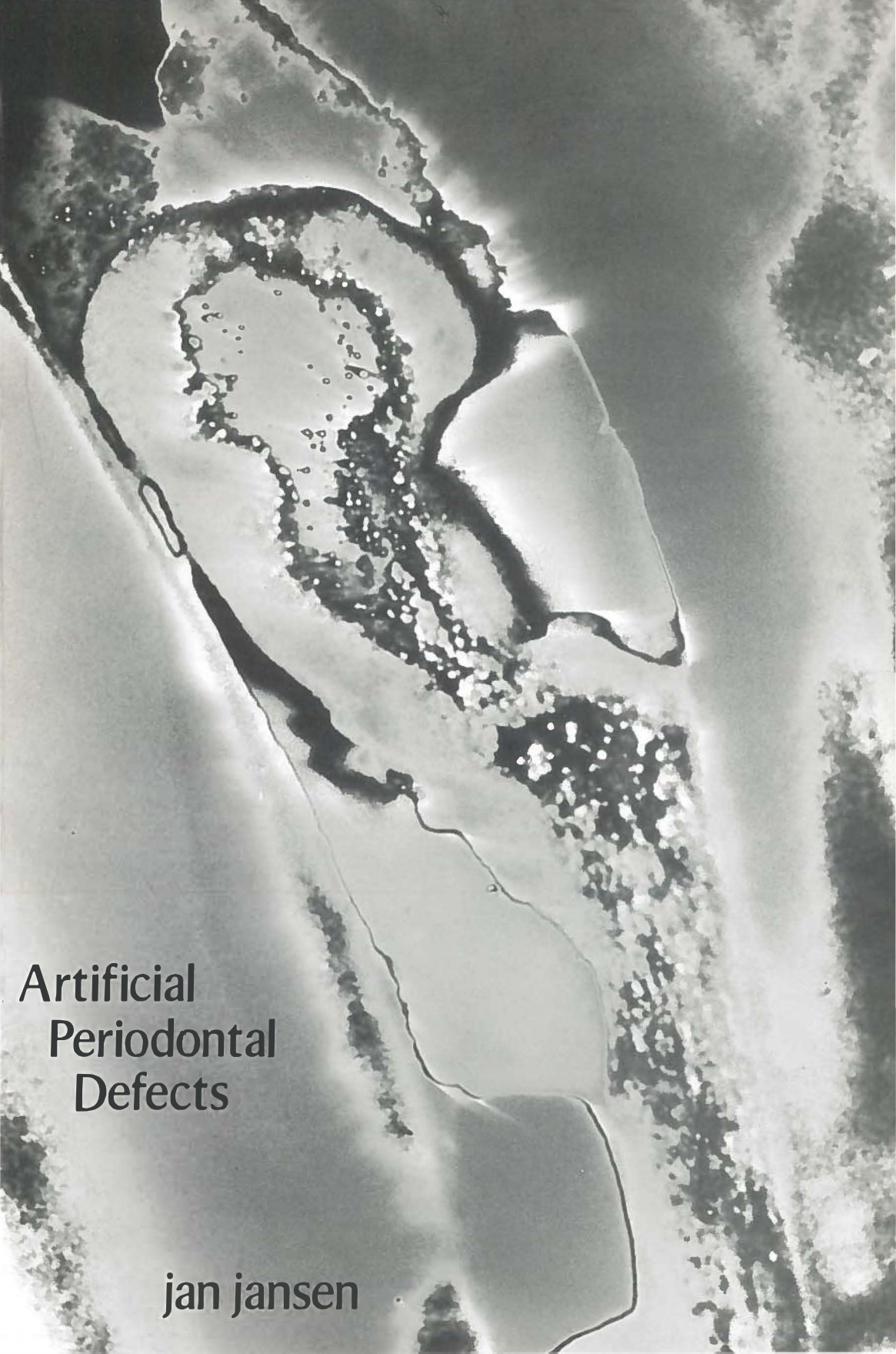

Artificial Periodontal Defects

jan jansen

ARTIFICIAL PERIODONTAL DEFECTS

JAN JANSEN

Dit proefschrift werd bewerkt in de kern voor vakverdieping Parodontologie (Vakgroep Parodontologie-Prothetodontie-Sosiodontie) van de Subfaculteit der Tandheelkunde, Rijksuniversiteit te Groningen.

Omslagfoto : Jan Schurer
(chapter 5, figure 2)

STELLINGEN

behorend bij het proefschrift

Artificial Periodontal Defects

1. De aanwezigheid van een lange epitheliale aanhechting na chirurgische behandeling van diepe pockets behoeft niet als een ongunstig resultaat te worden gezien, mits aan de eisen voor optimale plaquebeheersing wordt voldaan.

2. Een minimale breedte van aangehechte, gekeratiniseerde gingiva is geen vereiste om gingivitis of verlies van parodontaal steunweefsel te voorkomen.

3. Een niet-traumatische, ondubbelzinnige markering in het worteloppervlak van het niveau van de apicale begrenzing van het aanhechtingsepitheel zou de histologische interpretatie van experimenten die een verandering van dit niveau beogen aanzienlijk vereenvoudigen.

4. Het vinden van indicatoren die discrimineren tussen progressieve en niet-progressieve parodontale ontstekingen kan leiden tot een meer doelmatige definiëring van de behandelings-"behoefte", zowel voor de individuele patiënt als voor groepen van de bevolking.

5. De term plaquebeheersing geeft, beter dan de term plaquebestrijding, aan dat niet onder alle omstandigheden bij alle "patiënten" naar plaquevrije dentities gestreefd hoeft te worden om het natuurlijke gebit (of een essentieel deel daarvan) levenslang te kunnen behouden.

6. Het is thans voldoende bekend hoe parodontale afwijkingen voorkómen of behandeld kunnen worden. Verdere inspanningen dienen erop gericht te zijn wegen te vinden om deze kennis dienstbaar te maken aan de bevolking.

7. Het immobiliseren van, als gevolg van ver voortgeschreden parodontale afbraak, beweegbaar geworden gebitselementen als onderdeel van een parodontale therapie is obsoleet. De behandeling dient gericht te zijn op opheffen van de ontsteking van het parodontium.

8. De tandarts die geen aandacht aan het parodontium van de hem toevertrouwde patiënten besteedt, doet er goed aan een deugdelijke beroeps-W.A. verzekering af te sluiten.

9. Converserende tandheelkunde voorkomt veel conserverende tandheelkunde.

10. Zolang instellingen voor christelijk onderwijs afhankelijk zijn van overheidsgelden en een aanbod van niet-christelijke leerlingen, dienen ze zich m.b.t. het aannamebeleid van leerkrachten en leerlingen niet te onderscheiden van instellingen voor openbaar onderwijs.

11. Een neutronenbom kan weliswaar de promovendus vernietigen maar gelukkig niet zijn proefschrift.

12. Het poneren van stellingen bij een proefschrift is vaak pronken met andermans veren.

Groningen, 23 juni 1982

Jan Jansen

Rijksuniversiteit te Groningen

ARTIFICIAL PERIODONTAL DEFECTS

Proefschrift

ter verkrijging van het doctoraat in de
geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen
op woensdag 23 juni 1982 des namiddags te
4.00 uur

door

Jan Jansen
geboren te Deventer

Promotor : Prof. dr. T. Pilot

Referent : Dr. H.W.B. Jansen

Coreferent : Prof. dr. J. Theilade

Paranimfen : Nico Corba
Jan Tromp

Aan : Liesbeth
Marte
Anneleen
Sophie

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CONTENTS

Chapter 1

INTRODUCTION

Abstract. Some general aspects of the periodontium in health and disease are briefly outlined in this chapter. The importance of animal models in periodontal research is discussed. The literature on the use of animals with artificially created periodontal defects is also reviewed, particularly with reference to the role of plaque retaining ligatures. On the basis of this review and our experiences with artificial periodontal defects in dogs, a series of questions were formulated which indicated the need for specific research in this area. These questions provided the guidelines for the present investigations.

The periodontium in health and disease.

The supporting apparatus of the teeth, as defined by the term "periodontium", consists of alveolar bone, root cementum, the periodontal ligament and the investing sheath of the gingiva and oral mucosa. The gingiva acts as a seal between the oral environment and the environment of the root of the tooth. The gingiva is connected to the crown of the tooth by an epithelial attachment called the junctional epithelium. Apical to this epithelial junction the gingiva is connected to the root by collagen fibres which are inserted in a layer of cementum on the tooth. The roots of the teeth are firmly connected to the surrounding alveolar bone by the periodontal ligament fibres which are inserted into the root cementum on the one side and into the alveolar bone on the other.

The connective tissues around the roots of teeth are at risk to the external environment to a greater degree than in any other area of the body because, here, the overall epithelial covering of the body is breached by the tooth. In the absence of adequate oral hygiene bacteria of the commensal flora of the mouth colonize the tooth surface, readily forming a well-structured mass of densely packed microorganisms, held together in an intermicrobial substance. This is called dental plaque. When plaque is allowed to accumulate in the dentogingival area the bacterial products will diffuse through non-keratinised parts of the oral epithelium and the epithelial attachment and provoke an inflammatory reaction in the underlying connective tissues. The presence of inflammation in the connective tissues results in disintegration of the collagen bundles which, as the inflammation proceeds, are gradually replaced by a chronic cellular infiltrate. As long as the inflammation re-

mains confined to the gingiva, the disease is termed *gingivitis*. Extension of the inflammatory process into the deeper tissues *(periodontitis)* occurs with breakdown of the connective tissue attachment to the tooth, resorption of alveolar bone and an apical migration of the junctional epithelium. The microbial plaque will extent further apically on the root surface. The process of periodontal breakdown is assumed to be attended with exacerbations and periods of quiescence. The inflammatory infiltrate, that has taken the place of the degraded connective tissue structures consists mainly of mononuclear leucocytes with plasma cells being the predominant type. Since the periodontal tissues are no longer connected to the root surface a space exists between the inflamed soft tissues and the root surface, called a periodontal pocket. The assessment of the depth of such a pocket with the use of a periodontal probe is a clinical tool for estimating the apical extension of the disease process. In advanced cases of periodontitis pockets over 6 millimeters in depth are a common finding. Over the long term the result of progressive periodontitis may be the loss of the teeth and ultimately of the dentition.

This general description of the periodontal disease process does not mean to say that incipient gingivitis necessarily progresses in all cases to destructive periodontitis and eventually to tooth loss. Many factors governing the natural history of periodontal disease are at present not fully understood. There is considerable variation in structural, bacteriological and biochemical composition of the plaque with the site of formation, the duration of accumulation and the composition of the diet. There is also considerable variation in the pathogenicity of dental plaque. On the other hand there are many factors within the host, such as systemic disorders, immune responses or local factors, which may influence the susceptibility of individuals to periodontal disease.

Gaining an understanding of the etiology and pathogenesis of periodontal disease and the ways to prevent or treat the disease has been the goal of numerous studies in periodontal research. These studies have been performed both in humans and in experimental animals. Although the observations in humans have provided clear insight into many aspects of periodontal disease, there are several scientific or ethical reasons why animal models are to be prefered to human studies.

Animal models in periodontal research.

The 4 mean reasons why animal models are prefered to human studies in periodontal research are as follows:

- The results of studies in humans are mostly restricted to clinical or radiographical observations. Conclusions drawn from these data may be misleading because there is a lack of detailed information about the ultrastructural relationship of the soft periodontal tissues to the teeth or alveolar bone. This kind of information can only be obtained in microscopical or histological investigations, which, for ethical and practical reasons cannot be performed in man.
- When there is even a remote possibility of a health risk to a subject then preliminary testing on animals is mandatory.
- In animals it is possible to purposely perform procedures which are deliterious to health, e.g. the induction of periodontal disease in order to study the etiology, pathogenesis, therapeutical procedures or other factors which influence the disease process. These procedures obviously cannot be performed on man.
- Conclusions drawn from experiments may be more soundly based when the number of experimental variables is reduced. This demand can be more easily met in experimental animals than in man. It is for example possible to select a homogenous group of experimental animals with respect to age, phenotype and genotype, sex and the state of periodontal health or disease. Furthermore, experimental conditions such as housing, feeding and oral cleanliness can also be standardised. All these factors may reduce the number of experimental variables and thus facilitate conclusions about causal relationships.

Results obtained in animal studies should however always be applied to man with great care because the biology and physiology of the animal used may be quite different from man. Simplified experimental conditions may also be far from the "real life" situation thus limiting the extrapolation from animal to human.

Selection of experimental animals.

There are several criteria which can be used to select the appropriate animal for a specific experiment: availability, cost, background data, housing requirements and adaptability for experimental conditions (for review see Navia 1977). Rats, dogs and non human primates (squirrel and rhesus monkeys) are some of the laboratory animals most frequently used as models in periodontal research.

Rats have often been used in experiments aimed at identifying possible pathogenic plaque micro-organisms, because of the opportunity of mono-infecting germ-free animals (Heijl et al. 1980, for review see Garant 1976).

