, Eastman Dental Institute, London, for her guidance with the statistical analysis of the results obtained.

I would also like to thank my husband and friend Dr S Salt, for all his patience and moral support during the course of this study, as well as my friend Miss S Taylor for the long hours spent in the typing of this research report.

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ABSTRACT

The chacma baboon, *Papio ursinus*, displays a naturally-occurring site-specific gingivitis mainly localised to the labial surface of the anterior aspect of the jaws, but periodontitis, as defined by loss of periodontal attachment and periodontal pocket formation, is rarely found.

The aim of this study was to induce chronic periodontitis in four baboons. On elevation of buccal mucoperiosteal flaps, furcation defects were created in the first and second maxillary and mandibular molars. Mandibular defects were implanted with periodontal packs to further periodontal destruction. Furcation defects created in the maxillary molars were implanted with silk ligatures harbouring *Porphyromonas gingivalis*. Four weeks after healing a putative pathological strain of *Porphyromonas gingivalis* isolated from a human subject was inoculated into the maxillary and mandibular furcation areas of the first and second molars of the four baboons, which were selected from the primate colony of the Central Animal Service of the University of the Witwatersrand, Johannesburg. Inoculation of *Porphyromonas gingivalis* was carried out twice a month for a period of twelve months. Follow-up examinations included sulcular probing depths and samples of subgingival plaque, which were cultured to identify the newly established flora in the periodontal sulci of the experimental animals.

The results showed that it was possible to induce chronic periodontitis in all the experimental animals, confirmed through probing periodontal pocket depths which measured between 4 and 10mm, as well as through radiographic examinations which showed loss of alveolar bone in the furcation areas of the first and second molars. Microbiological analysis of subgingival plaque samples revealed that these animals harboured the putative periodontal pathogen *Porphyromonas gingivalis* in addition to other anaerobic organisms. Therefore, induction of periodontitis through surgical intervention and microbial inoculation was achieved for the first time in *Papio ursinus*, thus establishing these animals as models for regenerative procedures with reference to man. In fact, the animals were later used for periodontal regenerative procedures, and at the time of buccal flap elevation, there were substantial furcation defects present in the affected molars filled with exuberant granulation tissue.

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# INTRODUCTION

The chronic inflammatory periodontal diseases include gingivitis a. . periodontitis. Chronic gingivitis is defined as inflammation of the marginal gingival tissues due to the accumulation of dental plaque and is characterised clinicall y redness, swelling and bleeding of the tissues (Williams et al., 1996). Chronic periodontitis, on the other hand, is defined as plaque-induced inflammation of the periodontal tissues with resultant destruction of the periodontal ligament, loss of crestal bone and apical migration of the epithelial attachment (Williams et al., 1996).

The chronic inflammatory periodontal diseases are among the most widespread of human oral diseases with 10-15% of the population affected by severe disease expression (Loe et al., 1978; Brown et al., 1989; Miyazaki et al., 1991). For ethical reasons, initiation and progression of periodontal disease, as well as the safety and efficacy of tissue regenerative procedures cannot be studied in humans. This has led to a great interest in the use of animal models to study the pathogenesis of periodontal disease. Non-human primates have been found to be the best suited to studying periodontal diseases with relevance to man because their dental and periodontal anatomy is very similar to that of humans.

The Chacma baboon, *Papio ursinus*, accumulates large amounts of dental plaque and displays a naturally occurring site-specific gingivitis mainly localised to the labial and buccal surfaces of the anterior aspect of the jaws. This site-specific gingivitis is characterised

by erythema, oedema and enlargement of the gingivae, as well as spontaneous bleeding (Borland, 1994). Furthermore, the human periodontal pathogen, *Porphyromonas gingivalis*, in conjunction with other anaerobic pathogens, is frequently cultured from plaque samples of these animals (Ripamonti et al, 1986). The potential of *Porphyromonas gingivalis* to induce a burst of disease progression in both human and non-human subjects has been confirmed in many studies through serological and microbiological examinations (Mouton et al,1981; Holt et al, 1988; Schou et al, 1993).

Despite this abundant accumulation of plaque, severe gingival inflammation and the presence of *Porphyromonas gingivalis* in sulci, no periodontitis occurs in the baboon (Borland, 1994). Although increased probing depths may be encountered, these are related to gingival enlargement forming pseudopockets without an accompanying loss of attachment. For this reason, the baboon may be a useful model in the study of the host-tissue interactions during induction of periodontitis.

Presently, an animal model exists to experiment with novel regenerative procedures. This model is based on the surgical creation of osseous furcal defects, and then, on the induction of bone regeneration through the use of growth factors and bone morphogenetic proteins (Ripamonti and Reddi, 1997). Studies at the Bone Research Laboratory, University of Witwatersrand (Ripamonti et al, 1991; Ripamonti et al, 1992; Ripamonti and Reddi, 1994; Ripamonti et al, 1994; Ripamonti et al, 1996), have shown that naturally-sourced bone morphogenetic proteins, in conjunction with a collagenous matrix, induce cementum,

periodontal ligament and alveolar bone regeneration. However, the question still exists whether the same rate and degree of success in regeneration would be achieved when bone morphogenetic proteins are applied to tissues affected by chronic periodontitis.

Against this background, I proposed to induce chronic periodontitis in the Chacma baboon.

# CHAPTER ONE: REVIEW OF THE LITERATURE

## 1.1. CHRONIC INFLAMMATORY PERIODONTAL DISEASES

### 1.1.1. Pathogenesis

In periodontal health, there is a balanced relationship between the presence of bacteria in the form of a biofilm and the host immunity. Disease expression can therefore occur as a result of an alteration in this relationship, by either an alteration in virulence factors within the biofilm or changes to the host immunity (Genco and Slots, 1984). The clinical manifestations of chronic inflammatory periodontal disease are seen as a result of the host immune defence mechanism showing signs of inflammation represented by redness, swelling, oedema and inflammatory exudate. Clinical changes are detected as bleeding on probing and alterations in probing depths. If the balance between host and bacteria is not regained, further breakdown in the form of attachment loss and bone loss may be detected in susceptible individuals.

