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Induction of periodontitis in the chacma baboon.

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2014-04-30
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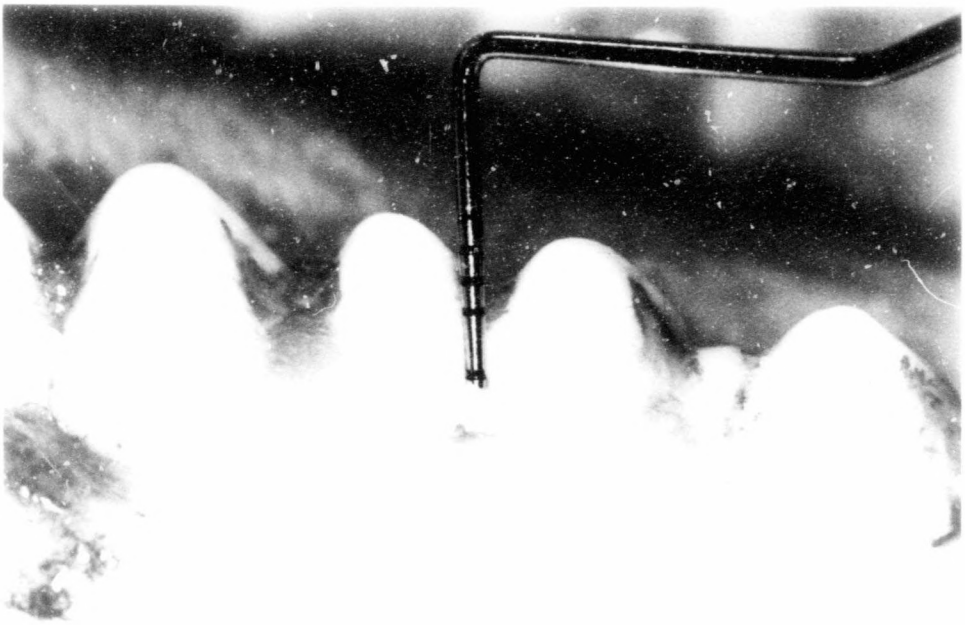


Figure 2: Clinical Photograph of Base Line Probing Depth I. I. 1308 (Week 0)

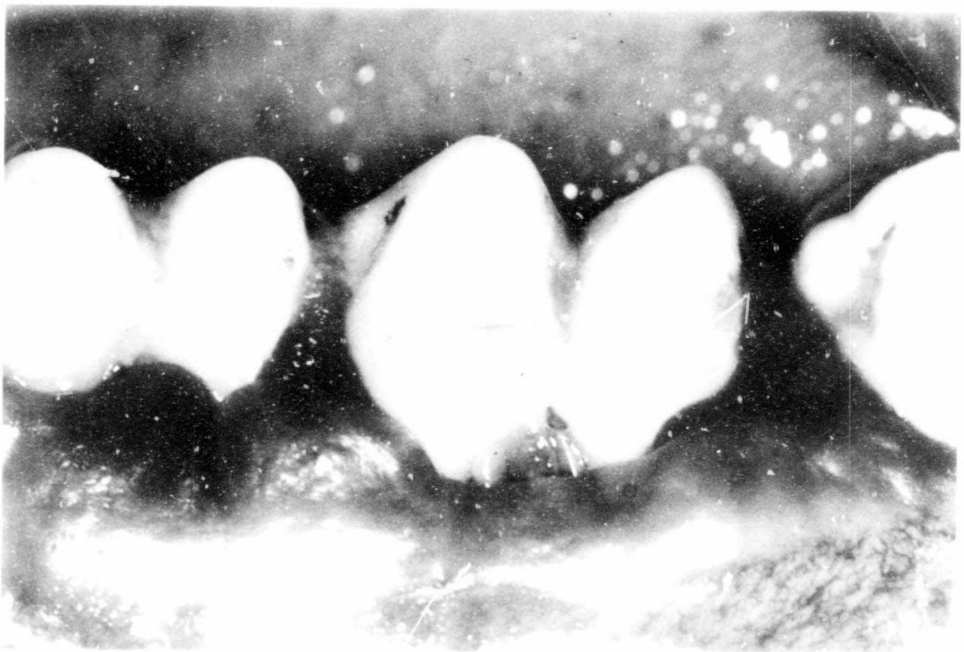


Figure 3: Clinical Photograph of Induced Chronic Periodontitis I. I. 1308 (Week 10)