Beagle dogs and monkeys appear to be the most appropriate animals for the evaluation of treatment procedures, preventive procedures or the influence of so-called "co-factors". In the absence of oral hygiene, both animals develop perio-

dontal breakdown with age, which is very similar to the disease in humans (Rosenberg et al. 1966, Hall, Grupe & Glaycomb 1967, Gad 1968, Arnold & Baram 1973, Avery & Simpson 1973, Hull, Soames & Davies 1974, Saxe et al 1976, Sorensen, Löe & Ramfjord 1980). Furthermore, beagle dogs and monkeys have been used extensively in periodontal research in the past. Comparison of the results of different studies are obviously more relevant when the same species is used.

Monkeys may be prefered by those who feel that the interpretation of results obtained on a primate is more reliable than on animals of lower species. The use of a large non-human primate has been especially advocated for the evaluation of treatment procedures (Ramfjord 1971). The anatomy of the teeth and the periodontium shows great similarity with man. Monkeys are however difficult to handle. Meticulous oral hygiene procedures are impossible without special precautions. Therefore, in most long term experiments in which oral hygiene procedures are included, these are never performed more frequently than 3 times a week (Caton & Zander 1979, Caton & Nyman 1980, Caton, Nyman & Zander 1980).

The beagle dog is selected because it is large enough for the various periodontal treatment procedures, which have to be completed. In contrast to the monkeys its ease in handling permits daily oral hygiene procedures without special precautions.This may be the reason for the selection of this animal for long term experiments in which the influence of dental plaque is investigated (Lindhe, Hamp & Löe 1975, van Dijk 1979). Although the histological aspects of the periodontal tissues are similar to man, the morphology of the dentitions is different: slender teeth with the widest curvature of the crowns beneath the gingival margin, absence of contact points in the premolar region, and absence of occlusal contact.

Naturally occurring periodontal disease in animals.

Experimental results obtained from the study of naturally occurring periodontal disease would be more suitable for extrapolating conclusions to the human situation. However, animals with the appropriate features of naturally occurring periodontitis may not be available in sufficient numbers. Page & Schroeder (1981) reported on the prevalence of spontaneous periodontitis in a dog colony. Only 15 out of over 2000 animals were found to have clinically apparent periodontitis, although gingivitis was universal. Furthermore, wide variation of the features of periodontal disease, both between animals and between different areas in the mouth have been reported (Hull, Soames & Davies 1974, Lindhe, Hamp & Löe 1975, Lang et al. 1979, Sorensen, Löe & Ramfjord 1980, Page & Schroeder 1981). It would be difficult, therefore, if not impossible, to select a homogenous group of experimental animals all with identical

features of naturally occurring periodontal disease. This will jeopardize the aim of using an experimental animal model, namely to reduce the number of experimental variables and to establish reliable control groups.

Finally, specific experimental procedures may require periodontal defects of well-defined size or morphology. For example new attachment procedures are preferably performed in intrabony defects whilst deep periodontal pockets are required in pocket elimination procedures. It is clear from the above that the requirement of an even distribution of size and morphology can only rarely be met in naturally occurring periodontitis.

In order to meet the requirement of availability and variability, different methods have therefore been developed to induce artificial periodontal disease.

Artificial periodontal disease.

Different aspects of periodontal disease may be required for different areas of research. One for example, is the activity of the disease process and another is the reaction of a stable periodontal lesion of given size and morphology to treatment procedures. In this section monkey- and dog models with artificially induced periodontal disease are defined, categorised and related to the type of study.

Artificial periodontitis is here defined as a progressive inflammatory *process* affecting all periodontal tissues and which is the result of experimental interference with the host parasite balance at the dentogingival junction. This interference may or may not be combined with, or preceeded by, mechanical injury to the marginal periodontal tissues. The destruction of the periodontal tissues as a result of artificial periodontitis or mechanical injury is termed here *artificial periodontal breakdown*.

Models with artificially induced periodontitis can be used in studies aimed at evaluating the effect of a variety of (co-)factors or drugs on the progression of periodontitis. Artificial periodontitis should resemble naturally occurring periodontitis as closely as possible i.e. it should exhibit:

- apical migration of pocket epithelium together with loss of connective tissue attachment,
- alveolar bone loss,
- chronic inflammatory infiltrate present beneath the pocket epithelium.

Artificial periodontal defects are defined here as stable lesions in the periodontium which remain after a process of artificial periodontitis or artificial periodontal breakdown. These lesions may be used in studies aimed at evaluating the effect of treatment procedures, provided that certain requirements are met:

- the established defects should not heal spontaneously: the apical positioning of the pocket epithelium and the alveolar bone loss should not be reversible,
- size and morphology of the defects should be suitable for the treatment procedures of choice.

The creation of both artificial periodontitis and artificial periodontal defects should be reproducible and predictable in order to obtain comparable experimental and control groups.

Artificial periodontitis.

Artificial periodontitis can be established by enhancing plaque accumulation in the dentogingival area by feeding the experimental animals a plaque promoting diet (Lindhe, Hamp & Löe 1975) and by placing plaque retaining ligatures around the necks of the teeth.

A study by Rovin et al. (1966) served as baseline for all the ligature application models. The authors demonstrated that in rats, ligature placement at the cervical margins of molar teeth resulted in inflammation of the supporting tissues within a few weeks as a consequence of the combined presence of micro-organisms and ligatures.

In squirrel monkeys Kennedy and Polson (1973) evaluated the effect of ligature placement over a period of 52 weeks. Histologic and histometric analysis revealed a progressive marginal periodontitis characterized by apical migration of the junctional epithelium. The experimental lesions were established with a high degree of reproducibility.

In beagle dogs, ligature placement in combination with the surgical removal of alveolar bone was used in a model for the evaluation of occlusal trauma and the progression of periodontal disease (Lindhe & Svanberg, 1974). The authors were able to produce periodontal lesions with features of progressive periodontitis in a period of 7 weeks.

Ericsson et al. (1975) further elaborated on this dog model. They created narrow bony defects at the mesial and distal aspects of the fourth lower premolars, after which copper bands were applied to prevent spontaneous healing of the wounds. These copper bands were removed after 3 weeks and replaced by cotton floss ligatures which were left in place for a further 30 weeks. This method resulted in rapidly progressing marginal periodontal breakdown with the characteristic features of periodontitis. There was no significant difference between the amount of periodontal breakdown on contralateral sides of the jaws.

Schroeder & Lindhe (1975) were able to demonstrate that it is possible to convert

a resting, apparently non-destructive, gingivitis in the beagle dog into a progressive and highly destructive periodontitis, simply by placing cotton floss ligatures around the teeth above the gingival margin.

Lindhe & Ericsson (1978) evaluated the effect of longstanding ligature placement (25-50 weeks) at the mesial and distal aspects of premolars in young beagle dogs with an initially healthy periodontium. The dogs were fed on a plaque promoting diet, and the ligatures were changed every month. It was demonstrated that this procedure resulted in conditions which promote development of gingivitis and rapid breakdown of the supporting tissues. No further periodontal breakdown was observed after ligature removal.

From the above studies it is concluded that application of plaque retaining ligatures around necks of teeth is a useful method to establish an animal model with experimental progressive periodontitis. The persistance of the plaque retaining ligatures in situ, however, seems to be a prerequisite for the maintenance of *progressive* artificial periodontitis (Lindhe & Ericsson 1978).

Artificial periodontal defects.

Artificial periodontal defects in monkeys and dogs have been created in various ways (for reviews see Ellegaard 1976, van Dijk 1979). Two different approaches can be followed. One is the surgical removal of a part of the coronal periodontium, after which either periodontal pack, steel wires, copper bands or ligatures are placed into the wound to prevent spontaneous healing and/or to collect plaque. The other is the non-surgical creation of periodontal defects by the application of ligatures or elastic bands around the necks of teeth.

Irrespective of the method used, a prerequisite is, as stated, that the established apical migration of the pocket epithelium and the alveolar bone loss is stable and not reversible after removal of the foreign bodies from the created defects, because a reversal would seriously interfere with the results obtained after experimental procedures.

Only a few authors among the many that have used artificial periodontal defects have evaluated their defects for stability. Ellegaard et al. (1973, 1974) created bifurcation defects and three-wall intrabony defects in rhesus monkeys surgically. Spontaneous healing was prevented by steel wires or periodontal pack which were left in place for 4 weeks in both types of defects. None of the bifurcation defects showed spontaneous healing in the first 18 weeks after pack or wire removal. However, in one fifth of the three-wall intrabony defects spontaneous healing occurred, probably due to early loss of steel bands or pack. Some reattachment was

observed in the deeper parts of the remaining defects.

In 1975 Caton & Zander developed a rhesus monkey model for testing periodontal treatment procedures. They were able to create deep periodontal defects (5-8 millimeters) in a period of 8-25 weeks using orthodontic elastic bands. The specimens which had the elastic bands for around 12 weeks showed the typical histological features of periodontal pockets. After removal of the bands no decrease in probing depths was observed and there was no evidence that the apical positioning of the pocket epithelium or the alveolar bone loss was reversible. In 20% of the defects a long junctional epithelium, typical for a resting lesion, had formed whilst the remaining pockets showed the histopathology of active periodontitis. It was concluded that the model was suitable for testing the effect of periodontal treatment procedures. In a subsequent study it was reported that almost identical lesions could be produced on contralateral teeth (Caton & Kowalski 1976).

Artificial periodontal defects in dogs have been evaluated for stability after ligature removal by Lindhe & Ericsson (1978), Hugoson & Schmidt (1978), Johansson, Nilvéus & Egelberg (1978) and van Dijk (1979).

Lindhe & Ericsson (1978) created periodontal breakdown by tying cotton floss ligatures around the necks of teeth at the cemento-enamel junction in beagle dogs. By changing the ligatures every 4 weeks and feeding a plaque promoting diet, as much as one third of the supporting tissues was broken down in a period of 25 weeks. Due to gingival recession the severely inflamed pockets were however extremely shallow (0.5 millimeter). When the ligatures were removed but plaque was allowed to accumulate for a further 25 weeks no further periodontal breakdown was observed but a resting, non-destructive lesion remained. No reversibility of the apical positioning of the junctional epithelium or the alveolar bone loss was observed. The authors concluded that the presence of the ligatures led to the establishment of extremely shallow pockets.

Hugoson & Schmidt (1978) reported radiographical and histological evidence of spontaneous regeneration, 6 months after silk ligature removal from experimental defects in beagle dogs in a plaque infected dentition. Without being specific about the type of improvement of the periodontal tissues they concluded that this phenomenon could seriously hamper adequate interpretation of results of treatment procedures in their model.

Johansson et al. (1978) created bifurcation defects in beagle dogs in a way similar to that described by Ellegaard (1973) in monkeys. The stability of the defects was evaluated 2 and 9 months after removal of the steel wires. In 3 out of 5 dogs no change was observed during this 7 month period. Bone repair could be

demonstrated in one dog together with a decrease in size of the infiltrated connective tissue, whilst another dog showed progressive periodontal breakdown.

A long term experiment was designed in our own laboratory in 1974 to evaluate the influence of oral hygiene on pocket elimination procedures in beagle dogs. In order to obtain deep pockets, artificial periodontal defects were created as follows: the supracrestal dentogingival fibres around the lower premolars were dissected and closely adapted copper bands were cemented around the teeth to prevent reattachment. After 3 weeks these bands were removed and cotton floss ligatures were tied around the teeth and forced as *deeply as possible into the defects*. These subgingival ligatures were left in place for 11 weeks. One week after ligature removal probing depths of approximately 4.5 millimeters were recorded (van Dijk 1979). Further observations on the control defects on which no surgery or oral hygiene procedures were performed revealed a decrease in probing depth of approximately 1 millimeter within 4 weeks which could not be accounted for by gingival recession. The decrease in probing depth could therefore either indicate that the created defects were reversible or that probing conditions (the resistance of the soft tissues to the periodontal probe) had changed upon ligature removal, probably due to a decrease of the inflammation in the tissues. Histometric analysis of the control defects 25 months after ligature removal revealed bone repair in spite of the absence of oral hygiene procedures.

Similar results were obtained in a pilot study, comparing the effect of cotton floss ligature placement with elastic band placement in the premolar regions of 4 mongrel dogs with plaque infected dentitions (Jansen, van Dijk & Pilot 1977). Probing depths, initially 4-5 millimeters, decreased by approximately 1 millimeter in a period of 3 weeks after ligature or elastic band removal; thereafter probing depths remained relatively stable. Furthermore, histological observations at varying intervals after ligature or elastic band removal revealed new cementum formation, new bone formation and the formation of a new periodontal ligament. However, the experimental design did not allow us to determine the exact nature of the healing phenomena.

The rationale for further studies.

From this survey it can be concluded that contradictory results on the establishment of non-reversible artificial periodontal defects have been reported. A series of studies, based on observations in our own dog model, was therefore designed to answer the following questions:

- do the decreasing probing depths observed after ligature removal from deep artificial periodontal defects in beagle dogs, reflect a coronal shift of the connective tissue attachment or are they the result of changing probing conditions as a result of a decrease of inflammation?
- should the histological changes (new cementum deposition, formation of new bone) as observed in the pilot study after removal of cotton floss ligatures or elastic bands from deep artificial periodontal defects, be interpreted as spontaneous healing and if so of what type (repair or regeneration) ?
- what are the consequences of these phenomena on the use of this dog model in experiments designed to evaluate the effect of treatment procedures ?