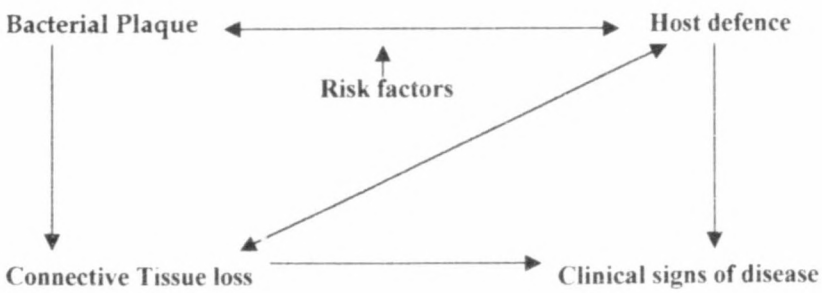



Figure 1. Plaque-Host Relationship

1.1.2. Clinical Methods of Assessing Disease Activity and Progression

A number of parameters have been used to detect and monitor periodontal disease activity over a period of time. These include bleeding on probing, probing attachment loss, probing depth and radiographs (Caton, 1989).

1.1.2.1. Bleeding on probing

Claffey et al (1990) found that 41% of the sites that bled upon probing at 75% or more of the examinations during a 42 month period underwent probing attachment loss during 0-42 months following initial periodontal therapy.

1.1.2.2. Probing depth and probing attachment loss

Probing of pockets has traditionally been the most critical portion of periodontal evaluation. Goodson (1986) stated that the 'gold standard' for measurement of periodontal disease activity is loss of probing attachment at a site. This is calculated from the record of recession (distance from the cemento-enamel junction to the gingival margin) and is added to the probing depth. Probing depth is defined as the distance from the gingival margin to the apical depth of periodontal probe tip penetration (Caton, 1989). The recording of probing depth is susceptible to variation (Watts, 1989), which may be influenced by the applied pressure of the clinician (Hunter, 1994). When significant pocketing and attachment loss are present, along with the clinical signs of inflammation, these probing measurements are interpreted to indicate the presence of periodontal disease.

1.1.2.3. Radiography

Radiographic evaluation is used to detect the severity and pattern of bone loss. However, in order for bone levels to be detected with conventional radiography, 30-50% of bone mineral loss or gain must occur before being visible (Jeffcoat, 1992). Nevertheless, conventional radiography is still the most widely used method in clinical practice. Evaluation of radiographic crestal lamina dura status is a valuable indicator for assessing periodontitis disease activity at interproximal sites as its presence is negatively correlated with disease activity (Rams et al., 1994).

1.1.2.4. Microbiological monitoring

Microbiological monitoring to identify known periodontal putative pathogens and their sensitivity to systemic antimicrobial therapy, by culturing, has been suggested by van Winkelhoff (1989). The timing between plaque collection and culturing, and the expertise of the laboratory is crucial for these tests to be meaningful.

## 1.2. ANIMAL MODELS

The practical difficulties associated with studying the pathogenesis of periodontal diseases in humans, as well as monitoring the safety and efficacy of regenerative surgical procedures, has led to a great interest in the use of animal models (Caton and Zander, 1975). However, following detailed clinical, histopathological and ultrastructural examinations, certain features of gingivitis and periodontitis in many laboratory animals

have differed significantly from those in humans, thus questioning the relevance of these models (Ammons et al., 1972; Schectman et al., 1972).

Amongst the animal models commonly used in an attempt to gain insight into the local and host factors involved in the periodontal diseases were rats, mice, other rodents, and dogs. Rats and mice offered advantages such as known age and genetic background, controllable microflora, a type of adult onset degeneration of the periodontal structures, resistance to other diseases, low cost, and ease of handling. However, these rodents have extremely small oral structures and continually growing incisors. Dogs, on the other hand, have a characteristic carnivorous tooth morphology, a different dental formula and a pronounced healing response following artificially created periodontal injuries (Miller et al., 1995).

Owing to the close anatomical and biological similarities between humans and non-human primates, non-human primates have been increasingly used for the study of periodontal diseases with relevance to man. With the exception of differences in the size of the dentition, especially the prominence of the canines; the dental formula of the permanent dentition; the presence of diastemata between anterior teeth, and an edge-to-edge relationship of the incisors, the dental anatomy of non-human primates is very similar to that of humans. Likewise, many clinical, histological, microbiological and immunological characteristics of spontaneous and experimental marginal inflammation in most non-human primates are similar to those in humans (Schou et al., 1993).

1.2.1. Clinical Manifestations of Periodontal Diseases

Schou et al. (1993) published a comprehensive review of the non-human primates which have been used in the study of periodontal disease pathogenesis. In their review, Schou et al. (1993) stated that the clinical manifestations of spontaneous gingivitis and periodontitis in chimpanzees (*Pan troglodytes*) (Page et al., 1975; Arnold and Baram, 1973), baboons *(Papio anubis)* (Avery and Simpson, 1973), as well as cotton-ear *(Callithrix jacchus)* (Ammons et al., 1972) and cotton-top marmosets *(Saguinus oedipus)* (Page et al., 1972) are quite similar to that of humans, with the exception of marked gingival telangiectasia in marmosets (Ammons et al., 1972; Page et al., 1972). However, significant differences have been reported in the prevalence and severity of the gingivitis, varying from localised in cotton-top marmosets (Ammons et al., 1972), young chimpanzees (Arnold and Baram, 1973) and baboons (Hodosh et al., 1971); slight in adult cynomolgus monkeys *(Macaca fascicularis)* (Wirthlin and Hancock, 1982); moderate in adult rhesus monkeys *(Macaca mulatta)* (Kenney, 1971) and young stump-tailed *(Macaca arctoides)* (Krygier et al., 1973) as well as young adult cynomolgus monkeys (Johnson and Hopps, 1975); to generalised in young adult and middle-aged baboons (Avery and Simpson, 1973) and rhesus monkeys (Caton, 1979; Kornman et al., 1980) as well as adult cynomolgus monkeys (Siegrist and Kornman, 1982; Johnson and Kenney, 1972; Nalbandian et al., 1985). Furthermore, the prevalence and severity of periodontitis were also variable. Only rarely was destructive periodontitis with bone loss reported in young adult baboons (Avery and Simpson, 1973; Hodosh et al, 1971), cotton-top marmosets (Ammons et al., 1972), and young stump-tailed (Krygier et al., 1973) and cynomolgus monkeys (Friskopp and Blomlof, 1988; Auskaps and Shaw, 1957). In contrast, the

majority of cotton-ear marmosets studied were found to have varying degrees of marginal inflammation, from mild gingivitis to extensive periodontitis, with increased tooth mobility and even tooth loss (Shaw and Auskaps, 1954). Some howler monkeys *(Alloutta caraya)* living in the wild also suffer from localised periodontitis, but none of the animals examined exhibited generalised alveolar bone loss (Hall et al., 1967). On the contrary, all skulls of adult mountain gorillas demonstrated alveolar bone loss and some even showed tooth loss (Lovell, 1990), indicating that periodontitis is also present in non-human primates living in the wild.