Figure 4. Clinical Photograph of Surgically Exposed Furcation Defects in Preparation for Regenerative Procedures ET 1398 (10.12.97)



Figure 5: Clinical Photograph of Base Line Probing Depth F.T. 1710 (Week 0)

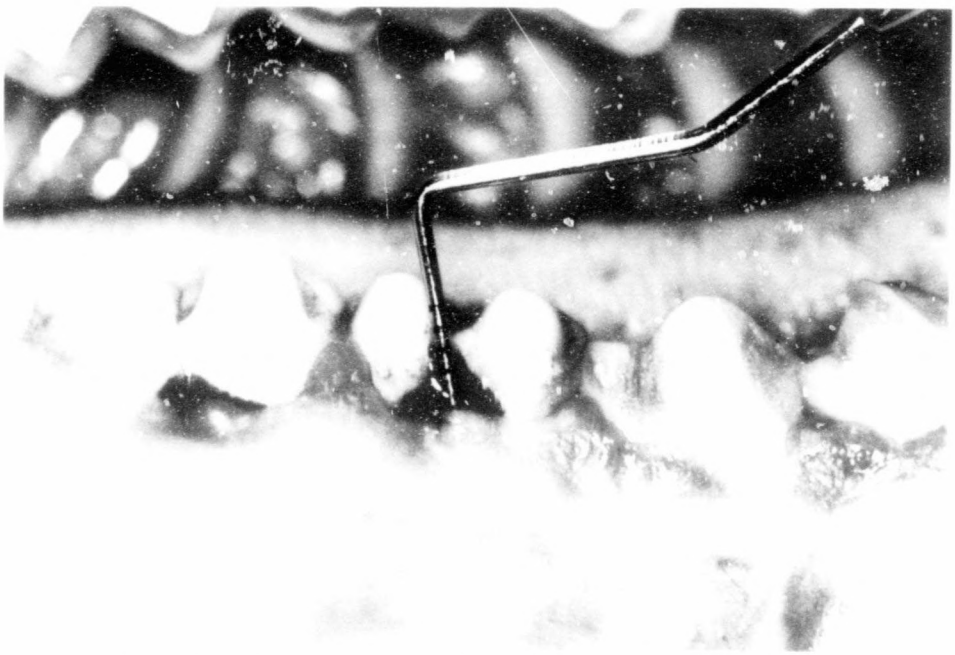


Figure 6: Clinical Photograph of Induced Chronic Periodontitis F.T. 1710 (Week 60)