To answer these questions the experiments should include:
- probing depth measurements before and after the establishment of the defects;
- the determination of possible probing errors in artificial periodontal defects;
- a histometric evaluation of the morphology of the artificial defects before and after their establishment;
- histopathological observations in artificial periodontal defects before and after their establishment.

The experiments were performed on 12 beagle dogs. Two different methods of creating artificial periodontal defects were evaluated in different areas of the mouth.

In the lower premolar region defects were created using the copper band-ligature method as described by van Dijk (1979). Observations on this model had raised the questions as formulated above.

In the upper incisor region defects were created by using orthodontic elastic bands as described by Caton & Zander (1975). This method was chosen because it had been used to establish a promising model for testing treatment procedures in the rhesus monkey.

The experimental design allowed for observations before and immediately after creation of the defects and at varying intervals up to 31 weeks thereafter.

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Chapter 2

HISTOLOGIC EVALUATION OF PROBE PENETRATION DURING CLINICAL ASSESSMENT OF PERIODONTAL ATTACHMENT LEVELS

An investigation of experimentally induced periodontal lesions in beagle dogs

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Abstract. The purpose of this study was to evaluate histologically the extent and characteristics of the penetration of a periodontal probe during clinical assessment of loss of periodontal attachment in 3 different situations:

(1) experimental mild gingivitis, (2) experimental severe periodontal inflammation and (3) experimental moderate periodontal inflammation.

Mild gingivitis was obtained by merely allowing plaque to accumulate on the teeth. Severe periodontal inflammation was created using copper bands for a period of 3 weeks followed by placement of cotton ligatures for another 11 weeks. Moderate periodontal inflammation was obtained by allowing the experimental defects to recover for a period of 3 to 31 weeks.

At different times gutta-percha imitations of a thin periodontal probe were inserted into the pockets using a gentle but unknown force. Histologic observation in 30 specimens showed that epithelium was always present around the probe tip, in most instances forming a continuous layer of epithelial cells. Histometrical analysis showed that in mild gingivitis the probe tip failed to reach the apical termination of the junctional epithelium ($\bar{x}$ = -0.84 mm).

In severe periodontal inflammation the tip of the probe went past this point ($\bar{x}$ = +0.50 mm), while in moderate periodontal inflammation the probe tip came closest to the apical termination of the junctional epithelium ($\bar{x}$ = -0.05 mm).

It is concluded that the epithelial lining of a pocket stays intact, even in severe periodontal inflammation where the probe tip is situated apical to the apical termination of the junctional epithelium, indicating that during clinical probing the periodontal tissues are compressed and displaced but not perforated. It is also concluded that in beagle dogs the extent of probe penetration in experimentally inflamed periodontal tissues is dependent upon the degree of inflammation.

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Introduction

The periodontal probe, inserted with a gentle force into a pocket, is the most widely used tool in the clinical assessment of the periodontal attachment level. The validity of such an assessment has been questioned during the last few years (Listgarten 1972, Armitage et al. 1977).

At least two aspects seem to be of special importance in this respect: the extent of probe penetration into a pocket and the reaction of the periodontal soft tissues on the presence of the probe in the pocket.

The extent of probe penetration has been shown to be influenced by the degree of inflammation of the periodontal tissues (Armitage et al. 1977, Spray & Garnick 1979, Robinson & Vitek 1979) and by the probing pressure applied on the probe tip (van der Velden 1979, van der Velden & Jansen 1980).

Inconsistent results have been reported with respect to the ability of pocket epithelium to withstand the forces induced by a periodontal probe. Armitage et al. (1977) evaluated microscopically the probing error in beagle dogs and reported that in several specimens of the periodontitis group the probe went through the pocket epithelium and was in direct contact with the connective tissue. Similar results were reported by Spray et al. (1978).

On the other hand it was recently suggested that the epithelial lining of an experimental periodontal defect was not perforated by a probe, even when high probing pressures (1.25 N) were applied (van der Velden & Jansen 1980).

The purpose of this study was to evaluate the presence of epithelium around a probe tip inserted with gentle but not standardized force into three different types of experimental periodontal defects.

Material and Methods

Method of creating defects. In 12 beagle dogs approximately 18 months of age and weighing 12-15 kg, periodontal defects were created around three upper right premolars P^1, P^2, P^3 using the method described by Lindhe & Svanberg (1974) and modified by van Dijk (1979). The dentogingival fibres around the teeth were severed down to the level of the alveolar bone. Copper bands were adapted as closely as possible to the teeth and then cemented. After 3 weeks the copper bands were replaced by cotton ligatures, tied around the teeth and secured as deeply as possible into the defect. Eleven weeks later the ligatures were removed (end of the active phase of creating defects). In 2 dogs the sulci of the premolars of the contralateral sides, where no periodontal defects were created, were also used. Only plaque was allowed to accumulate.

The dogs were sacrificed after a period of 0, 3, 7, 15 or 31 weeks. This approach would result in three distinguishable degrees of inflammation in the periodontal tissues:

Mild gingivitis, without loss of attachment (no defects created but only plaque accumulation).

Severe periodontal inflammation, with loss of attachment (defects at the end of the active phase plus the weeks immediately thereafter, 0-3 weeks).

Moderate periodontal inflammation, with loss of attachment (defects in the healing phase, 3-31 weeks).

Method of probe placement. Since the evaluation was meant to be a check on clinical probing without standardized pressure, only a gently unknown pressure was used in the experiment.

Because of the striking similarity and easiness in histological processing, gutta-percha points nr. 70 (Produits Dentaires S.A. Vevey, Switzerland) were used as immitations of a University of Michigan probe (terminal diameter of 0.35 mm) (Hu Friedy, Chicago, Ill. U.S.A.).

Care was taken to insert the "probes" parallel to the long axis of the root and to keep the tip of the gutta-percha point in contact with the tooth surface. The probes were inserted with care until a definite resistance was felt. They were then held in place and fused to the teeth using a droplet of Histacryl (histoacryl^{R} blau, B. Braun, Melsungen A.G., Germany).

Only interproximal measurements were performed and only one gutta-percha point was placed between 2 premolars. This resulted in a maximum of 4 probes per dog. Two days before placement of the gutta-percha probes and sacrifice of the animals, clinical probing depths were performed on the same sites so that the 3 histological levels of inflammation could be correlated with the clinical measurements.

Histologic preparation. The dogs were killed by a perfusion technique. The sequence was as follows: Nembutal anesthesia, intravenous heparin injection, opening of the thorax, positioning of canula in the left atrium, clamping of the aorta descendens, opening of the right atrium, perfusion with 3 l saline followed by 3 l 10% neutral formalin.

The head was dissected and small blocks were sawed containing 2 neighbouring halves of teeth together with the interdental periodontium. These small blocks were then decalcified in a solution of 25% formic acid, containing 10% sodium citrate, dehydrated and embedded in paraffin. Mesiodistal section of 8 μm thickness were cut

and stained with hematoxylin-eosin. Only those sections judged to have been correctly orientated and containing the midsection of the gutta-percha points were used.

Analysis of sample. Thirty different sections out of 40 proved to be suitable and were used for analysis: 4 in the mild gingivitis group, 11 in the severe periodontal inflammation group and 15 in the moderate periodontal inflammation group.

From black and white photographs of the selected sections (magnification 35 X), the distance from the tip of the probe to the most apical termination of the junctional epithelium (ATJE) and the distance from the middle of the probe tip to the root surface were measured (Fig. 1).

Furthermore, the presence of epithelium around the probe tip was independently evaluated by 2 investigators (H.W.B. J. and R. L.) using a light microscope at a magnification of 250 X.

(++) Continuous layer of epithelium present around the probe tip.

(+) Epithelial cells present around the probe tip but there are one or more sites where these cells do not form a distinct continuous layer.

(-) Distinct area where no epithelial cells are present but where the probe is in direct contact with connective tissue.

Epithelial cells were differentiated from connective tissue cells or inflammatory cells on the basis of difference in staining and the presence of continuity of neighbouring cells, constituting thin cell layers. The typical form of epithelial cells could not be used as a criterion because the cells underneath the probe tip were often extremely compressed.

Results

Mild gingivitis group. Mean probing depth in the 4 selected sites was 2.00 mm (s.d. = 0.40 mm).

Histologic characteristics: few inflammatory cells were present in the connective tissue beneath the sulcular and junctional epithelium. This epithelium showed virtually no rete peg formation. Well-organized collagen bundles in the connective tissue beneath the junctional epithelium could be identified. There were few blood vessels of normal width present and no apical migration of junctional epithelium had taken place (Fig. 2a).

Epithelium was present around the probe tip in all 4 cases observed. In one section both examiners had some doubt about the continuity of the present epithelial

cells (score +, Fig. 2b), the remaining 3 sections were found to be positive (score ++). In *all* specimens, the probe tip failed to reach ATJE. The mean distance from probe tip to ATJE was $\bar{x}$ = -0.84 mm (s.d. = 0.37). Fig. 2a shows the characteristic relationship. In all 4 cases the probe tip ended at the transition from sulcular to junctional epithelium.

Severe periodontal inflammation group. Mean probing depth in the selected sites was 3.09 mm (s.d. = 0.91 mm).

Histologic characteristics: cell-rich, collagen-poor connective tissue, abundance of inflammatory cell infiltrate beneath and in the sulcular and junctional epithelium, many extensive rete pegs, many dilated blood vessels, and an apical migration of junctional epithelium along the cementum. In no instance was the probe tip in direct contact with the underlying connective tissue. There was always an epithelial lining present around the probe tip, although this epithelium was stretched, often resulting in very thin, flattened cells (Fig. 3b).

While one examiner had doubt about the continuity of the epithelial lining in 2 sections (score +), all other sections were found positive (score ++) by both examiners.

In this group the probe tip was generally situated apically to ATJE ($\bar{x}$ = +0.50 mm, s.d. = 0.43 mm). In only one specimen out of the 11 the tip failed to reach ATJE.

The probe tip was in most instances situated at a distance from the root surface ($\bar{b}$ = 0.97 mm, s.d. = 0.58 mm) and some tissue was always found between the probe tip and the root surface. Junctional epithelium was present on the root surface, not detached from it by the penetrating probe (Fig. 3a).

Moderate periodontal inflammation group. Mean probing depth in the 15 selected sites was 3.40 mm, s.d. = 0.50 mm.

Histologic characteristics: more inflammatory cells were present than in the gingivitis group, but less than in the severe inflammation group. There were few partially organized collagen bundles in the cell-rich connective tissue. Rete peg formation of the sulcular epithelium was less pronounced than in the severe inflammatory group. There was an apical migration of the junctional epithelium and a few enlarged blood vessels, but not as many as in the severe inflammation group (Figs. 4ab). As in the former 2 groups, in this group again no probe tip was in direct contact with connective tissue but a thin layer of stretched epithelium was always present in between. This epithelium formed in the majority of cases a continuous

layer (score ++). In only 3 out of 30 observations in this group was a score (+) given by one examiner.

In this inflammation group the extent of the penetration of the probe tip indicated ATJE more closely than in the 2 other groups. The mean deviation from this point was $\bar{x}$ = -0.05 mm, s.d. = 0.34 mm. In 9 cases the tip failed to reach ATJE, in the remaining 6 specimens the tip was situated apical to AJE. The terminal part of the probe was in contact with the root surface in almost all specimens ($\bar{6}$ = 0.49 mm, s.d. = 0.28 mm).

Discussion

Three different inflammatory conditions were identified in this experiment and were histologically classified as: a) mild gingivitis without apical migration of junctional epithelium, b) severe periodontal inflammation and c) moderate periodontal inflammation, the latter two accompanied by loss of connective tissue attachment.

The presence of leucocytes just beneath the sulcular epithelium in the mild gingivitis group indicated that the tissues could not be classified as absolutely healthy (Lindhe & Rylander 1975), although in other studies on dogs the presence of a few leucocytes was considered normal (Hamp & Lindberg 1977, Attström 1970).

In the severe periodontal inflammation group all histological symptoms of severe periodontitis as shown in dogs by Hamp & Lindberg (1977) and Armitage et al. (1977) and in man by Zachrisson & Schultz-Haudt (1968) were present: extensive anastomosing epithelial proliferations into the connective tissue, dense inflammatory cell infiltrate and loss of collagen structure. On this basis this group was termed "severe periodontal inflammation group" according to Zachrisson & Schultz-Haudt (1968). The moderate periodontal inflammation group was obtained by allowing a created defect to recover from the presence of a plaque-loaded ligature for different periods of time (3-31 weeks). Histologically, all symptoms present were less pronounced than in the severe inflammation group.

In no specimen had the probe tip cleary perforated the epithelial lining of the periodontal defect. The probe was thus never in direct contact with the underlying connective tissue, not even when severe inflammation was present and the probe tip was located apical to ATJE. This means that even in the case of severe periodontal inflammation not only is an epithelial lining of the defect present, but this epithelium resists forces induced by gentle probing.

This finding is in agreement with van der Velden & Jansen (1980) who under experimental conditions similar to the severe inflammation group in the present study, but using a probe twice as thick, also reported that perforation of the

epithelium rarely occurred, even when very strong probing forces were used. However, Armitage et al. (1977) as well as Spray & Garnick (1979) suggested frequent perforation of the epithelial lining of the pocket wall in naturally occurring periodontitis, using a probe of the same size as the one used in the present experiment. Weaker probing forces in this experiment as a possible explanation for the discrepancy is not very likely because in this study the probe tip extends on average more apically than in the afore-mentioned studies, so one would expect even more cases of epithelial perforation. Differences in severity of inflammation of pocket walls (e.g. ulceration), as suggested by van der Velden & Jansen (1980), is more likely.