1.2.1.1. Age-related disease expression

As in humans, where the prevalence and severity of marginal inflammation increases with age (Loe et al., 1978; Schei et al., 1959), a similar increase in gingivitis with age and duration of captivity has been reported in baboons (Avery and Simpson, 1973), bush babies *(Galago senegalensis)* (Grant et al., 1973), chimpanzees (Page et al., 1975; Arnold and Baram, 1973), cynomolgus (Johnson and Hopps, 1975) and squirrel monkeys (Clark et al., 1988). Avery and Simpson (1973) examined a large population of baboons of different ages maintained in captivity for varying periods and found that the mean pocket depth was significantly higher for middle-aged animals than for the younger animals; radiographically, horizontal and vertical bone loss was quite prominent; and there were numerous clinically discernible furcation defects ranging from incipient to Class III involvement. A generalised alveolar bone loss was observed in old chimpanzees (Page et al., 1975; Arnold and Baram, 1973), and bone loss of approximately one-half to three-quarters of the root length was observed in a cotton-ear marmoset held in captivity for

several years (Page et al., 1972). However, a survey carried out by Ammons et al (1972) of defleshed jaws from 60 marmosets of known origin and history has demonstrated only a 0.5% prevalence of bony lesions exhibiting a morphology which may indicate the presence of a form of periodontal disease.

1.2.2. Histological Manifestations of Periodontal Diseases

The histological manifestations of spontaneous gingivitis and periodontitis in baboons (Avery and Simpson, 1973; Hodosh et al., 1971; Simpson and Avery, 1974), bushbabies (Grant et al., 1973), chimpanzees (Page et al., 1975; Arnold and Baram, 1973), howler monkeys (Hall et al., 1967), cynomolgus monkeys (Nalbandian et al., 1985; Brecx et al., 1986; Bosshardt and Schroeder, 1988; Brecx and Patters, 1985), squirrel monkeys (Heijl et al., 1976), as well as cotton-ear (Page et al., 1972) and cotton-top marmosets (Schectman et al., 1972) are quite similar to those of humans (Page & Schroeder, 1976). Characteristic features are progressive rete ridge formation, ulceration, development and apical migration of the pocket epithelium with wide intercellular spaces and emigrating polymorphonuclear leukocytes, increased infiltration of inflammatory cells in connective tissue, vascular proliferation, destruction of collagen fibres, and resorption of alveolar bone. The inflammatory infiltrate of the connective tissue in spontaneously-occurring periodontal disease varies significantly between different species. In baboons (Avery and Simpson, 1973; Hodosh et al., 1971; Simpson and Avery, 1974), bushbabies (Grant et al., 1973), howler monkeys (Hall et al., 1967), cynomolgus monkeys (Nalbandian et al., 1985; Brecx et al., 1986), and chimpanzees (Page et al., 1975), the inflammatory infiltrate contains the same inflammatory cells as in that of humans ( Seymour et al., 1983;

Zachrisson, 1968; Lindhe et al., 1980; Listgarten et al., 1978) with a predominance of lymphocytes and macrophages in the early lesion and plasma cells in the established lesion (Page et al., 1975; Nalbandian et al., 1985; Simpson and Avery, 1974; Brecx et al., 1986). In contrast, cotton-top (Schectman et al., 1972) and cotton-ear marmosets (Page et al., 1972) exhibit a predominance of macrophages and almost an absence of lymphocytes and plasma cells. Based on these differences, marmosets are only slightly related to the human condition and are considered inappropriate as models for studies of periodontal disease pathogenesis with relevance to human inflammatory and immune responses (Schou et al., 1993).

1.2.3. Microbiological Manifestations of Periodontal Diseases

Although periodontitis is a bacterially induced chronic inflammatory disease that results in the destruction of the supporting tissues of the teeth, its multidimensional nature and its bacterial complexity have made it difficult to definitively prove that specific micro-organisms initiate the disease process. Although it has been estimated that more than two hundred different microbial species inhabit the subgingival area of periodontally diseased sites, only a limited number of these species have been implicated as putative periodontal pathogens (Newman et al., 1976; Tanner et al., 1979; Loesche et al., 1982; Slots et al., 1986; Delaney and Kornman, 1987; Slots et al., 1985; Slots and Genco, 1984). At present, *Actinobacillus actinomycetemcomitans* is the only accepted etiologic agent for localised juvenile periodontitis (Slots et al., 1986; Slots and Genco, 1984). The association of specific pathogens with the most common form of periodontal disease, adult periodontitis, is more confounding because of the complexity of the microbiota, the apparent transient

nature of disease progression, and the variability in microbiological data (Slots et al., 1986). Several clinical studies have indicated that proportional increases of a few specific micro-organisms may be associated with progression of periodontitis (Tanner et al., 1984; Socransky and Haffajee, 1986). *Porphyromonas gingivalis* is repeatedly isolated from advanced periodontitis lesions in adults (Tanner et al., 1979; Slots et al., 1986; Loesche et al., 1985) and has been associated with the progression of periodontitis in humans and non-human primates (Slots et al., 1985; Kornman et al., 1981; Loesche et al., 1985; Slots and Hausmann, 1979). Further, a majority of patients classified as having adult periodontitis exhibit elevated peripheral blood antibody levels to *Porphyromonas gingivalis* (previously known as *Bacteroides gingivalis*) (Ebersole et al., 1986; Mouton et al., 1981). Holt et al (1988) implanted a rifampin-resistant strain of the putative periodontal pathogen *Porphyromonas gingivalis* into the periodontal microbiota of *Macaca fascicularis* and found an increase in the systemic levels of antibody to the micro-organism accompanied by rapid and significant bone loss. *Porphyromonas gingivalis* therefore is capable of functioning as a primary pathogen in the periodontally susceptible individual, and the potential of microbial changes to induce a burst of disease progression is thus confirmed.