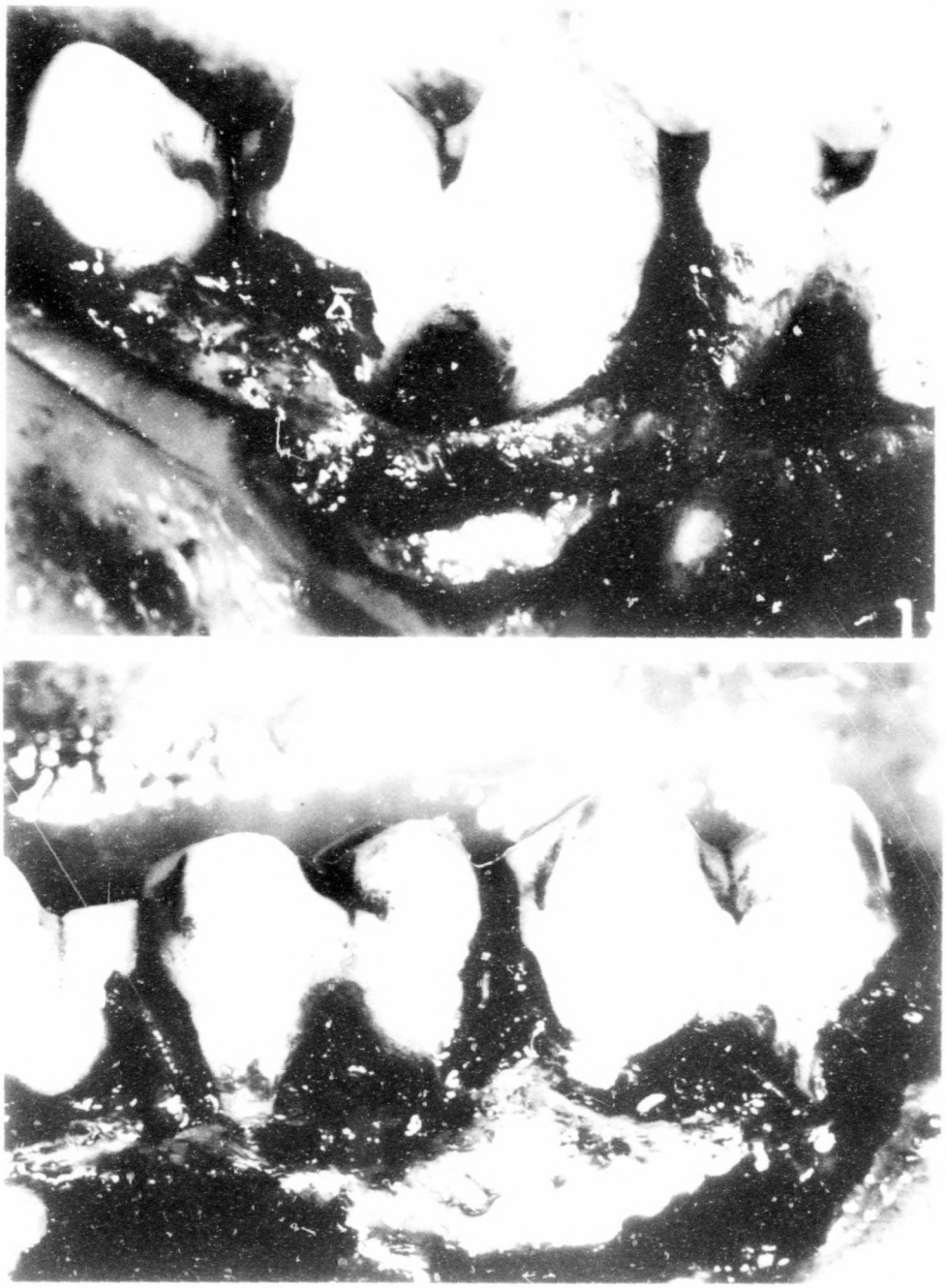


Figure 7. Clinical Photographs of Surgically Exposed Furcation Defects in Left and Right Mandibular Molars in Preparation for Regenerative Procedures [J. C. Tu et al. 12:97]



Figure 8. Radiographs of the Right and Left Mandibular Molars ET 1710 for Week 0 (23/11/95)

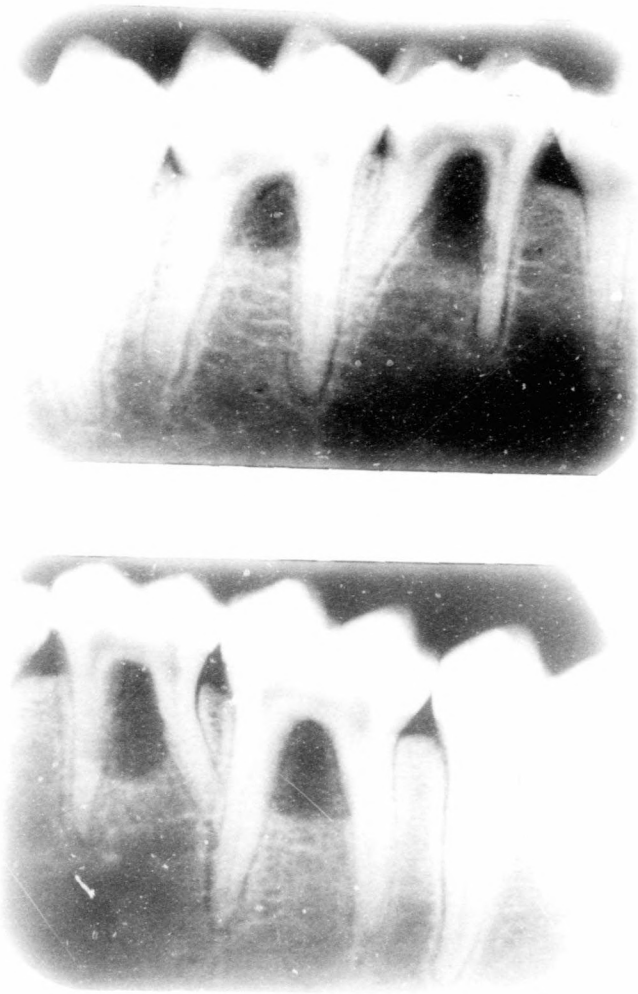


Figure 9. Radiographs of the Right and Left Mandibular Molars ET 1710 for Week 4 (22.12.95)