In the present study, a distinct relationship was found between the degree of inflammation and the extent of probe penetration. In slightly inflamed gingiva the probe tip failed to reach ATJE, while in severe periodontal inflammation the probe tip was located apical to ATJE.

A number of other studies confirm this findings (Armitage et al. 1977, Robinson & Vitek 1979, Spray et al. 1978, Spray & Garnick 1979). This indicates that healthy or slightly inflamed periodontal tissues seem to offer too much resistance to a probe inserted with a gentle force, to correctly indicate the connective tissue attachment level or ATJE. Severely inflamed tissues, on the other hand, seem to be too loosely arranged to stop the probe at ATJE. Probably some loss of integrity of the inflamed tissues, as was the case in the moderate periodontal inflammation group in the present study or the experimental gingivitis group in the experiment of Armitage et al (1977), provides the best condition of more closely measuring connective tissue attachment level through clinical probing.

In the present experiment a gentle but unknown probing force was used. According to Gabathuler & Hassel (1971) probing forces that represent "gentle probing" will only rarely exceed 40 ponds (= 0.40 N). Therefore it is assumed that in our experiment no forces exceeding 40 ponds were used.

It has recently been suggested by van der Velden & Jansen (1980) that with the use of a thin probe, the probing force per unit area at the end of the probe tip is much greater than with the use of a thick probe. In evaluating probing forces in relation to probe penetration in experimentally induced periodontal defects, they found that with the use of their probe (terminal diameter of 0.63 mm) it took forces over 0.75 N to consistently pass ATJE. In the severe periodontal inflammation group of the present study, which corresponds closely to van der Velden & Jansen's experimental pockets, a gentle probing force, probably not exceeding 0.40 N, was sufficient to consistently pass the ATJE, using a probe 0.35 mm in diameter.

Acknowledgments

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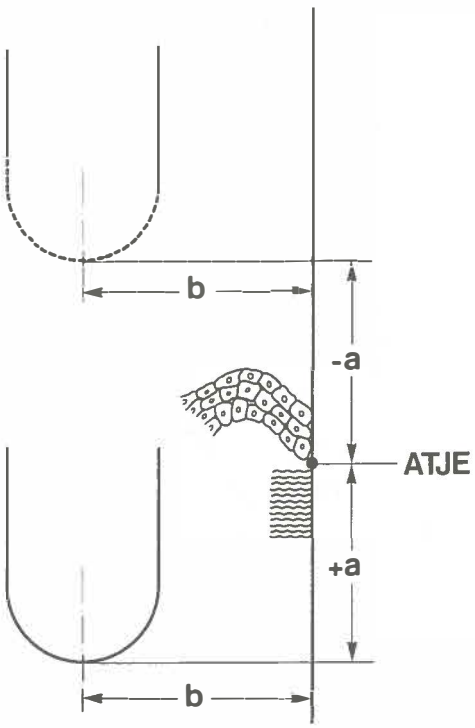
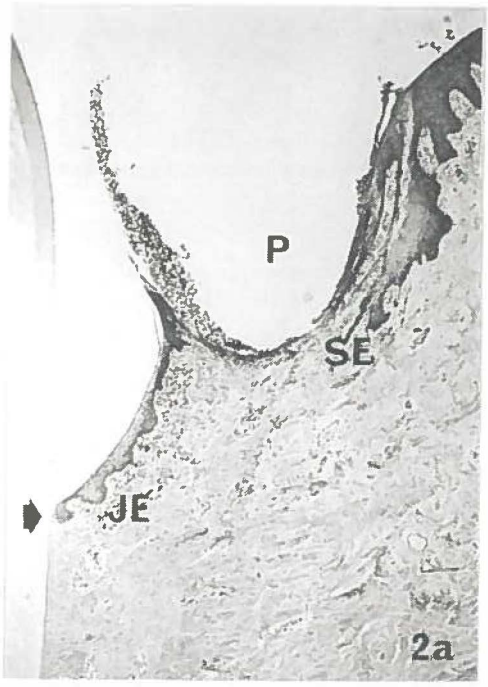


Fig. 1. Schematic representation of the distance (a) from the probe tip to the most apical termination of the junctional epithelium (ATJE) and the distance (b) from the middle of the probe tip to the root surface.



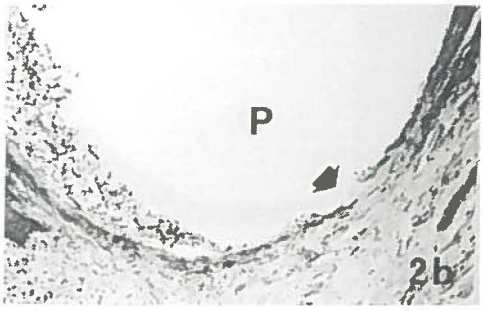
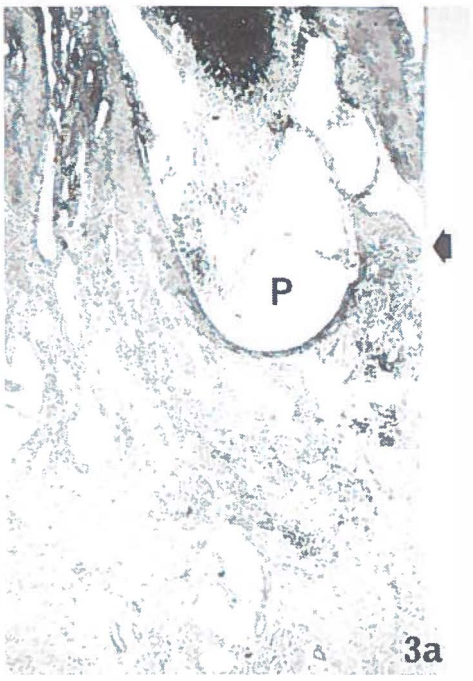
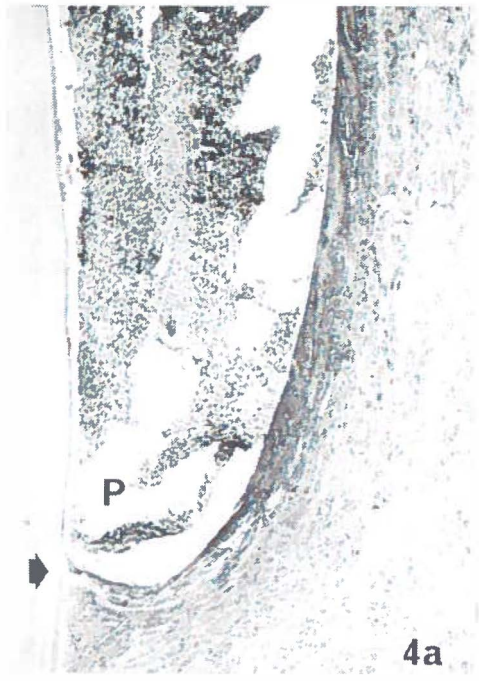
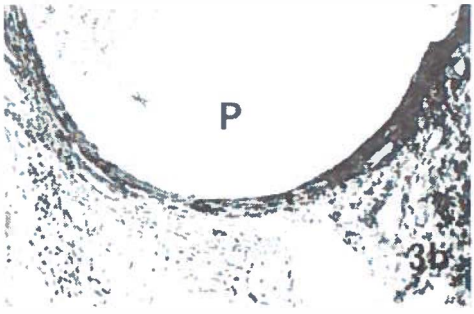


Fig. 2a. Representative section of the mild gingivitis group with the "probe" (P) in situ. The probe tip rests at the transition from sulcular epithelium (S.E.) to junctional epithelium (J.E.) and fails to reach the apical termination of junctional epithelium (arrow). X 100.

Fig. 2b. Higher magnification of the area around the probe tip. Score + was given for the presence of the epithelium in this area, because of lack of continuity in the indicated area (arrow). X 250.







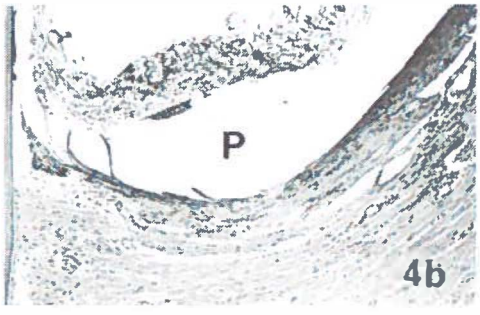


Fig. 3a. Section of the severe periodontal inflammation group. The probe tip is located apical to the apical termination of the junctional epithelium (arrow) and situated at a certain distance from the root surface. X 100.

Fig. 3b. Higher magnification of the probe tip area of Fig. 3a showing clearly the presence of continuous layer of epithelium around the probe (score ++). X 250.

Fig. 4a. Representative section of the moderate periodontal inflammation group. The probe tip is situated at the apical termination of the junctional epithelium (arrow). The probe (P) is in contact with the root surface. X 100.

Fig. 4b. Higher magnification of the probe tip area of Fig. 4a showing a very thin flattened layer of epithelium lining the probe tip (score ++). X 250.

Chapter 3

PREDICTABILITY OF PROBING DEPTHS IN LIGATURE-INDUCED PERIODONTAL DEFECTS IN BEAGLE DOGS

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Abstract. The aim of the present study was to evaluate the predictability of a method of creating periodontal defects in dogs. The dentogingival fibres around the lower premolars of 12 beagle dogs were cut to the level of the alveolar bone and closely adapted copper bands were cemented to the teeth. After 3 weeks the copper bands were replaced by cotton ligatures which were left in place for 11 weeks. After this active phase the ligatures were removed, and the defects were allowed to heal for varying periods up to 30 weeks. Probing depths were recorded at different stages of the experiment.

Indications for predictability were: 1^{o} the magnitude of the standard deviations of the mean probing depths and 2^{o} the differences between results from contralateral areas. Relatively deep defects were obtained immediately after creation, but the magnitude of the standard deviation of the mean indicated wide variations in probing depths. Following ligature removal probing depths decreased but were of a more uniform depth. These results indicate that variation of probing depths in artificial periodontal defects is rather high immediately after creation but decreases drastically during the healing phase. It was concluded that in young adult beagle dogs predictable periodontal defects, 3.0 - 3.5 millimeters in depth, can be produced artificially provided that the initial deeper artificially created defects are allowed to heal for at least 3 months.

Introduction

Artificially created periodontal defects in animals have been used to test therapeutic procedures for several decades. The ultimate aim of animal experiments is the reproduction of the human situation. An artificially created periodontal defect should therefore resemble a human pocket as closely as possible, clinically with regard to probing depth and/or attachment level and histologically with regard to

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epithelization of the defect, composition of inflammatory infiltrate, loss of connective tissue attachment, loss of alveolar bone and changes in the cementum.

Deep periodontal pockets respond differently to therapeutic and preventive procedures than do shallow pockets or sulci. This has been shown in clinical trials (Knowles et al. 1979) and in animal studies (Lang et al. 1979). If an animal model is used to test periodontal procedures, it is necessary to create pockets of distinguishable depths. A problem in creating deep pockets is, however, that artificial periodontal defects tend, to some extent, to heal spontaneously, resulting in shallower pockets than those required (Hugoson & Schmidt 1978, Jansen et al. 1979, van Dijk 1979).

A further prerequisite when using an experimental model is that it must be reproducible and that the artificial periodontal defects should be entirely predictable. Little attention has as yet been paid to this aspect (Lindhe, Hamp & Löe 1975, Ericsson et al. 1975, Caton & Kowalski 1976).

The aim of this experiment, which is part of a more extensive study on the behaviour of artificially created periodontal defects in beagle dogs, was to evaluate probing depths in ligature-induced periodontal defects. The results should shed more light on the predictability of probing depths and thus ensure more efficient use of the experimental animal model.

Material and Methods

Twelve beagle dogs, approximately 18 months old and weighing $\pm$ 15 kg were used. Throughout the experiment the dogs were fed on a diet which favors gross plaque accumulation on the teeth, and no oral hygiene procedures were performed. Periodontal defects were created by using a technique adopted from Van Dijk (1979) who modified the method described by Lindhe & Svanberg (1974). The dentogingival fibres around the necks of the lower right premolars $_2P$, $_3P$ and $_4P$ were cut to the level of the alveolar bone, and closely adapted copper bands were cemented to the teeth with the use of copper cement. After 3 weeks the copper bands were replaced by cotton ligatures which were forced down as deep as possible. These ligatures were left in place for 11 weeks. After this period the defects were allowed to heal for periods varying up to 30 weeks.

Pairs of dogs were scheduled for sacrifice and subsequent histological preparation 0, 2, 6, 14 and 30 weeks after removal of the cotton ligatures from the defects. The experimental design is shown in Fig. 1.

Probing depth measurements were performed at baseline immediately after the removal of the ligatures and 2, 6, 14 en 30 weeks thereafter.