A microbiological study carried out by Leggott et al (1983) in rhesus monkeys showed bacterial compositions similar to those observed in humans with healthy gingivae as well as with gingivitis. In cases of spontaneous gingival inflammation, the proportion of motile rods and spirochetes and the total number of bacteria in the subgingival plaque were greater than under healthy conditions. In the case of established gingivitis, the total

number of bacteria and the proportion of anaerobic species were greater than in gingival health. In cynomolgus monkeys with gingivitis, a predominance of Gram-positive cocci and rods, and Gram-negative rods were characteristic (Kornman et al., 1981). Zappa et al (1986), showed that loss of connective tissue attachment and loss of crestal bone are negatively correlated with coccoids, whilst periodontal destruction is positively correlated with the total number of bacteria in the plaque sample, and with spirochete species. In fact, these authors found spirochetes to have the strongest positive correlation with loss of attachment and loss of crestal bone. However, the role of spirochetes in destructive periodontitis has been questioned since this organism may increase due to a more favourable environment following periodontal breakdown caused by other subgingival plaque bacteria (Socransky et al., 1963). Microbial changes similar to those described for rhesus and cynomolgus monkeys are also observed in subgingival plaque from human teeth with gingivitis, but greater numbers of *Actinomyces* species are commonly found in humans (Slots et al, 1978). *Actinobacillus actinomycetemcomitans* is primarily isolated from humans with juvenile periodontitis and from more than 25% of patients with adult periodontitis (Slots et al., 1978). In young cynomolgus monkeys, however, *Actinobacillus actinomycetemcomitans* was frequently isolated from subgingival plaque of sites with localised gingivitis and no alveolar bone loss, indicating that the mere presence of these bacteria does not cause overt periodontitis in this animal species or that the simian strain of *Actinobacillus actinomycetemcomitans* was less virulent (Taichman et al., 1984).

Among the most extensively studied of the putative anaerobic bacterial pathogens that inhabit the oral environment is *Porphyromonas gingivalis*, a Gram-negative, anaerobic

non-motile short rod (Cutler et al, 1995). *Porphyromonas gingivalis* can be cultured from the gingival sulcus, tongue, buccal mucosa and tonsillar area in healthy humans as well as in those with periodontitis (van Steenbergen et al., 1993).

The association of *Porphyromonas gingivalis* with periodontitis is based on microbiological and serological studies in humans and in non-human primates (Mouton et al., 1981).

1.2.4. Plaque-Host Relationships

As in humans, increased plaque accumulation and gingivitis can be induced rapidly in rhesus (Listgarten and Ellegaard, 1973), stump-tailed (Krygier et al., 1973) and cynomolgus monkeys (Johnson and Hopps, 1975), through a change to a soft diet (Mashimo et al., 1979). In these species, the histological manifestations of experimental gingivitis (Krygier et al., 1973; Slots and Hausmann, 1979) are similar to gingivitis in humans (Loe et al., 1965; Listgarten and Hellden, 1978; Theilade et al., 1966; Savitt and Socransky, 1984; Slots et al., 1978; Seymour et al., 1983; Zachrisson, 1968; Page and Schroeder, 1976). Although an increase in the size of the inflammatory cell infiltrate in connective tissue predictably follows plaque accumulation, the time frame for histological changes may vary (Listgarten and Ellegaard, 1973; Johnson and Hopps, 1975). The inflammatory cells were dominated by lymphocytes in early lesions in rhesus (Listgarten and Ellegaard, 1973) and stump-tailed (Krygier et al, 1973) monkeys, while an increased number of plasma cells was observed in established lesions of rhesus (Listgarten and Ellegaard, 1973), stump-tailed (Krygier et al., 1973), and cynomolgus monkeys (Johnson

and Hopps, 1975). Johnson and Hopps (1975) found notably numerous macrophages in early lesions in cynomolgus monkeys, but all cells containing phagocytosed material were classified as macrophages regardless of cell morphology, suggesting that the number of macrophages was most likely overestimated (Schou et al., 1993). Inter-species variability also existed in the composition of the supragingival plaque in monkeys placed on a soft diet. The supragingival plaque from rhesus monkeys contained significantly higher numbers of *Prevotella* and *Porphyromonas* species, *Fusobacterium* species, *Veillonella* species, and *Streptococcus mutans* than supragingival plaque from cynomolgus monkeys. Significantly higher numbers of *Streptococcus mitis* and unidentified *Streptococci* were observed in cynomolgus monkeys (Loftin et al., 1980).

## 1.3. EXPERIMENTALLY-INDUCED PERIODONTAL DISEASES

Experimental periodontitis has been induced in non-human primates by a variety of techniques including placement of silk sutures or orthodontic elastics at the cemento-enamel junction, or surgical removal of alveolar bone. Subsequent placement of mechanical devices, such as wires or metal bands, allows for accumulation of supra and subgingival plaque, thus inducing and maintaining an established and destructive lesion. Frequently, the diet is simultaneously changed to a soft diet to increase plaque accumulation at the desired sites.

### 1.3.1. Ligature-Induced Periodontal Diseases

Placement of silk ligatures in stump-tailed (Slots and Hausmann, 1979), cynomolgus

(Kornman et al., 1981; Kiel et al., 1983; Brecx et al., 1985; Alvares et al., 1981; Manti et al., 1984), and squirrel (Meitner, 1975; Kennedy and Polson, 1973; Polson et al, 1986; Kantor, 1980) monkeys resulted in increased plaque accumulation, gingival inflammation, pocket formation, and alveolar bone loss of identical depth on contralateral tooth surfaces. Application of ligatures to several adjacent teeth in squirrel monkeys led to a horizontal loss of bone, but when applied to a single tooth, angular bone loss occurred (Polson, 1974; Polson et al., 1974; Kantor, 1980). Thus, both horizontal and angular bone loss can be induced in non-human primates (Schou et al., 1993). When antibacterial therapy was instituted during the presence of silk ligatures, no loss of attachment and alveolar bone was identified (Polson et al., 1986; Zappa and Polson, 1988; Hausmann et al., 1979), indicating that the attachment and bone loss was due to the altered subgingival plaque and not to mechanical trauma from the ligature (Schou et al., 1993).

1.3.1.1. Clinical and radiological features

The clinical and radiological changes after ligature placement have been studied in cynomolgus monkeys by Kornman et al (1981). Gingival inflammation increased 1 to 3 weeks after placement of silk ligatures, but no bone level or pocket depth change occurred. However, an increased pocket depth was observed after 1 week in a study carried out by Alvares et al (1981). Active periodontitis including clinical increased pocket depth and radiographic evidence of bone loss developed 4 to 7 weeks after ligation, accompanied by a Gram-negative anaerobic flora with *Porphyromonas gingivalis* as the dominant cultivable organism, while the condition stabilised between 8 and 17 weeks with decreased inflammation, no further alveolar bone loss and a decrease in the levels of

*Porphyromonas gingivalis* (Kornman et al., 1981). Brecx et al (1985), on the other hand, found radiographically progressive bone loss in the interproximal regions that had a ligated tooth surface in the same monkey species following 8 weeks of ligation, and furcation involvement 9 weeks after ligature placement.