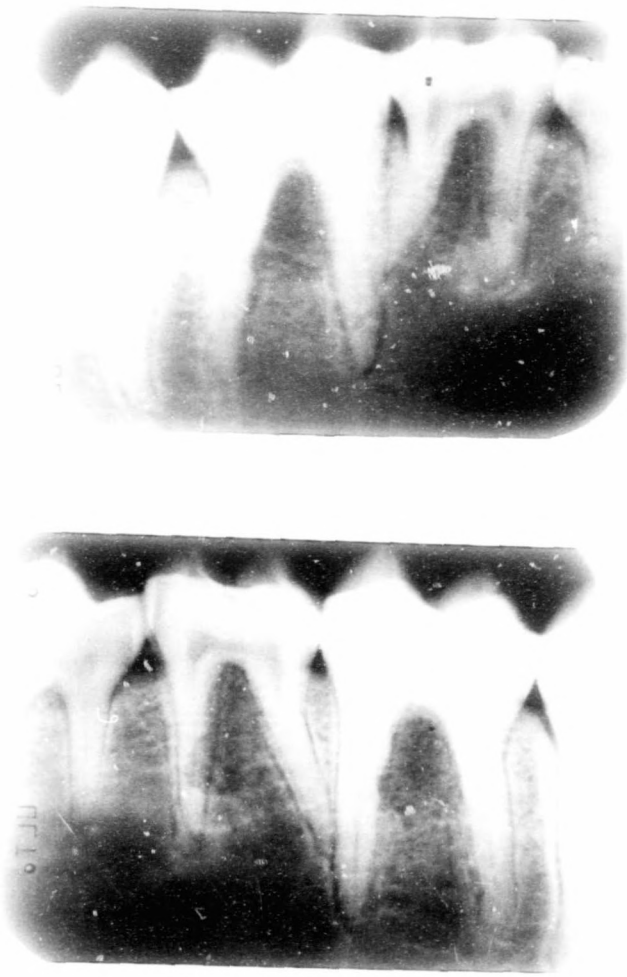


Figure 10. Radiographs of the Right and Left Mandibular Molars ET 1710 for Week 7 (08/01/96)

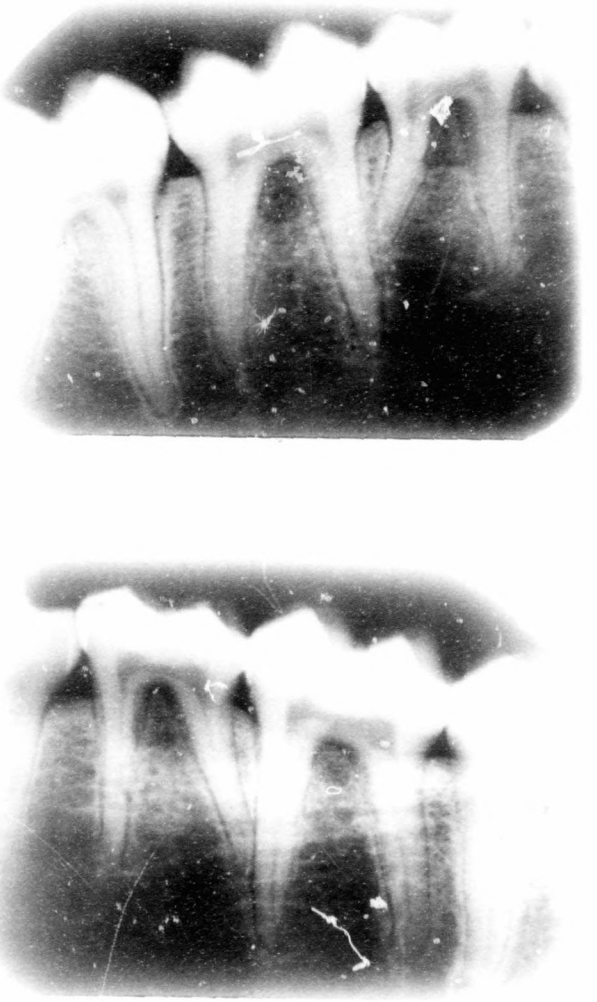


Figure 11. Radiographs of the Right and Left Mandibular Molars ET 1-10 for Week 60 (10/01/97)