In this experiment probing depth was defined as: the extent of penetration into the sulcus or pocket of a University of Michigan O probe (terminal diameter 0.35 millimeters) inserted with a non-standardized light hand pressure until definite resistance was felt. All measurements were made to the nearest half millimeter. Only mesial and distal surfaces of the experimental teeth were investigated.

The depths measured were devided into classes as follows: class I (probing depth $\leq$ 2.5 millimeters) was chosen because it was assumed to be within the range of clinically normal depths after 1 - 1.5 year of plaque accumulation (Lindhe, Hamp & Löe 1973, 1975, Schroeder & Lindhe 1975). Therefore, pockets with probing depths exceeding 2.5 millimeters were considered to be deepened. A distinction was made between moderately deep defects: class II (2.5 < probing depth < 5.0 millimeters) and deep defects: class III (probing depth $\geq$ 5.0 millimeters), because 5 millimeters is often considered to be the borderline between a conservative and surgical approach in periodontal therapy.

In order to investigate the predictability of making defects in the same dog, periodontal defects were created in 10 out of the original 12 beagle dogs using the same technique at the contralateral (left) premolars 14 weeks before sacrifice, thus allowing left-right comparison at baseline and at the end of the active phase.

Two parameters were used as being indicative of the predictability of probing depths:

1°. the magnitude of the standard deviations of the mean probing depths and

2°. differences in probing depths from contralateral areas.

Means of probing depths were compared using Student's t test for each area separately in order to avoid the potential problem of dependency of observations (Pilot et al. 1980).

Results

The frequency distribution of probing depth in classes, mean probing depths and standard deviations are presented in Fig. 2 for all defects after different periods of time. Baseline measurements were dominated by class I defects. During the active phase of the experiment class II and III defects predominated and at the end of this phase deep defects (class III) constituted a large proportion of those measured. In the healing phase, however, class II defects constituted more than 80% of the registrations at all stages. After 14 weeks defects that did not fall within the class II range were exceptional.

Mean probing depths and standard deviations for separate areas are presented in Table 1. After a considerable increase in mean probing depth during the creation

phase a rapid decrease was noted during the healing phase, especially during the first 2 weeks after elimination of the ligatures.

At baseline standard deviations of the mean were approximately 0.50 millimeter in all areas (Table 1). At the end of the active phase, deviations in probing depth were often 2 to 3 times as high, whilst in the following period the standard deviations returned to baseline levels or even lower in most instances.

It can be seen from Fig. 2 that the frequency distribution of classes of probing depths at baseline and at the end of the active phase were almost the same in the left and right quadrants. Two dogs did not, however, follow this general pattern. In dog number 9 defects were created on the right side of 7 and 8 millimeters depth whilst on the contralateral side no such exceptional depths were recorded. In dog number 5 similar but less pronounced differences were also found. A comparison between the mean probing depths of separate areas in the left and right jaw in this group of dogs revealed no significant differences in any area (Table 1).

Discussion

In this study the probing depth of a periodontal defect was chosen as the clinical parameter, because it was felt that the depth of the defect is more relevant than the loss of periodontal attachment when a treatment procedure is tested in a model.

Most authors producing experimental periodontal breakdown in dogs record shallow pockets (Ericsson et al. 1975, Schroeder & Lindhe 1975, Lindhe & Ericsson 1978, Hugoson & Schmidt 1978). Ligatures, in particular placed coronally to the gingival margin, often produce considerable recession, leading to extensive loss of attachment but extremely shallow pockets (Lindhe & Ericsson 1978). The fact that the cotton ligatures were situated subgingivally in this experiment, immediately after placement as well as at the end of the active phase of creating the defects, probably explains why relatively deep pockets were obtained in a period of 14 weeks.

At baseline standard deviations were approximately 0.5 millimeters thus demonstrating very little variation between animals. At the end of the active phase standard deviations varied from 0.8 to 1.6 millimeters. This means that the degree of variation was high at this stage. The results of 3 dogs with exceptional reactions did, however, contribute to these high values. In 1 dog it was impossible to create defects of any considerable depth because of the gingival recession, and in 2 dogs very deep defects of 7 and 8 millimeters were measured on only one side whilst the contralateral side reacted "normally".

In spite of these individual variations, no statistically significant differences ($P <$

0.05) were found in any area between left and right, which provides some support for predictability at this stage (Table 1).

During the healing phase predictability was estimated through the values of the standard deviations, since the present experimental design did not allow for left-right comparison during this phase. Following ligature removal standard deviations of mean probing depths in separate areas decreased gradually to baseline levels or even lower, indicating that aberrant high values regressed to the mean.

The observation that, following ligature removal, mean probing depths decreased suggests a gain in periodontal attachment, but can also be explained by probing errors due to differences in the inflammatory state of the tissues (Armitage et al. 1977). Recently Jansen et al. (1980) in evaluating probing errors in experimental periodontal defects in beagle dogs, demonstrated that in defects at the end of the creation phase, the probe went past the apical termination of the junctional epithelium; in the healing phase the probe tip on average indicated the connective tissue attachment level correctly. This would suggest that probing depth measurements obtained at different time points during the present experiment should be interpreted with care.

The ultimate values for mean probing depths varied from 3.0 to 3.5 millimeters after 30 weeks of healing (Table 1). Lindhe et al. (1975) demonstrated in beagle dogs that accumulation of plaque for a period of 48 months resulted in pockets with the same mean probing depth (3.4 millimeters). However, they reported that the magnitude of the standard deviation of the mean showed that "the rate of periodontal tissue breakdown varies considerably from one dog to the other and from one experimental period to the next". Their standard deviation for probing depth after 48 months of plaque accumulation was comparable to our standard deviations in the present study at the end of the active phase but was much higher than the standard deviations after 14 and 30 weeks of healing.

Hull, Soames & Davies (1974), investigating naturally occurring periodontal disease in a beagle dog colony aged between 1 and 8 years, found that "the amount of bone loss in beagle dogs increased with age, but to a degree which varied considerably, despite the fact that all dogs were maintained under identical conditions and were fed identical diets throughout their lives". Recently Sorensen et al. (1980) reported on naturally occurring periodontal disease in a cross sectional study on 74 beagle dogs from 1 to 12 years of age. They found only slight to moderate increase in pocket depth with age but even in the oldest age group mean pocket depth did not exceed 3.5 millimeters. This increase in probing depth was not found in all areas. These studies indicate that the accumulation of plaque alone without intervention of

e.g. ligature placement is neither a quick nor a reliable method of obtaining pockets of a predictable depth in dogs. The experimental periodontal defects in the present study were, after a period of healing, at least as deep as plaque induced periodontal defects after 4 years but showed considerably less variation in depth.

From the results of the present experiment in young adult beagle dogs it can be concluded that predictable periodontal defects, 3-3.5 millimeters in depth, can be produced provided that the initial artificially created defects are allowed to heal for at least 3 months.

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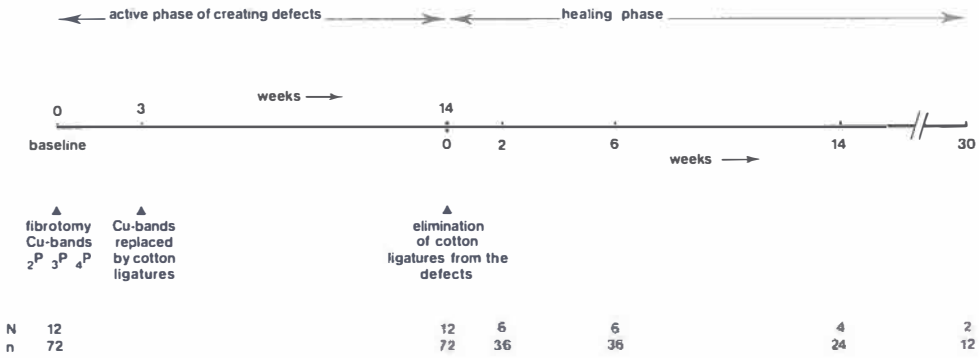

Fig. 1. Design of the experiment for the lower right premolars. N = number of dogs available for probing depth registration at different stages, n represents the total number of defects. Around contralateral teeth of 10 dogs defects were created but no healing phase was included (see text).

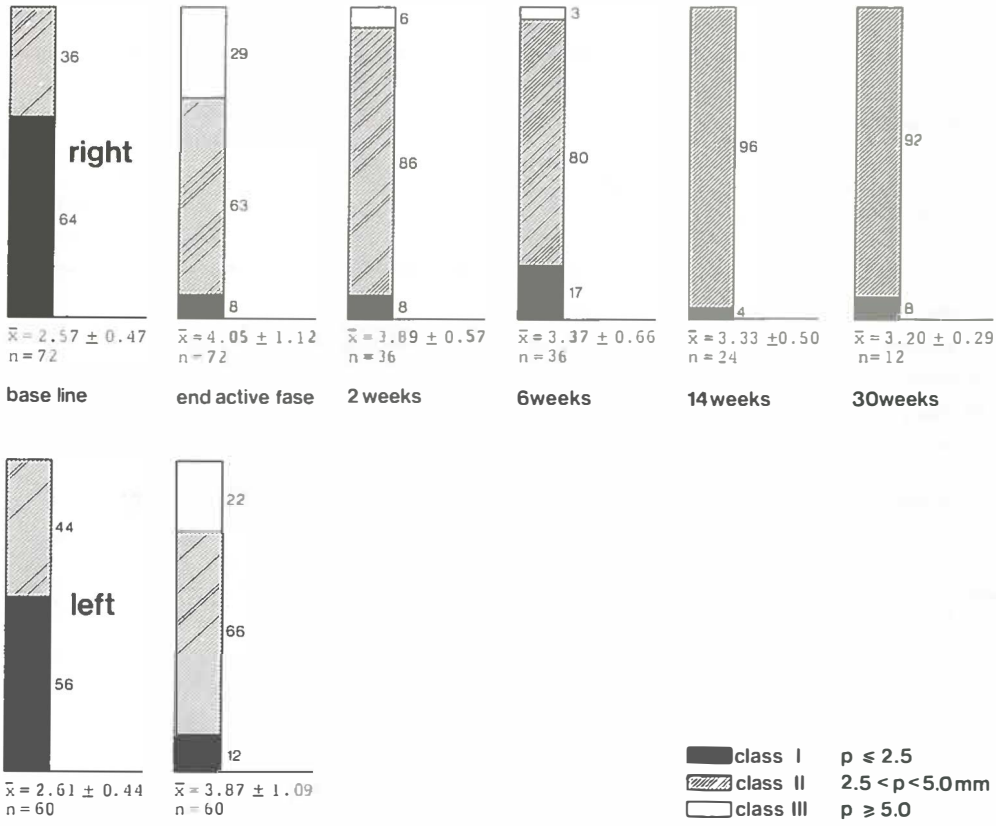

Fig. 2. Frequency distribution of classes of probing depths at different stages of the experiment for defects in the lower right premolar region (upper bars) and in the lower left premolar region (lower bars). Mean probing depths ($\bar{x}$) and standard deviations are presented at the bottom of each bar for all defects (n) available for registration in that phase of the experiment.

Table 1

Mean probing depths($\bar{x}$) for separate areas and standard deviations (s.d.) of the defects at different stages of the experiment.

right side of the jaw							
phase of the experiment	number of dogs	$_4P$ dist.	$_4P$ mes.	$_3P$ dist.	$_3P$ mes.	$_2P$ dist.	$_2P$ mes.
		$\bar{x}$±s.d.	$\bar{x}$±s.d.	$\bar{x}$±s.d.	$\bar{x}$±s.d.	$\bar{x}$±s.d.	$\bar{x}$±s.d.
baseline	12	2.92±0.51	2.50±0.64	2.45±0.50	2.50±0.37	2.50±0.37	2.50±0.37
end active phase	12	4.75±0.92	3.66±0.86	3.92±1.60	4.04±1.27	3.62±1.00	4.33±1.11
2 weeks of healing	6	4.25±0.42	3.50±0.43	3.25±0.41	3.00±0.63	2.83±0.75	3.58±0.80
6 weeks of healing	6	3.66±0.40	3.33±0.60	3.50±0.70	3.25±0.41	3.00±0.70	3.50±1.18
14 weeks of healing	4	3.37±0.48	3.12±0.25	3.62±0.47	3.37±0.48	3.25±0.86	3.25±0.50
30 weeks of healing	2	3.25±0.35	3.00±0.00	3.50±0.00	3.25±0.35	3.25±1.06	3.00±0.00

left side of the jaw		P_4 dist.	P_4 mes.	P_3 dist.	P_3 mes.	P_2 dist.	P_2 mes.
base line	10	2.85±0.47	2.80±0.42	2.50±0.47	2.70±0.48	2.40±0.46	2.55±0.28
end active phase	10	4.45±0.79	4.20±1.13	3.55±0.98	3.65±0.85	3.45±0.85	3.95±1.01

Chapter 4

HISTOMETRICAL ANALYSIS OF LIGATURE-INDUCED PERIODONTAL DEFECTS IN BEAGLE DOGS

Longitudinal evaluation following ligature removal

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Abstract. The aim of the present study was to investigate the sequence of events that takes place in ligature-induced periodontal defects following removal of the ligature.