1.3.1.2. Microbiological features

When active periodontitis was present in cynomolgus monkeys after 4 to 7 weeks of ligation, Gram-negative anaerobic rods dominated with *Porphyromonas gingivalis* as the most prevalent species (Kornman et al., 1981; Kiel et al., 1983). It thus stands to reason that the subgingival microflora of ligature-induced periodontitis in *Macaca fascicularis* closely resembles that reported for human periodontal diseases and the episodic pattern of clinical attachment loss is associated with levels of Gram-negative anaerobes, primarily *Porphyromonas gingivalis*. Slots and Hausmann (1979) reported a significant positive correlation between a number of black-pigmented organisms including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Bacteroides macacae*, and the loss of alveolar bone height in stump-tailed monkeys. In addition, the radiographic evidence of bone loss that followed *Porphyromonas gingivalis* implantation to ligated sites in cynomolgus monkeys indicates that this organism is an etiological factor in ligature-induced periodontitis in non-human primates (Holt et al., 1988). This was confirmed by a decrease in the proportion of *Porphyromonas gingivalis* with concomitant termination of alveolar bone loss after 8 to 17 weeks of ligation resulting from elevated antibody levels to this organism (Kornman et al., 1981).

1.3.1.3. Histological features

Brecx et al (1985), made histological observations on ligature-induced periodontitis in two adult female cynomolgus monkeys and found the following features to be most prominent: supragingival and subgingival plaque with dense accumulations of bacteria around and within the ligatures; apical migration of the junctional epithelium; loss of collagen and connective tissue attachment along the ligated and the adjacent non-ligated tooth; and crestal osteoclastic bone resorption on the side of the septum closest to the ligated tooth, as well as on the side adjacent to the non-ligated tooth. Because the 'range of effectiveness' of plaque in inducing bone resorption is about 2,5mm, Page & Schroeder (1982) showed that the apical progression of organisms on a single tooth surface can induce pocket formation and alveolar bone loss around the adjacent tooth if the interdental bone has a mesiodistal width less than 2,5mm. The inflammatory infiltrate of cynomolgus monkeys was dominated by lymphocytes and plasma cells (Brecx et al., 1985), while the inflammatory infiltrate of squirrel monkeys was different, with a predominance of polymorphonuclear leukocytes and macrophages and few lymphocytes and plasma cells (Heijl et al., 1976). Even though most of these studies in squirrel monkeys are only 14 day studies and/or studies of the connective tissue apically to the pocket epithelium, Schou et al (1993) concluded that the histological features of squirrel monkeys are also poorly related to human periodontal conditions.

1.3.2. Orthodontic Elastic-Induced Periodontal Diseases

1.3.2.1. Clinical, histological and radiological features

Placement of orthodontic elastics at the cemento-enamel junction resulted in pronounced

plaque accumulation, increased gingival inflammation, progressing periodontal pocket depth with irreversible apical migration of the junctional epithelium, and horizontal as well as angular alveolar bone loss in rhesus (Caton and Zander, 1975; Yukna, 1976; Caton and Nyman, 1980; Carson et al., 1978) and cynomolgus monkeys (Siegrist and Kornman, 1982; Blomlof et al., 1989; Nyman et al., 1981). The morphology and extension of the defects on contralateral tooth surfaces were identical when evaluated histologically (Caton and Kowalski, 1976). The time required to produce periodontal pocket depths of 5 to 8mm with orthodontic elastics varied both between different rhesus monkeys and between different teeth, from 66 to 84 days for maxillary central incisors to 167 to 176 days for maxillary first molars (Caton and Zander, 1975; Caton and Kowalski, 1976). As with silk ligatures, orthodontic elastics can be used to study horizontal and angular bone loss separately (Caton and Zander, 1975).

1.3.2.2. Microbiological features

After the induction of periodontitis by means of orthodontic elastics, a highly complex subgingival microflora seemed to be present in cynomolgus monkeys, consisting of 14% Gram-positive cocci, 6% Gram-negative cocci, 3% surface-translocating bacteria and motile rods, 12% spirochetes, 13% Gram-positive rods (predominantly *Actinomyces* species), and 53% Gram-negative rods (predominantly black-pigmented *Prevotella* and *Porphyromonas* species), (Siegrist and Kornman, 1982). This is similar to the composition of the subgingival flora described for advanced periodontal lesions in humans (Listgarten and Hellden, 1978; Savitt and Socransky, 1984; Tanner et al., 1984; Tanner et al., 1979; Slots, 1977; Loesche et al., 1985).

### 1.3.3. Surgically-Induced Periodontal Diseases

Surgical alveolar bone removal followed by placement of different mechanical devices also results in increased plaque accumulation, gingival inflammation, and periodontal pocket formation in rhesus (Ellegaard et al., 1973; Ellegaard et al., 1974; Ramfjord, 1951), stump-tailed (Nery et al., 1986), and cynomolgus monkeys (Wirthlin and Hancock, 1982). Surgical approaches have been used to attempt the standardisation of the severity of the lesion in order to better evaluate different periodontal therapies (Schou et al., 1993).

## 1.4. LIGATED VS NON-LIGATED SITES

### 1.4.1. Clinical and Histological Features

Clinical and histologic features of non-ligated sites in the presence of ligated teeth have also been studied in cynomolgus monkeys by Kiel et al (1983). These authors found that no clinical changes occurred after 8 to 12 weeks in non-ligated quadrants in monkeys with ligature-induced periodontitis in contralateral quadrants. Likewise, no histological changes were identified, and gingivitis present at non-ligated quadrants with ligated teeth in contralateral quadrants and gingivitis in animals without ligated teeth showed identical histology (Nalbandian et al., 1985; Brecx et al., 1986). Therefore, non-ligated teeth can be used as controls for ligated teeth (Schou et al., 1993).

### 1.4.2. Microbiological Features

In addition to their study of histologic characteristics of non-ligated sites in the presence of ligated teeth, Kiel et al (1983) also studied the ability to disrupt the resident microflora

of the non-ligated sites in monkeys by exogenous infection with a suspected periodontal pathogen, *Porphyromonas gingivalis*. In this investigation, the non-ligated sites underwent changes in their subgingival microflora when ligature-induced periodontitis was produced in contralateral areas. These changes consisted primarily of increases in the proportion of Gram-negative surface translocating bacteria and motile rods with a distribution pattern similar to that seen in ligated sites in the same animal just prior to the development of high proportions of *Porphyromonas gingivalis*, increased pocket depth, and radiographic evidence of bone loss. The ability to infect a non-ligated site with *Porphyromonas gingivalis* appeared to be augmented by a microflora that was characterised by elevated Gram-negative surface translocating bacteria and motile rods. It was not possible to recover high numbers of *Porphyromonas gingivalis* four weeks after implantation unless implantation occurred at a time when Gram-negative surface translocating bacteria and motile rods were increased. This suggests that it may be possible to infect certain healthy periodontal sites in the human patient if large amounts of plaque, rich in *Porphyromonas gingivalis*, are transferred into active sites during certain routine dental procedures, such as periodontal probing (Barnett et al., 1981). In view of these findings, therefore, inoculation and subsequent development of periodontitis in apparently healthy sites of patients with active periodontitis, seems possible.