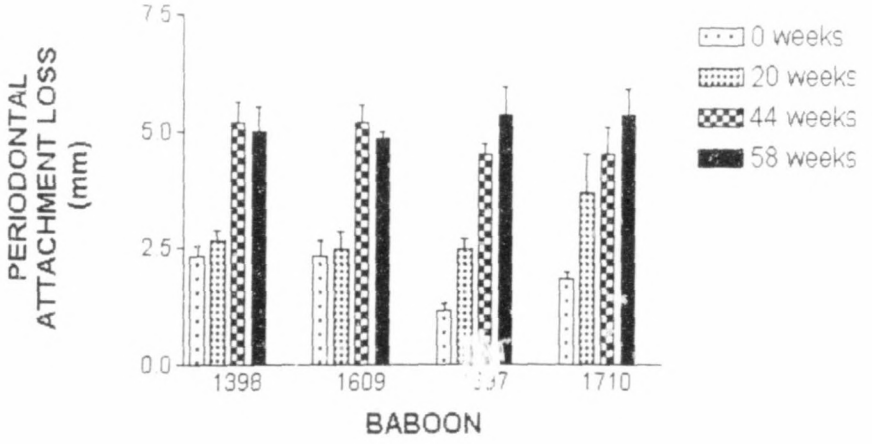


Figure 12. Progression of Periodontal Attachment Loss: Right Mandible

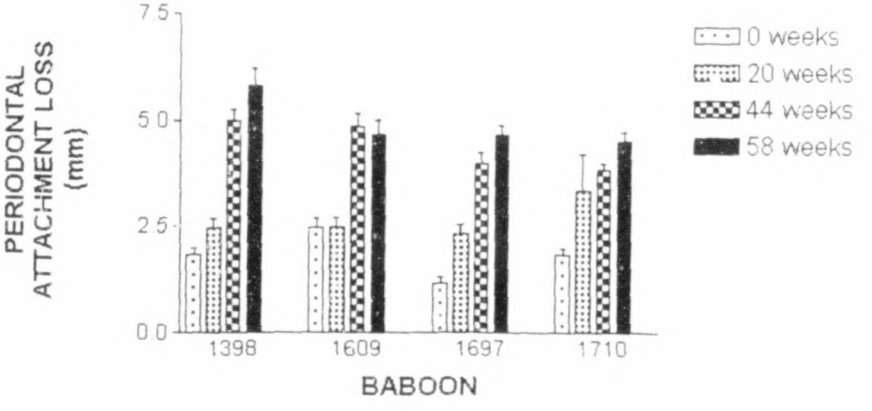


Figure 13. Progression of Periodontal Attachment Loss: Left Mandible

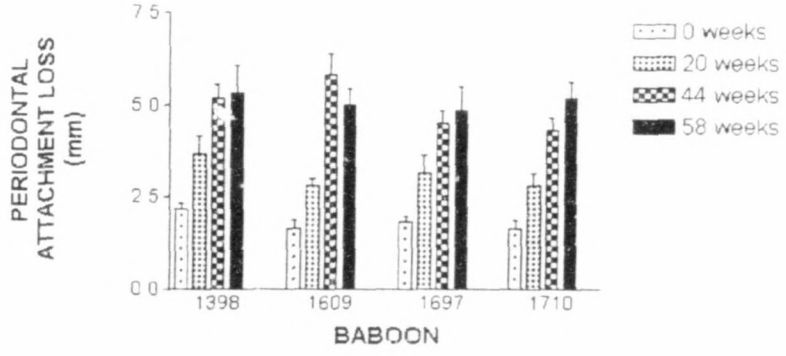


Figure 14. Progression of Periodontal Attachment Loss: Right Maxilla

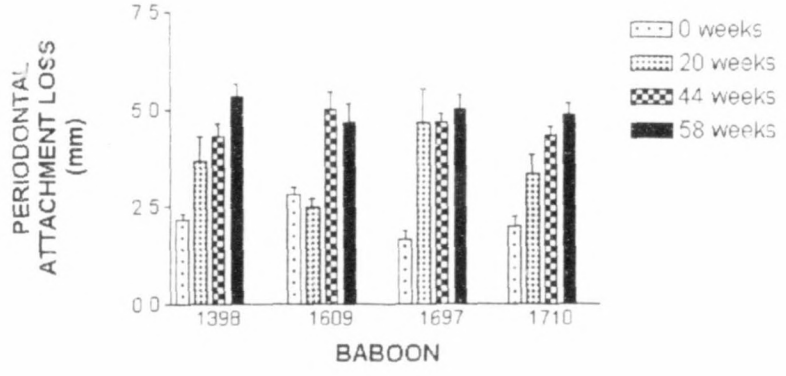


Figure 15. Progression of Periodontal Attachment Loss: Left Maxilla

TABLE 1 Subgingival plaque cultures: ET 1398

	December 1995				July 1996				January 1997			
	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn
Fusobacterium species	+	-	-	+	-	-	-	-	-	+	-	-
Veillonella species	-	-	-	-	-	+	+	+	-	-	+	-
Prevotella species	+	+	+	+	+	-	+	+	-	-	-	-
Clostridium species	-	-	-	-	-	+	-	-	-	-	-	-
Porphyromonas gingivalis	+	+	+	-	+	+	+	+	+	+	+	+

TABLE 2 Subgingival plaque cultures: ET 1609

	December 1995				July 1996				January 1997			
	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn
Fusobacterium species	-	-	-	-	-	-	-	-	+	-	-	-
Veillonella species	-	-	-	-	-	-	-	-	-	-	-	+
Prevotella species	+	-	+	-	+	+	+	+	-	+	-	+
Clostridium species	-	-	-	+	-	+	-	+	-	+	-	-
Porphyromonas gingivalis	+	+	+	+	+	+	+	+	+	+	+	+

TABLE 3. Subgingival plaque cultures: ET 1697

	December 1995				July 1996				January 1997			
	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn
Fusobacterium species	-	+	-	-	-	+	-	-	nd	nd	-	-
Veillonella species	-	-	-	-	-	+	-	-	nd	nd	+	-
Prevotella species	-	+	+	+	-	+	+	-	nd	nd	-	-
Clostridium species	+	-	+	+	-	-	-	+	nd	nd	-	+
Porphyromonas gingivalis	+	+	+	+	+	-	+	-	nd	nd	+	+

TABLE 4. Subgingival plaque cultures: ET 1710

	December 1995				July 1996				January 1997			
	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn	R Mx	L Mx	R Mn	L Mn
Fusobacterium species	+	nd	-	-	-	-	-	-	+	-	-	-
Veillonella species	-	nd	-	-	-	-	-	-	-	+	-	+
Prevotella species	+	nd	+	+	+	+	+		-	-	-	-
Clostridium species	-	nd	+	+	+	+	+	+			+	-