In 8 beagle dogs the dentogingival fibres around 6 lower premolars ($_2P_2$, $_3P_3$, $_4P_4$) were cut to the level of the alveolar bone. Closely adapted copper bands were then cemented around the teeth. After 3 weeks the copper bands were replaced by cotton floss ligatures which were left in place for 11 weeks.

The dogs were sacrificed on different dates so that it was possible to analyse defects 3, 7, 15 or 31 weeks after ligature removal. Contralateral defects one week after ligature removal served as controls. Micrographic colour slides of the histological sections were analyzed using a Ferranti-Cetec digitizer.

Mean values for loss of attachment in the 15 and 31 weeks specimens were greater than in the control defects, suggesting that apical positioning of the junctional epithelium was irreversible. However, alveolar bone height tended to increase with experimental time, indicating bone repair. In the 15 and 31 weeks specimens junctional epithelium was occasionally found between the root surface and the alveolar bone.

It was concluded that the model does not show all the histological characteristics of naturally occurring periodontitis even in those dogs examined 31 weeks after removal of ligatures.

Introduction

Artificial periodontal defects in animals are widely used in periodontal research. The use of plaque retaining ligatures or bands has been shown to induce periodontal breakdown effectively (Kennedy & Polson 1973, Caton & Zander 1975, Caton & Kowalski 1976, Schroeder & Lindhe 1975, Lindhe & Ericsson 1978, Schroeder &

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Lindhe 1980). Additional procedures obviously enhance the process of degradation of periodontal tissues e.g. surgical removal of alveolar bone (Lindhe & Svanberg 1974, Ericsson et al. 1975) or dissection of dentogingival fibres (Van Dijk 1979). Frequent renewal of ligatures has also been reported to increase the amount of periodontal breakdown (Lindhe & Ericsson 1978, Nyman et al. 1980).

When the influence of co-factors or systemic disorders on the progression of periodontal disease is to be tested, a model in which the disease promoting ligatures are left in place during the entire experimental period has been advocated (Kennedy & Polson 1973, Lindhe & Svanberg 1974, Ericsson et al. 1975).

When therapeutic or preventive procedures are tested, the ligatures or bands are often removed from the periodontal defects a certain period of time before the start of the actual experiment (Hugoson & Schmidt 1978, Caton & Zander 1976, 1979, Van Dijk 1979, Caton & Nyman 1980, Nyman et al. 1980). The reason to include such a "resting period" is that the periodontal tissues are given time to recover from the presence of the irritating band or ligature and change from an acute defect (Lindhe & Ericsson 1978, Schroeder & Lindhe 1980) into a more chronic lesion with the characteristics of naturally occurring periodontitis. The loss of connective tissue attachment and the alveolar bone loss created in the experimental lesion should not be spontaneously reversible. The monkey model as described by Caton & Zander (1975) and Caton & Kowalski (1976) appears to meet these requirements.

Conflicting results have, however, been reported in the dog model. Reversibility of artificial periodontal defects upon ligature removal was reported by Linghorne & O'Connell (1955), Hugoson & Schmidt (1978) and Jansen et al. (1979), while Lindhe & Ericsson (1978) reported that a resting non-reversible lesion resulted after ligature removal. Johansen, Nilvéus & Egelberg (1978) reported both reversibility and progression of their furcation defects.

These conflicting results indicate the need for further research into the dog model. The present longitudinal study in beagle dogs was therefore undertaken to investigate the sequence of events that take place in ligature-induced periodontal defects following ligature removal, using histometrical analysis.

Material and Methods

Eight beagle dogs 1 to 1.5 years of age were used. Throughout the experiment the dogs were fed on a soft diet which favoured plaque accumulation. No oral hygiene procedures were performed, and no attempt was made to bring the gingivae, which were mildly inflamed at the start of the experiment, into a healthy condition.

Periodontal defects were created as described earlier (Van Dijk 1979, Jansen & Pilot 1981). The dentogingival fibres around 6 lower premolars ($_2P_2$, $_3P_3$, $_4P_4$) were cut to the level of the alveolar bone. Closely adapted copper bands were then cemented around the teeth to the full depth of the defects. After 3 weeks the copper bands were replaced by cotton floss ligatures which were tied around the toothnecks and forced down subgingivally as far as possible. These ligatures were left in place for 11 weeks. Upon removal of the ligatures a notch was made on the mesial and distal root surfaces to indicate the apical extent of the soft tissue defect. A small excavator was placed in the defect using light hand pressure until definite resistance was felt. The notch was then made in the root surface with one bucco-lingual stroke.

Pairs of dogs were scheduled for sacrifice 3, 7, 15 or 31 weeks after removal of the ligature on the right side of the jaw. Contralateral defects were created 15 weeks before the sacrifice date. One week before sacrifice the ligatures were removed on this side and a notch prepared. These defects on the left side of the jaw, examined one week after ligature removal, served as controls.

The dogs were sacrificed and prepared for histological analysis using the method described by Van Dijk (1979). At the end of the experimental period, the animals were anaesthesized with sodium pentobarbital and given an intravenous injection of heparin to prevent blood clotting. The thorax was opened, and a canula was placed in the left atrium. The vascular system was then perfused with saline followed by a fixative, consisting of 90 grams of sodium chloride and 300 milliliters of glacial acetic acid in 10 liters of 10% formalin.

Segments of the jaws involved in the experiment were removed, sectioned into smaller blocks, fixed in a 10% buffered formalin solution for 2 weeks, washed with tap water and decalcified in a solution of 25% formic acid and 10% sodium citrate in water. After embedding in paraffin, 8 μm thick sections were cut and stained with haematoxylin and eosin according to Delafield.

Three sections 40 μm apart were selected from the midsagittal region of each interproximal block. Micrographic colour slides were made and projected with a total magnification of 70 on a Ferranti-Cetec digitizer table connected to a Digital P.D.P.-11/0.5. computer.

The following points were digitized (Figure 1): Cemento-enamel junction (CEJ), the apical termination of the junctional epithelium (ATJE), the apical extension of the notch and the most coronal extension of the alveolar bone opposing the root surface at a distance similar to that of the normal width of the periodontal ligament.

These points were then projected on a tangent along the root surface which parallels the vertical tooth axis. The distance between the projected points were then computed. The mean of the three sections was taken for further analysis. In this way the following distances were calculated: CEJ to ATJE (loss of attachment), alveolar bone to CEJ, bone to ATJE (width of the zone of connective tissue) and notch to bone.

Approximately 25% of all measurements were repeated 2 weeks after the first assessment. Of the 75 repeat scores, 40 were in full agreement, 24 differed 0.1 millimeter and 11 differed by 0.2 millimeter. This means that 85% of the registrations were reproducible within an accuracy of 0.1 millimeter.

To be able to relate the experimental recordings to the situation in unaffected beagle dogs of the same age, 2 additional animals were sacrificed and the periodontium of their lower right premolars was analyzed as described above. This situation will be referred to as baseline.

Seventy-seven out of the 96 possible defects were available for analysis, 40 experimental and 37 control defects. Nineteen specimens were eliminated because these were either incorrectly sectioned or the CEJ could not be detected.

The number of data at each time point was limited. In every evaluation phase only 2 dogs with at most 6 defects on each side of the jaw were available. Furthermore, these defects cannot be considered strictly independent, mandatory for statistical analysis (Pilot et al. 1980). Therefore results are presented as mean values per side of the jaw for each dog separately, and no effort was made to subject the results to statistical analysis.

Results

The measurements of the baseline sections revealed no loss of attachment. The mean distance from the ATJE to the alveolar bone level was 0.64 millimeter (S.D. = 0.21). The results of the measurements on the control and experimental sides of dogs number 1-8 are presented in Table 1. Loss of attachment in control defects was approximately 2 millimeters, the alveolar bone level was situated more than 3 millimeters apical to the CEJ. As a result, the width of the connective tissue (distance ATJE-bone) was approximately 1.2 millimeters. In all but one dog (no. 1) the mean values for loss of attachment at the experimental sides were greater than those of the controls. Mean loss of attachment seemed to increase with increasing experimental periods.

In the 15 and 31 weeks specimens the mean values for bone loss (distance "CEJ-bone") were smaller on the experimental side than on the control side. The

notch in the root surface (made immediately after removal of the ligatures from the defects) could be identified in 65 of the 77 defects (29 control and 36 experimental). Twenty-seven of the 29 notches were situated coronal to the alveolar bone level in the control (Fig. 2a). The alveolar bone in the experimental defects was on average still situated apical to the notches in dogs 1 and 2 (experimental period 3 weeks), but in the remaining 6 dogs the alveolar bone was situated coronal to the notch (Fig. 2b). From the mean values per dog it appeared that alveolar bone height tended to increase with experimental time (Table 1, distance "notch-bone"). As a result the width of connective tissue (Table 1, distance "ATJE-bone") became smaller during the experimental phase and was eventually less than half that found in the baseline sections. In the 15 and 31 weeks specimens junctional epithelium was occasionally found between the root surface and alveolar bone.

Discussion

Although gingivitis was present in all dogs at the start of the experiment, baseline measurements revealed no loss of attachment, and the alveolar bone was situated about 0.6 millimeter apical to the apical termination of the junctional epithelium. Similar findings have been reported by Lindhe, Hamp & Löe (1975) and Lindhe & Ericsson (1978).

Mean loss of attachment on the experimental side of the jaw was greater than on the control side. The difference was most pronounced in defects 31 weeks after ligature removal. This result suggests that, after removal of the ligatures, loss of connective tissue attachment (apical positioning of the junctional epithelium) is not reversible but progressive. Progressive loss of attachment is a characteristic feature of naturally occurring periodontitis in man (Löe et al. 1978) and in dogs (Lindhe et al. 1975, Hamp & Lindberg 1977). Thus, when loss of attachment only is considered, the present dog model seems to meet the requirements for artificial periodontal defects.

Mean distance from CEJ - bone in experimental defects 15 and 31 weeks after ligature removal was smaller than that in the control side. This observation suggests repair of bone. The finding that in all but one control defect the alveolar bone was situated apical to the notch (Table 1, Fig. 2a) and that in the experimental defects the bone level became situated coronal to the notch in the majority of specimens (Table 1, Fig. 2b) further substantiates the hypothesis that bone repair occurs after ligature removal.

Alveolar bone repair can occur upon reduction of periodontal inflammation (Kantor, Polson & Zander 1976, Rosling, Nyman & Lindhe 1976, Rosling et al. 1976,

Polson & Heijl 1978). In an earlier publication (Jansen, Pilot & Corba 1981) it was reported that the histopathological characteristics of this type of defect changed from severe to moderate upon ligature removal in spite of the absence of oral hygiene procedures. This change in inflammatory state may well explain why bone repair occurred in the present material. Similar findings can be found in the literature. Hugoson and Schmidt (1978) reported bone repair in experimental bone defects in beagle dogs after ligature removal. The repair was most evident in teeth exposed to mechanical plaque control but could also be demonstrated in plaque--infected defects. Johansen et al. (1978) evaluated experimental furcation defects in beagle dogs 2 and 9 months after ligature removal. In 3 out of 5 dogs no change in bone loss was observed and in one dog bone repair was demonstrated in the 9 month period after ligature removal. In this dog the area of infiltrated connective tissue was smaller in the 9 month specimens than in the 2 month specimens. Recently, bone repair was demonstrated in experimental periodontal defects in the squirrel monkey after ligature removal and the institution of oral hygiene procedures (Kantor 1980).

Absence of bone repair upon ligature removal from experimental periodontal defects has, in contrast, been reported by Caton & Zander (1975) and Lindhe & Ericsson (1978). Caton & Zander (1975) evaluated elastic induced periodontal defects in Rhesus monkeys up to 380 days following ligature removal. They concluded that there was no evidence that either the apical migration of the junctional epithelium or the loss of alveolar bone was reversible. The overall histopathology of the "long-term elastic-off" specimens was that of active periodontitis and only in a minority of cases could a long junctional epithelium be seen. It appears that elastic induced defects in monkeys results in persistance of severely inflamed tissues which may prevent alveolar bone repair.

Lindhe and Ericsson (1978) evaluated the effect of ligature removal from experimental defects in beagle dogs and concluded that "in dogs where ligatures were removed but plaque accumulation continued for another 6 months an apparently resting, non-destructive lesion attained". No significant change in bone level was reported during this period. It may well be that the persistance of inflammation in a shallow pocket, as was the case in their material, prevented bone repair. Another explanation may be the size of the defect. In their material the zone of connective tissue found between the apical termination of the junctional epithelium and the alveolar bone at the time of ligature removal was much smaller than in the present material. It can be speculated that a wider zone of connective tissue preserves a space in which osteogenic cells are still present. These cells may, upon ligature removal and subsequent reduction of the inflammation, produce new alveolar bone in

the deeper part of the lesion.

The notch in the root surface was made to indicate the apical extent of the pocket epithelium (bottom of the defect) at the time of ligature removal. From Table 1 it can be calculated that the notch was made just above the alveolar bone level (distance notch-bone control defects) and about one millimeter apical to the apical termination of the junctional epithelium (distance ATJE-bone control defects). It can therefore be concluded that the notch does not accurately indicate the bottom of the pocket but rather the alveolar bone level. The severely inflamed tissues obviously did not act as a barrier to the notch instrument, although it was inserted with light hand pressure. This finding is of importance for the qualitative interpretations of the tissue reactions because it means that tissues coronal to the notch have not necessarily been exposed to the oral environment.