## 1.5 SPECIES SPECIFIC PERIODONTAL DISEASE PROGRESSION

Schou et al (1993) have shown that ligature-induced periodontitis in the non-human primate has provided a model for studying microbial changes associated with the initiation

and progression of periodontitis. Monitoring this period is not possible in humans, due to the low incidence of initiation and our inability to identify initiation prior to extensive clinical destruction. Evidence shows that the acute and rapid nature of induced periodontitis in squirrel monkeys and marmosets produce rapid microbial changes associated with progression of this disease, thus making accurate observations difficult (Polson et al., 1976; Zappa et al., 1986; Heijl et al., 1976). *Macaque* species have a more extended period of disease induction that allows easier observation of their more complex microbial interactions. The time frame for microbial changes in non-human primates depends on the monkey species and size, previous history of periodontal disease, and the method of disease induction (Schou et al., 1993).

## 1.6 THE BABOON AS AN ANIMAL MODEL

Comparatively few studies have been conducted on baboon species (genus *Papio*, superfamily *Cercopithecoidea*, family *Cercopithecidae*, subfamily *Cercopithecinae*), especially on the species indigenous to Southern Africa, the Chacma baboon (*Papio ursinus*), (Roth, 1965). Possible reasons for this include the expense of housing medium to large primates (10 to 40kg), the problems of dealing with aggressive animals as well as their limited availability (Miller et al., 1995). The following features favour the use of the baboon as a model in periodontal research: large oral cavity with easy access for surgical procedures and for the assessment of various oral indices (Avery and Simpson, 1973); identical dental formula (Reed, 1967); dentition similar to humans, particularly in the occlusion of the posterior teeth and in the size and shape of both anterior and posterior

crowns (Virgadamo et al., 1972); the presence of an inflammatory infiltrate which microscopically appears to be identical to that seen in the human (Avery and Simpson, 1973); the accumulation of copious plaque as in humans (Avery and Simpson, 1973); and remission of inflammation after removal of local irritants (Avery and Simpson, 1973).

1.6.1. Clinical and Radiological Findings

Although studies done by Hodosh et al (1971) have demonstrated the presence of gingivitis in varying degrees of severity and commonly localised gingival enlargement, the occurrence of periodontal disease in *Papio anubis* was infrequent and was not characterised by deep pocket formation, extensive bone loss, tooth mobility and/or suppuration. In fact, only 3 out of the 40 baboons studied showed clinical evidence of only localised periodontitis, characterised by pockets ranging from 3 to 5mm in depth with some suppuration on palpation. Avery and Simpson (1973), on the other hand, reported that severe gingival inflammation in this species resulted in oedema, erythema, enlargement, increased crevicular fluid flow, and spontaneous bleeding, that progressed to pocket formation with marginal bone loss and gingival recession as seen in human periodontitis. Furthermore, the gingival inflammation was related to extensive accumulation of plaque and calculus, and histologically resembled established periodontal lesions in humans. Miller et al. (1995) observed the periodontal health of baboons from the species *Papio anubis* and *Papio cynocephalus*, and confirmed that these baboons do develop periodontitis and that the disease in these animals has an increased prevalence and severity with age. Inflammatory gingival enlargement previously described by Hodosh et al (1971), was also evident in the experiment carried out by Miller et al (1995),

especially in the maxillary anterior regions. In addition, furcation involvement of at least class I severity was noted in 68.8% of the animals examined, with bilaterally symmetrical lesions in the molar region. Tal and Lemmer (1982) have noted symmetrical distribution of furcation involvement in human populations. Oschenbein (1986) examined baboon molars radiographically and found them to have short root trunks in comparison with human molars. This, in combination with horizontal bone loss, allows for rapid involvement of the furcation area. Clinically, baboon molars also have a large cervical bulge superior to the furcation entrance which may precipitate early furcation bacterial invasion as described in human populations (Oschenbein, 1986). Furcation invasion is indicative of loss of attachment, bone loss and evidence of increased severity of disease (Larato, 1970; Tal, 1982). The finding of furcation involvement adds additional support to the finding of increased severity of this periodontal disease with age in these animals, as well as suggests clinical similarity between baboon and human periodontal disease (Miller et al., 1995).

1.6.2. Histological Findings

Simpson and Avery (1974) investigated the ultrastructural features of inflamed pocket epithelium in *Papio anubis*. These authors found the most characteristic features included increased width of intercellular space, loss of junctional complexes between epithelial cells, and regional discontinuity of the basal lamina. Numerous lymphocytes, monocytes, macrophages, and occasional lymphoid blastic cells infiltrated the connective tissue adjacent to the pocket epithelium in the early gingival inflammatory lesions; while that in the established gingival inflammatory lesions had a prominent plasma cell infiltrate. The

following ultrastructural features of inflamed baboon gingiva are essentially identical to those of human inflamed gingiva.

In view of the fact that the ideal animal for investigation of the host tissue response should exhibit a high prevalence of spontaneous periodontal disease with a natural history and clinical and histopathologic manifestations comparable to the human form of the disease, or alternatively, have the capacity to be induced in a relatively simple way to develop chronic inflammatory periodontal disease (Ammons et al., 1972), the above results further substantiate the usefulness of the baboon as an animal model for the study of periodontal disease.

## 1.7 THE CHACMA BABOON

Very limited information is available on the clinical, radiographic, histological and immunological features of the periodontium of *Papio ursinus* (Chacma baboon). Preliminary studies by Ripamonti et al (1986) have shown this species to suffer from naturally-occurring gingivitis, but despite the presence of the putative human periodontal pathogen *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, no destructive lesions occurred. Borland (1994) evaluated the periodontal status of 20 captive Chacma baboons and confirmed the finding that all of the animals had naturally-occurring, site-specific gingivitis, mainly localised to the labial surfaces of the anterior aspect of the jaws. Periodontitis as defined by loss of periodontal attachment and periodontal pocket formation was, however, not found in any of the 20 animals. The

observation that *Papio ursinus* has inflamed sites harbouring *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* without a destructive lesion may establish this primate as an attractive model for the study of experimentally induced periodontal microbial-host interactions.