Porphyromonas gingivalis	+	nd	+	+	+	+	+	+	-	+	-	+

LEGEND TABLES 1-4: + = positive culture

- = absence of organism

nd = not determined

CHAPTER SIX: DISCUSSION

The prevalence of spontaneous gingivitis and periodontitis in non-human primates has been assessed in only a few studies. Cohen and Goldman (1960) observed alveolar bone loss in 1 out of 17 skulls from *Macaca cynomolgus*. Hall et al (1967) studied 106 formalin fixed heads and 65 defleshed skulls of wild howler monkeys, but only found pockets in 16 of the wet specimens with no evidence of alveolar bone loss of the type seen in human periodontitis. In a study of 40 healthy baboons of the species *Papio anubis* aged 7 to 10 years, Hodosh et al (1971) observed periodontitis in 3 of the animals, although gingivitis was present in 33 of the animals. Avery and Simpson (1973) reported a higher prevalence in baboons of the species *Papio ursinus*. All of the young animals they studied had severe gingivitis and 3 middle-aged animals had periodontitis. However, the young and adult animals were selected from large colonies in which the actual prevalence of periodontitis is not known. Borland (1994) found abundant accumulation of plaque, severe gingival inflammation and the presence of *Porphyromonas gingivalis* in sulci of 20 captive baboons of the species *Papio ursinus*, however no periodontitis was present. The results of a study conducted by Miller et al (1995) were in agreement with those of Avery and Simpson (1973) and showed that some baboons of the species *Papio anubis* and *Papio cynocephalus* do develop periodontitis and that the disease has increased prevalence and severity with age. Therefore, gingivitis in varying degrees of severity is often seen, but spontaneous destructive periodontitis with periodontal pocket formation, suppuration, bone loss and tooth mobility, has rarely been demonstrated in non-human primates.