The 15 and 31 weeks specimens were characterized by bone repair and a narrow or sometimes non-existent zone of connective tissue. These are features that are frequently found as a result of periodontal therapy (Lindhe & Ericsson 1978, Caton & Zander 1976, Caton & Nyman 1980, Caton et al. 1980). If a model is used to evaluate therapeutic or preventive procedures, it should have all histopathological characteristics of naturally occurring periodontitis. The present dog model does not seem to meet this requirement in full.

Acknowledgments

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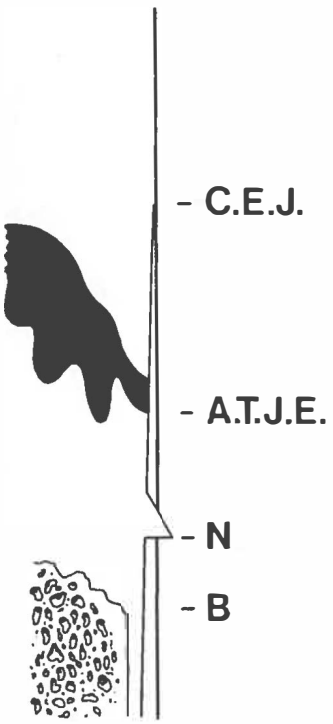

Fig. 1. Schematic representation of the points that were digitized.
ATJE: apical termination of the junctional epithelium.
CEJ : cemento-enamel junction.
N : apical extension of the notch.
B : most coronal extension of the alveolar bone opposing the root surface at a distance similar to that of the normal width of the periodontal ligament.

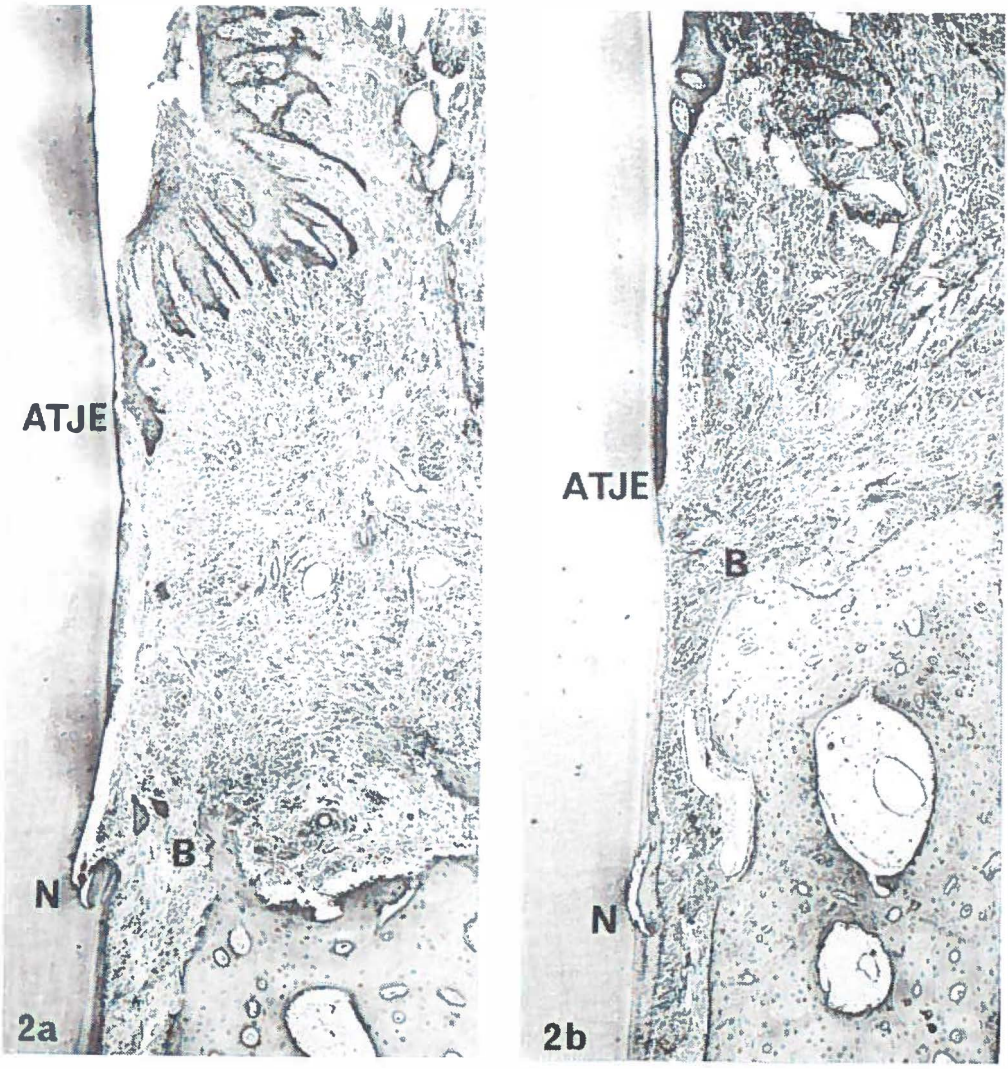

Fig. 2a. Control defect, one week after ligature removal.

Fig. 2b. Experimental defect, 15 weeks after ligature removal.
ATJE: apical termination of junctional epithelium.
B : most coronal level of alveolar bone opposite the root surface at a distance similar to that of the normal width of the periodontal ligament.
N : notch.
Original magnification X 40.

Table 1

Mean values and standard deviations in millimeters for the left (control) and the right (experimental) side of the jaw for each dog separately

dog nr.	weeks after ligature removal		loss of attachment (C.E.J.-A.T.J.E.)		bone level (C.E.J.-bone)		bone level (notch-bone)		width of connective tissue (A.T.J.E.-bone)	
	contr.	exp.	contr.	exp.	contr.	exp.	contr.	exp.	contr.	exp
1	1	3	2.10±0.35	1.91±0.37	3.35±0.36	3.01±0.42	-0.32±0.15	-0.08±0.42	1.25±0.17	1.10±0.58
2	1	3	1.77±0.49	2.00±0.49	2.95±0.51	3.32±0.44	-0.13±0.32	-0.10±0.37	1.17±0.09	1.32±0.16
3	1	7	1.76±0.45	2.15±0.10	3.10±0.26	3.15±0.20	-0.35±0.07	+0.20±0.20	1.33±0.23	1.00±0.14
4	1	7	1.80±0.18	2.25±0.65	3.05±0.32	2.91±0.49	+0.04±0.20	+0.66±0.47	1.25±0.18	0.66±0.18
5	1	15	2.90±0.31	3.33±0.75	4.54±0.51	3.76±0.61	-0.14±0.20	+1.10±0.51	1.64±0.32	0.44±0.45
6	1	15	1.85±0.15	2.16±0.23	2.90±0.23	2.54±0.36	-0.55±0.34	+0.52±0.61	1.05±0.13	0.38±0.24
7	1	31	1.96±0.43	2.42±0.12	3.16±0.31	2.75±0.05	-0.06±0.15	+0.67±0.51	1.20±0.14	0.32±0.17
8	1	31	2.15±0.55	2.82±0.61	3.67±0.57	3.00±0.24	-0.30±0.14	+1.00±0.96	1.52±0.40	0.17±0.41

Chapter 5

ARTIFICIAL PERIODONTAL DEFECTS AROUND INCISOR TEETH OF BEAGLE DOGS

A clinical and histometrical analysis

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Abstract. The aim of the present experiment was 1. to investigate if it is possible to create deep periodontal defects around incisor teeth of beagle dogs with the use of orthodontic elastic bands, 2. to investigate if this method is reproducible and predictable and 3. to evaluate if such defects possess the histopathological characteristics of naturally occurring periodontitis.

In 8 beagle dogs the dentogingival fibres around 4 upper incisors, $3\underline{1}3$ and $2\underline{1}2$, were cut to the level of the alveolar bone. Orthodontic elastic bands were placed in these deepened sulci and removed after 8 weeks. Clinical data (probing depth and gingival recession) were recorded at the start of the experiment, immediately following removal of the elastic bands, and 2, 6, 14 and 30 weeks thereafter. The dogs were sacrificed on different dates so that it was possible to analyse defects histometrically 3, 7, 15 or 31 weeks after elastic removal. Contralateral defects one week after elastic band removal served as controls. Micrographic colour slides of the histological sections were analysed using a Ferranti - Cetec digitizer.

Immediately after elastic band removal mean values for probing depth and gingival recession were approximately 5.5 millimeters and 2.0 millimeters respectively. Histometrical assessments revealed a loss of attachment of approximately 3.0 millimeters.

The differences in probing depths between contralateral defects *in the same dog* were small indicating rather high reproducibility of the method. Because of wide variations in probing depth and histometrical dimensions *between* dogs, it was concluded that the present method is not fully predictable.

Upon elastic removal values for probing depth decreased with approximately 1.5 millimeters, the result being a moderately deep defect of around 4 millimeters. Histometrically there was no evidence that the apical positioning of the junctional epithelium was reversible after elastic removal. However, alveolar bone repair could be demonstrated in the 7, 15 and 31 weeks specimens.

It was concluded that because of this bone repair the present defects do not possess all histopathological characteristics of naturally occurring periodontitis.

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Introduction

When in periodontal research a dog model is used, premolars are usually selected as experimental teeth. However, the anatomy in this area, such as the broad interdental alveolar bone septum and the absence of contacts between neighbouring teeth only allows for the institution of shallow or moderately deep pockets, due to recession of the interdental gingiva (Lindhe & Ericsson 1978, Jansen & Pilot 1981). In the beagle dog incisor teeth often have contact points, and the interdental periodontium is narrow. Furthermore these teeth are single-rooted, and the roots have a conical shape. These anatomical features would favour a rapid artificial periodontal breakdown with the formation of deep pockets provided that the marginal gingiva does not retract. Deep experimental pockets would be a valuable extension to the dog model, not only in evaluating preventive or therapeutic procedures but also e.g. to study microbiological aspects of subgingival plaque.

In monkeys, Caton & Zander (1975) very elegantly produced deep periodontal defects in incisor teeth with the use of orthodontic elastic bands. They concluded that the created loss of attachment and the alveolar bone loss was irreversible upon elastic band removal. Furthermore, Caton & Kowalski (1976) indicated that these defects were fully reproducible.

The aim of the present experiment was:

1. to investigate if it is possible to create deep periodontal defects around incisor teeth of beagle dogs with the use of orthodontic elastic bands,
2. to investigate if this method is reproducible and predictable,
3. to evaluate if such defects posses the histopathological characteristics of naturally occurring periodontitis.

Material and Methods

Eight beagle dogs, approximately 18 months old and weighing $\pm$ 15 kg. were used. Throughout the experiment the dogs were fed on a diet which favours gross plaque accumulation on the teeth, and no oral hygiene procedures were performed.

Before the start of the experiment small amalgam fillings were made at the level of the gingival margin to serve as reference points for gingival recession. Periodontal defects were created using a modification of the method described by Caton & Zander (1975). The dentogingival fibres around upper incisors $^{3}I^{3}$ and $^{2}I^{2}$ were cut to the level of the alveolar bone. Orthodontic elastic bands (Unitek, Englewood Cliffs N.J. 1/8 inch) were placed in these deepened sulci below the gingival margin and removed after 8 weeks. Upon removal of the elastic bands a notch was made with the use of a small excavator on the mesial and distal root surfaces to indicate

the apical extent of the soft tissue defects. The instrument was placed into the defects with light hand pressure until definite resistance was felt. The notch was then made in the root surface with one bucco-lingual stroke.

Histological design:

Pairs of dogs were sacrificed 3, 7, 15 or 31 weeks after elastic band removal from the right side of the jaw (Fig. 1). Contralateral defects were created 9 weeks before sacrifice was planned. One week before sacrifice the elastic bands were removed from this side and notches prepared. These defects on the left side of the jaw, one week after ligature removal, served as controls. The dogs were sacrificed and histometrically analysed as described in detail by Jansen, Van Dijk & Pilot (1981).

Three sections 40 μm apart were selected from the midsagittal region of each interproximal block. Micrographic colour slides were made and projected with a total magnification of 70 on a Ferranti-Cetec digitizer table connected to a Digital P.D.P.-11/0.5 computer.

The following distances were calculated for each section:

- the distance from the cemento-enamel junction to the apical termination of the junctional epithelium (CEJ-ATJE),
- the distance from the cemento-enamel junction to the alveolar bone level (CEJ-bone),
- the distance from the notch to the alveolar bone level (notch-bone),
- the distance from the ATJE to the alveolar bone level (ATJE-bone).

The mean of the 3 sections was taken for further analysis.

Two additional beagle dogs of the same age in which no periodontal defects had been created, were sacrificed, and the dimensions of the periodontium of the upper incisors were analysed as described above (baseline situation).

Clinical design:

Clinical recordings were made before the start of the experiment (baseline) immediately after removal of the elastic bands and 2, 6, 14 or 30 weeks thereafter (Fig. 1). The reason for collecting the final clinical data one week prior to sacrifice was that it was expected that probing immediately prior to sacrifice would disturb the histological picture. Only mesial and distal surfaces of the experimental teeth were investigated.