## CHAPTER TWO: STATEMENT OF THE PROBLEM

In a number of studies at the Bone Research Laboratory, it has been shown that bone-derived bone morphogenetic proteins (BMPs) in conjunction with collagenous matrix, can induce cementum, periodontal ligament and alveolar bone regeneration in surgically created furcation defects in the baboon (Ripamonti et al., 1994). These studies evaluated healing on root surfaces which had been surgically exposed and root planed, and had not been affected by periodontal disease. Whilst the regeneration of connective tissue attachment and alveolar bone seems to be independent of the character of the root surface if the exposed root had been adequately decontaminated (Nyman et al., 1982), the contention still exists that regenerative procedures should ideally be tested in experimental models with root surfaces exposed by disease. This has important clinical implications, since regenerative procedures in man are performed on periodontal tissues that have been affected by naturally-occurring chronic periodontitis. *Papio ursinus* could therefore serve as an excellent model of induced periodontitis for experimental regenerative procedures related to man. However, at present, there is no data available on the induction and maintenance of periodontitis in any baboon species.

## CHAPTER THREE: THE AIM OF THE STUDY

The present study was undertaken to induce chronic periodontitis in 4 captive baboons of the species *Papio ursinus*, the presence of which would be determined clinically through gingival pocket depths of 4mm or more, as well as microbiologically through the presence of *Porphyromonas gingivalis* in the subgingival flora of these animals. A further objective was to determine whether the severity of periodontal disease could be correlated to the presence of these black-pigmented Bacteroides (*Porphyromonas gingivalis*)

# CHAPTER FOUR: MATERIALS AND METHODS

## 4.1. SELECTION OF ANIMALS

### 4.1.1. Selection of Species

The genus *Papio*, superfamily *Cercopithecoidea*, family *Cercopithecidae*, subfamily *Cercopithecinae*, has been assigned to the Savannah baboons and includes 5 species of which the Chacma baboon, *Papio ursinus*, is a natural inhabitant of major vegetational zones of Southern Africa (Roth, 1965). Thus this baboon is readily available for experimental use, and the four clinically healthy adult male baboons selected for this study were chosen from the primate colony at the University of the Witwatersrand, Johannesburg.

### 4.1.2. Selection Criteria

The criteria for selection were: skeletal maturity as confirmed by radiographic evidence of closure of the distal epiphyseal plate of the radius and ulna (Ripamonti et al., 1994); erupted third molars (Reed, 1965); site-specific gingivitis but no loss of periodontal attachment (Borland, 1994); and, normal haematological and biochemical profiles (Melton and Melton, 1982).

## 4.2. ETHICS APPROVAL

The research protocol was approved by the Animal Ethics Screening Committee of the University (AESC No 95/107/5) before the project was started in September 1995, when the experimental animals were chosen.

## 4.3. HOUSING CONDITIONS

Following standard quarantine procedures, the animals were housed individually in suspended wire-mesh cages in the primate unit of the Central Animal Service of the University. Animals were caged in rooms kept under slight negative pressure (-25kPa) with controlled ventilation (18 filtered air changes per hour), temperature of 22 degrees Celsius, humidity (between 30 and 50%), and photoperiod (lights on 06h00 to 18h00).

## 4.4. DIET

The diet of the baboons consisted of a balanced protein-fat-carbohydrate diet with vitamins (thiamine, riboflavin and nicotinic acid) and mineral supplements (Ca:P=1,2:1), and a soft dietary intake of sweet potatoes, pumpkins and oranges mixed in a ratio of 3:1 with a protein-vitamin-mineral dietary supplement (Dreyer and De Bruyn, 1968). The baboons had access to tap water ad libitum.

## 4.5. BASELINE DATA

### 4.5.1. Introduction

In November 1995, baseline data were collected before any surgical procedures were undertaken. The animals were immobilised by intramuscular injection of ketamine hydrochloride (Ketalar, Parke-Davis; 10mg/kg body weight). General anaesthesia was induced by intravenous injection of sodium pentobarbitone (Sagatal, Maybaker; 8mg/kg body weight). The animals were weighed, clinical photographs and radiographs were taken, subgingival plaque samples for micro-organism identification were collected, and sulcular probing depths were measured.

### 4.5.2. Radiographic Examination

The radiographs were posterior periapicals of each quadrant and were taken using the long-cone parallel technique with a 50kVp dental x-ray unit. The exposure time was set on 0,8s at 7,5mA (Philips). Kodak Ultraspeed (R) DF58 (Eastman Kodak Co, Rochester, New York) film was used and the radiographs were developed manually. They were then viewed with the aid of a roentgenographic viewer at a 10x magnification to verify absence of bone loss.

### 4.5.3. Microbiological Examination

The subgingival plaque samples were collected by placing a sterile calcium alginate swab (Calgi Swab, Spectrum Laboratories, Dallas) into the pocket or sulcus of designated test sites for 60 seconds. These samples were then suspended in individually labelled

McCartney's bottles containing Robertson's chopped meat and carbohydrate (CMC) broth to enable transportation. Once in the laboratory, the Robertson's cooked meat broth bottles were incubated for 48 hours at 37 degrees Celsius, then suspensions were vortexed using a Whirlimixer (Fisons, power output 50W, frequency 50-60Hz) on maximum setting for 10 seconds. The broths were subbed onto 10% blood agar plates and incubated in an anaerobic chamber at 37 degrees Celsius (Anaerobic System, Forma Scientific) for 48 hours. The chamber had the following gas concentrations: carbon dioxide 10,5%, hydrogen 4,7%, and the balance made up of nitrogen (Holdeman et. al., 1977). At 48 hours, colonies were picked off onto 10% blood agar, chocolate agar and blood agar. The 10% blood agar plates were again incubated anaerobically; the chocolate agar under 5% carbon dioxide; and the blood agar aerobically; all at 37 degrees Celsius. The original plates were reincubated for 5 days thereafter, for pigmenting bacilli. After 48 hours, the plates that had no growth on the blood and chocolate agar, but growth on the 10% blood agar, indicated anaerobic colonies.

The anaerobic colonies were then selected for Gram-typing. According to the Gram-type, the anaerobes were then classified according to species. If there was any suspicion of the Gram-type, sensitivity testing was done to clarify whether the organism was Gram-positive or Gram-negative.

As far as pigmenting bacilli were concerned, these were subcultured for purity, and once pure, were identified according to methods described in all of the following texts: Manual of Clinical Microbiology (1991); Colour Atlas and Textbook of Diagnostic Microbiology

(1992); Principles and Practice of Infectious Diseases (1995); and, Manual of Clinical Microbiology (1995).