Most periodontal procedures in use today have been developed empirically, and only in the last 3 decades systematic attempts have been made to define the effects of various types of therapy (Caton and Zander, 1975). This type of analysis is hampered by the ethical and practical difficulty of obtaining histologic and other material from human subjects. As a consequence, an animal model, which has the characteristics of the human periodontal defect, is essential if the detailed results of various types of treatment are to be defined and understood. A number of animal model systems have been developed for testing periodontal treatment procedures. Ramfjord, in 1951, surgically detached periodontal tissues from the root surface and removed alveolar bone in rhesus monkeys, followed by the insertion of copper bands to establish disease. He found that while in place, the bands caused some decrease in pocket depths, but on removal of the bands, pockets were assumed to have completely epithelialised with no histologic evidence for this. Ellegaard et al (1973) produced experimental bifurcation defects in rhesus monkeys and left a steel wire or periodontal pack in the wound for 4 weeks, maintaining a stabilised defect that was demonstrated to be epithelialised following histological examination. Caton and Zander (1975) produced periodontal defects in rhesus monkeys through the use of orthodontic elastics, characterised by irreversible apical positioning of the junctional epithelium and both horizontal and angular alveolar bone loss. No studies to date have been performed on the induction of periodontitis in the Chacma baboon, *Papio ursinus*. Therefore, this is a unique animal model that combined surgical and microbiological approaches in an effort to induce chronic periodontitis in this baboon species for the purpose of testing novel periodontal regenerative procedures.

In the present study, the dual induction of periodontitis through surgical intervention and microbial inoculation has led to loss of periodontal attachment in all four animals investigated. Probing depths ranged from 4-10mm, and because the measurements were taken from the cemento-enamel junction to the subepithelial connective tissue attachment, values of 4mm or more were diagnosed as destructive periodontal lesions (Caton, 1989). The clinical features of periodontal disease in *Papio ursinus* were found to be quite similar to those in the human and consisted of severe gingival inflammation resulting in oedema, erythema, hyperplasia and spontaneous bleeding. As the disease progressed, pocket formation and marginal bone loss occurred, resulting in gingival recession and osseous defects resembling those found in advanced human periodontal disease. Gingival inflammation was related to the accumulation of extensive plaque and calculus. Furthermore, there was clinical and radiographic evidence of alveolar bone loss in the furcation areas of all the experimental animals. Furcation involvement is indicative of loss of attachment, loss of alveolar bone and increased severity of disease (Larato, 1970; Tal, 1982).

This study is consequently in agreement with Avery and Simpson's findings that baboon molars are subject to rapid furcation invasion due to their shortened root trunks when compared to root trunks of human molars (Oschenbein, 1986). Clinically, baboon molars also have a prominent cervical bulge superior to the furcation entrance that may help precipitate early furcation involvement as described in human populations. In addition, furcation involvement in this experiment was found to be bilaterally symmetrical

suggesting a further similarity of baboon periodontal disease to human periodontal disease (Tal and Lemmer, 1982).

The evidence of attachment loss and severe furcation involvement with abundant granulation tissue was confirmed during surgical application of regenerative procedures 6 months after completion of *Porphyromonas gingivalis* inoculation, thus indicating the irreversible nature of the experimental periodontal lesions created. The question of reversibility of an experimental periodontal pocket is important because the human periodontal pocket is not spontaneously reversible (Caton and Zander, 1975). In addition, the experimental periodontal lesion that could spontaneously revert would seriously confuse the evaluation of treatment, which has as its purpose the reversal of the pocket. Pocket reversal occurs when the apical level of the junctional epithelium assumes a more coronal position, the alveolar bone height increases, and the connective tissue attachment is re-established to the previously exposed root surface. Previous models utilising surgically produced periodontal lesions in animals, which were not allowed to epithelialise, demonstrated reversibility (Hiatt, 1970; Linghorn and O'Connell, 1950). Those defects that were allowed to epithelialise either showed partial reversibility (Ramfjord, 1951; Hurt, 1963; Yuktanandana, 1959) or were not examined for this point. The results of the present study indicate that the surgically- and microbiologically-produced periodontal lesion is not spontaneously reversible and is, therefore, a suitable model for treatment evaluation.