### 4.5.4 Periodontal Probing Attachment Loss

The periodontal probing attachment loss was recorded at 3 sites (mesiobuccal, midbuccal and distobuccal) for the first and second molars of each quadrant, and was calculated from the apex of the periodontal probe penetration to the cemento-enamel junction (CEJ). A manual periodontal probe with William's markings (Hu-Friedy XP 23/QOW, USA) was used, and probing depths were rounded off to the nearest millimetre.

## 4.6 INDUCTION OF PERIODONTITIS

To induce periodontal disease, a dual approach was followed which included mechanical creation of bony defects as well as introduction of periodontal pathogens to specified areas. Bony lesions were created surgically in the mandible, followed by placement of periodontal packs impregnated with the putative organism *Porphyromonas gingivalis*. In the maxilla, silk sutures impregnated with the same organisms were applied into smaller defects.

### 4.6.1 Surgical Procedures

The animals were anaesthetised with intravenous thiopentone (100 mg) and maintenance was achieved by inhalation of halothane 1%. Full thickness mucoperiosteal flaps were raised buccally between second premolar and third molar. Class II furcation defects with

a vertical height of 6mm were surgically created in the first and second mandibular molars of both the left and the right side. Before the flaps were readapted using nonresorbable sutures (Vicryl, Ethicon), periodontal packs were placed into the bony defects to maintain and harbour micro-organisms in the operated area.

Smaller Class II furcation defects of approximately 2mm vertical height were surgically created in the first and second maxillary molars of both the left and the right side. In these teeth, before readaptation of the flaps, selected micro-organisms grown on silk sutures were placed within the defects and sutured with 3,0 Vicryl sutures.

In the ideal animal model of periodontal disease, identical or very similar lesions should be produced on contralateral teeth so that the effect of therapeutic procedures can be tested on one side with the other acting as an untreated control (Caton and Zander, 1975). For this reason, baseline data rather than contralateral quadrants served as controls in this experiment.

## 4.7 ASSESSMENT OF THE CONTINUALLY INOCULATED PERIODONTIA

*Porphyromonas gingivalis* was originally cultured on blood agar and incubated in a candle jar to create a micro-aerophilic environment for 24 hours at 37 degrees Celsius. This culture was then subbed into a serum broth medium and further incubated for 24 hours in a candle jar. 1,8x10 to the eighth CFU (Colony Forming Units)/ml of *Porphyromonas gingivalis* were delivered into the maxillary and mandibular defects once every 2 weeks

using a micropipette. Sulcular probing depths were read once every 2 weeks and radiographic changes were examined once every 2 months. Once a month, subgingival plaque samples were collected for identification of the newly acquired flora.

## 4.8 STATISTICAL ANALYSIS

The data were analysed using the Special Packages for the Social Sciences (SPSS Inc., Chicago, USA). Levels of statistical significance and P-values were determined using a 4-way analysis of variance (ANOVA) procedure with the following interactions: baboon; date; jaw; side, and site. A correlation analysis was performed to investigate the effect of anaerobic micro-organisms within the gingival tissues on the amount of periodontal attachment loss. A 2-sample t-test was used to calculate significant differences between the mean periodontal probing depths and the presence or absence of anaerobic organisms.

# CHAPTER FIVE: RESULTS

## 5.1. CLINICAL EVALUATION

All four baboons demonstrated copious amounts of supra- and sub-gingival plaque and calculus mainly localised to the facial and interproximal surfaces of the teeth, the accumulation of which was encouraged by a change to a soft diet. The marginal gingiva and interdental papillae of the anterior teeth were grossly inflamed and exhibited marked hyperplasia, erythema and oedema. The posterior segments showed acute gingival inflammation, suppuration on palpation and bleeding on probing, progressing to attachment loss and pocket formation. There appeared to be a direct correlation between the degree of soft tissue involvement and the deposits on tooth surfaces. The areas of greatest pocket depth were the molar regions, which also had the most deposits and the most severe inflammation.

Probing periodontal pocket depths from the cementoenamel junction to the tip of periodontal probe penetration revealed loss of periodontal attachment over a 60 week period with pocket depths ranging from 4 to 10mm. Furcation involvement was also clinically discernible in these animals (Figures 6 and 7).

## 5.2. MICROBIOLOGICAL EVALUATION

All animals studied harboured a subgingival microflora considered to be pathogenic for humans (Persson et al, 1993). *Porphyromonas gingivalis* was the dominant organism recovered among other anaerobic species, which included Fusobacterium, Veillonella, Prevotella and Clostridium. Results of the microbiological analysis are shown in Tables 1, 2, 3 and 4.

## 5.3. RADIOGRAPHIC EVALUATION

Radiographs of right and left mandibular first and second molars of baboon ET 1710 at time periods 0, 4, 7, and 60, are presented in Figures 8, 9, 10 and 11.

At the baseline examination, a week prior to the surgical procedures, there was evidence of continuity of the lamina dura at interproximal sites signifying health of the dental attachment apparatus. The lamina dura was dense and there was evidence of a widened periodontal ligament space at the coronal and apical areas of all the teeth studied. The interdental crest was seen to approximate the cemento-enamel junction in all areas. No furcation involvement was present.

Four weeks post surgery, the created furcal defects were clearly visible and were further propagated over a 58 week period through inoculation of *Porphyromonas gingivalis*. Bone loss was of the generalised horizontal type accompanied by isolated infrabony

defects. This resulted in inconsistent margins, interproximal craters, and involved furcations on many posterior teeth.

## 5.4. STATISTICAL EVALUATION

The mean values for the probing of periodontal attachment loss in each animal at week 0, 20, 44 and 58, are presented in Figures 8, 9, 10 and 11. The 4-way ANOVA procedure showed statistically significant differences ($p<0.017$) in periodontal attachment loss over a 60 week time period. The degree of significance and the date when significant breakdown occurred varied between the baboons, although periodontal attachment loss was significant in all the animals when compared to base-line data. The time periods of most significance were weeks 20, 44 and 60 ($p<0.001$).

Periodontal attachment loss was positively correlated with the presence of *Porphyromonas gingivalis* ($p<0.031$) in baboon ET 1398. However, multiple regression analysis performed on the remaining baboons was not statistically significant despite the fact that all of the animals harboured the putative periodontal pathogen *Porphyromonas gingivalis*. These results correspond to the

2-sample t-test data obtained comparing the effect of the presence of anaerobes on mean periodontal probing depths.

**Author** Ksiezycki-Ostoya B K
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