The microbiological analysis showed that Gram-negative anaerobic organisms with predominantly the black-pigmented *Porphyromonas gingivalis*, which is associated with early-onset forms of destructive periodontitis in humans (Slots et al., 1980; Zambon et al., 1983), were repeatedly cultured from the newly established microenvironment in the gingival sulci of the animals studied. Four sites per animal were sampled at any given time period (total of 112 samples). Rarely, did a sample not harbour the micro-organisms under investigation, possibly because the organism was present in insufficient numbers for detection. Three samples were accidentally damaged; that for the maxillary left quadrant in baboon ET 1710 at week 4, and that for the maxillary right and left quadrant in baboon ET 1697 at week 60. Inherent difficulties in the culturing of anaerobic organisms may also have contributed to certain samples yielding negative cultures. Nevertheless, implantation of *Porphyromonas gingivalis* in a large back-ground of the normal microbiota demonstrated that there was a direct connection between the implanted *Porphyromonas gingivalis* and clinical characteristics of periodontitis. The *Porphyromonas gingivalis* emerged from this complex plaque ecological niche in such a way that it produced a 'burst' of bone loss indicating its ability to induce progression of periodontitis.

The successful induction of periodontitis in this animal model has important implications for future studies with relevance to tissue regeneration and tissue engineering. The regeneration of the periodontium reduced by periodontitis is an object central to periodontal treatment. Such regeneration requires the formation of new cementum upon a root surface denuded by intective-inflammatory disease, deposition of bone, insertion of

functionally oriented new connective tissue fibres into both the new bone and the new cementum, and organisation of a competent gingival unit (Melcher, 1976; American Academy of Periodontology, 1986). While regeneration of the periodontal tissues is the ultimate goal of the treatment of periodontitis, regeneration as defined above, is not a predictable healing outcome in both pre-clinical and clinical studies (Wirthlin, 1981; Bowers et al, 1982; Lynch, 1992). Rather, healing following surgical procedures devised for periodontal tissue regeneration proceeds predominantly via repair phenomena, in which the newly formed tissue does not fully restore the architecture of the lost tissue (Melcher, 1976; American Academy of Periodontology, 1986).

Tissue engineering is an emerging field of science developing techniques for fabrication of new tissues for replacement based on principles of cell and developmental biology and biomaterials (Ripamonti and Reddi, 1997). Bone has considerable potential for regeneration and therefore is a prototypic model for tissue engineering. Bone derived BMP's, with a collagenous matrix as carrier, induced cementum and alveolar bone regeneration in surgically created furcation defects in this primate (Ripamonti et al, 1994). However, the efficacy of bone-derived and recombinant human (rh) BMPs must be evaluated in a pertinent animal model with experimentally-induced chronic periodontitis (Ripamonti and Reddi, 1997). This may help to design therapeutic strategies based on cell biology for optimal outcome in the periodontal patient.

CHAPTER SEVEN: CONCLUSION

It is apparent from the foregoing discussion that *Papio ursinus* , normally resistant to spontaneous periodontitis, does exhibit a chronic form of periodontal disease following surgical and microbiological intervention. True attachment loss was attained as a result of an alteration in both the macro- and the micro-environments of the experimental animal. Surgery provided furcal bony defects into which the human putative periodontal pathogen, *Porphyromonas gingivalis*, was delivered to propagate the infectious nature of the disease. These experimental periodontal lesions were further shown to be irreversible thus establishing this animal as a suitable model for testing novel regenerative procedures as part of periodontal treatment.

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Name of thesis Induction Of Periodontitis In The Chacma Baboon Ksiezycki-Ostoya B K 1999

PUBLISHER:
University of the Witwatersrand, Johannesburg
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