Probing depth was assessed with the use of a University of Michigan O probe with a terminal diameter 0.35 millimeter which was inserted with light hand pres-

sure. Recession of the clinical margin was measured using the same probe. The level of the gingival margin was related to the apical border of the amalgam filling which indicated the original position of the gingiva. All clinical measurements were made to the nearest half millimeter.

The clinical data were subjected to student's t test to analyse differences between mean values at different intervals using the dog as a unit.

The number of histometrical data at each time point was limited. In every evaluation phase only 2 dogs with at most 3 defects on each side of the jaw were available. Furthermore these defects connot be considered strictly independent, mandatory for statistical analysis (Pilot et al. 1980). Therefore, results are presented as mean values per side of the jaw for each pair of dogs, and no effort was made to subject the histometrical results to statistical analysis.

Results

In this experiment it was impossible to create periodontal breakdown at the distal aspect of $^{3}I^{3}$. Data from this area were not included in the present material in order not to disguise the effect of elastic band placement at the mesial aspect of $^{3}I^{3}$ and the distal and mesial aspect of $^{2}I^{2}$.

Clinical results: The elastic bands were situated deep subgingivally, both immediately after placement and after 8 weeks (Fig. 2). The clinical aspect of a defect at the end of the active phase is shown in Figure 3. Data for probing depths and gingival recession are presented in Table 1. Probing depth at baseline varied from 2.0 to 3.0 millimeters. At the end of the active phase of creating defects, after 8 weeks, the probing depths most commonly recorded were 5.0, 5.5, 6.0 and 7.0 millimeters. Without exception the deepest defects were found at the mesial aspect of $^{3}I^{3}$ ($\bar{x}$ = 6.21 mm SD = 0.96 mm) and the shallowest at the distal aspect of $^{2}I^{2}$ ($\bar{x}$ = 4.71 mm SD = 0.85 mm). Mean probing depth at the mesial aspect of $^{2}I^{2}$ was $\bar{x}$ = 5.59 millimeters SD = 0.63 millimeter.

Differences between dimensions in contralateral defects *in one dog* at the end of the active phase were small. Out of 24 defects, 9 contralataral defects differed 0.0 millimeter or 0.5 millimeter in probing depth, 12 contralateral defects differed 1.0 or 1.5 millimeters, and only 3 contralateral defects differed 2.0 millimeters. *Between* dogs the variation in clinical data in the same area was much greater. Probing depth values at the mesial aspect of $^{3}I^{3}$ ranged from 4.5 - 8.0 millimeters, at the distal aspect of $^{2}I^{2}$ from 3.0 - 5.5 millimeters and at the mesial aspect of $^{2}I^{2}$ from 4.5 - 7.0 millimeters.

Two weeks after elastic band removal mean values for probing depths had decreased by approximately 1.5 millimeters. Mean values for probing depth in the experimental phase remained greater than at baseline ($p < 0.05$). Six weeks after elastic band removal a decrease in gingival recession was noted, indicating a crownwise shift of the gingival margin; however, the differences at different intervals were not significant.

Histometrical results: Measurements of the baseline sections in the 2 additional dogs revealed negligable loss of attachment ($\bar{x}$ = 0.08 mm SD = 0.13 mm). The mean distance from the ATJE to the alveolar bone level was 1.28 millimeters (SD = 0.40 mm).

The results of the measurements in the control and experimental sides are presented in Table 2. Mean loss of attachment on the control sides was slightly over 3 millimeters. Mean values for loss of attachment on the experimental sides were at all times greater than those of the controls.

The mean distance from the CEJ to the alveolar bone on the experimental side was at all intervals smaller than on the control side. This difference was most pronounced in the 31 week specimens. The alveolar bone in the control specimens was situated apical to the notch in the root surface, but in the experimental specimens the alveolar bone was situated coronal to the notch, except in dogs 1 and 2 (3 weeks after elastic band removal). From the mean values per pair of dogs it appeared that alveolar bone height tended to increase with experimental time (Table 2, distance notch-bone). As a result the distance from the ATJE to the bone became gradually smaller and was in the 31 week specimens half that found in the baseline sections.

Discussion

The present experiment has demonstrated that it is possible to create deep periodontal defects at the mesial aspect of $^{2}_{I}{}^{2}$ and $^{3}_{I}{}^{3}$ and at the distal aspect of $^{2}_{I}{}^{2}$. These defects were characterized by probing depths of approximately 5.5 millimeters and a gingival recession of approximately 2.0 millimeters.

Differences in probing depth between contralateral defects in the same dog were small, indicating that the method in itself is rather *reproducible*. However, there was much variation in probing depths in the same area between dogs. This finding indicates that the method does not *predictably* result in defects of equal size in a group of dogs.

In the first weeks after elastic band removal probing depth values decreased

sharply and from 7 weeks on alveolar bone repair could be observed. Both, reduction of probing depth and alveolar bone repair after creation of an artificial periodontal defect in the dog have been reported earlier (Jansen & Pilot 1981, Jansen et al. 1981, Van Dijk 1979).

The decrease in *probing depth* values does not reflect a true reduction in *pocket depth:* the loss of connective tissue attachment did not seem to be reversible (Table 2, distance CEJ-ATJE), and the gingival margin did not further retract. The decrease in probing depth may, however, be explained by different probing conditions as a result of difference in inflammation before and some time after elastic band removal. A less severely inflamed lesion would then result in a lower probing depth value (Armitage, Svanberg & Löe 1977, Robinson & Vitek 1979, Jansen, Pilot & Corba 1981). Indeed, most of the long term experimental specimens in the present study revealed a long junctional epithelium together with less severe inflammation in the deeper part of the lesion than was found in the control specimens (Figure 4 a, b). This may also explain the alveolar bone repair because it has been reported that reduction of periodontal inflammation stimulates bone repair (Kantor, Polson & Zander 1976, Rosling, Nyman & Lindhe 1976, Kantor 1980).

In contrast to the present dog model it was reported for the rhesus monkey that after elastic band removal probing depths did not decrease and the alveolar bone loss was irreversible (Caton & Zander 1975). At least part of this discrepancy may be explained by the fact that in contrast to the dog most of the long term "elastic-off" specimens in the rhesus monkeys still showed the histopathological characteristics of active periodontitis. The question why the periodontal tissues in dogs obviously react differently to elastic band removal than in monkeys remains obscure but difference in species and anatomical dimensions can not be excluded.

In spite of qualitative similarities, the *amount* of periodontal breakdown in the present defects around incisors was substantially greater than that found in ligature-induced defects in the premolar region in beagle dogs (Jansen & Pilot 1981, Jansen et al. 1981). The difference may be explained by differences in technique and different anatomical dimensions.

In contrast to premolars, roots of incisor teeth converge in an apical direction. It can be expected that in this situation an elastic band under tension easily moves in an apical direction. Furthermore, the amount of periodontal tissue to be broken down around premolar teeth, including the bifurcation area, is substantially greater than that around incisor teeth with a very thin interseptal bone crest. The broad interdental bone crest between the third incisor and the cuspid may explain why it was impossible to create defects at the distal aspect of $\frac{3}{1}\frac{3}{3}$.

Another important difference is the width of connective tissue attachment in the normal unaffected situation which is twice as wide in the incisor region than in the premolar region as reported earlier (Jansen et al. 1981). Since this attachment was severed at the start of the experiment the initial defect was already much deeper in the incisor region.

The application of the present dog model in periodontal research should be advocated with great care. Reduction of severity of periodontal inflammation and alveolar bone repair are often objectives of periodontal therapy. Since both phenomena seem to occurr in the present defects, it will be difficult, if at all possible, to demonstrate these effects of periodontal therapy.

Acknowledgments

The author would like to express his gratitude to the staff of the Central Experimental Animal Laboratory of the University of Groningen for their skillfull assistance during the actual experiment and to the staff of the Laboratory of Oral Histology of the University of Groningen, Head Dr. H.W.B. Jansen, for the preparation of the sections.

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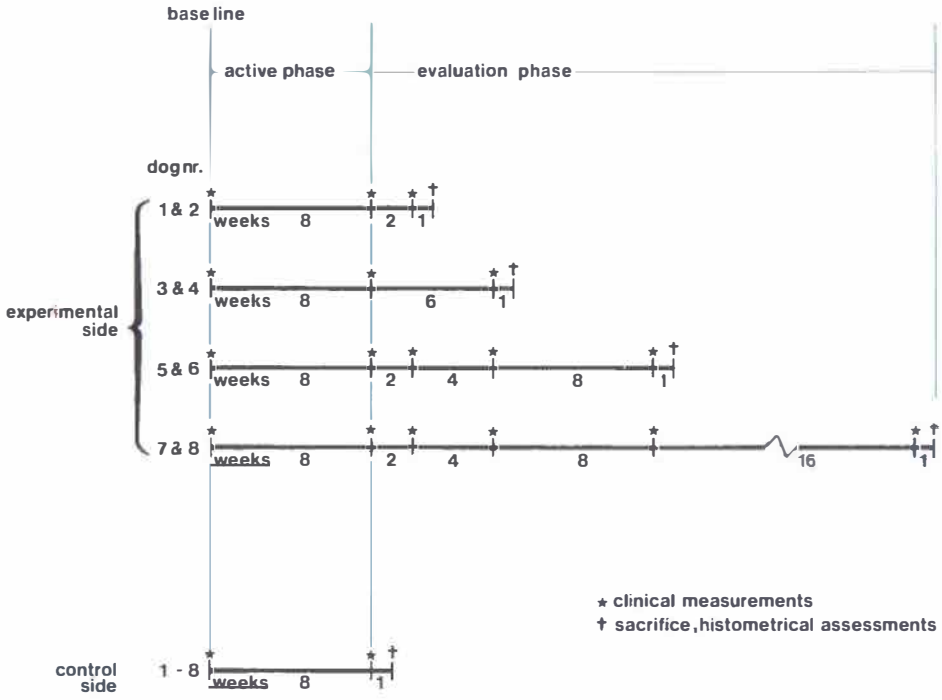
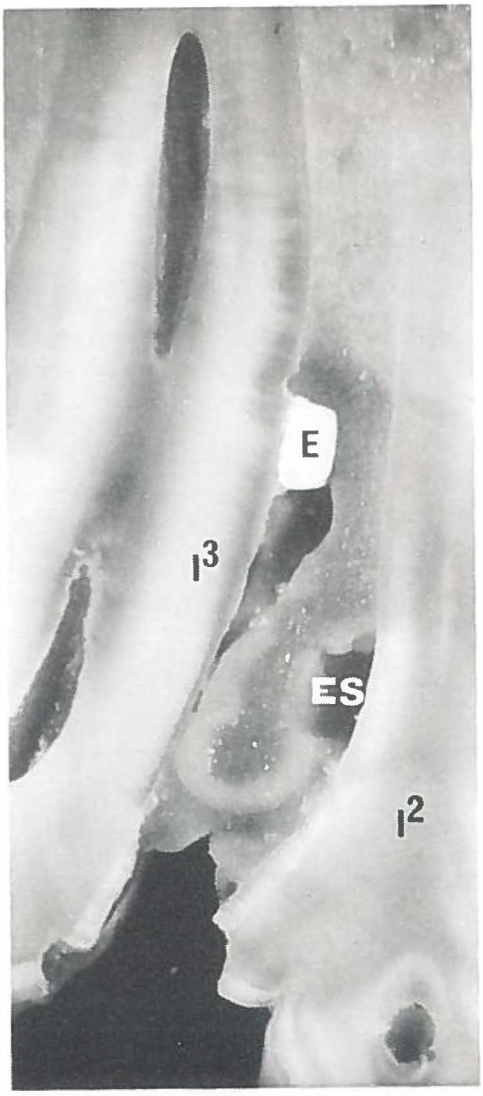


Fig. 1. Schematic representation of the design of the experiment.



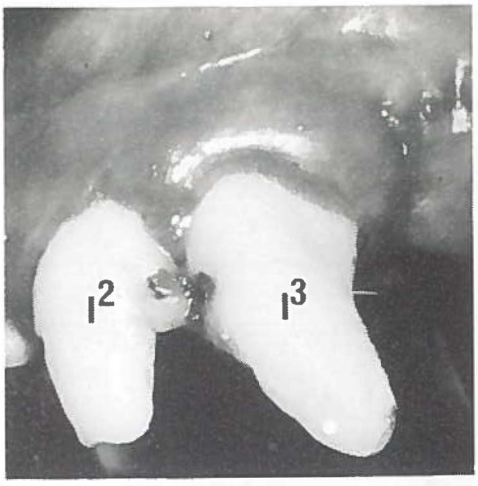


Fig. 3. Clinical aspect of an elastic-induced defect at the end of the active phase.

Fig. 2. Specimen (before decalcification) of an interdental area between I^2 and I^3 at the end of the active phase of creating defects. The interdental area does not provide enough space for 2 elastic bands to be situated next to each other. Since the elastics were first placed around $^3I^3$ and subsequently around $^2I^2$ the elastics at the mesial aspect of $^3I^3$ were always situated more apical than at the distal aspect of $^2I^2$. This explains why deeper defects were created at the mesial aspect of $^3I^3$. The dog from which the above section was taken was not included in the present experiment. E = elastic band. ES = space formarly occupied by an elastic band.

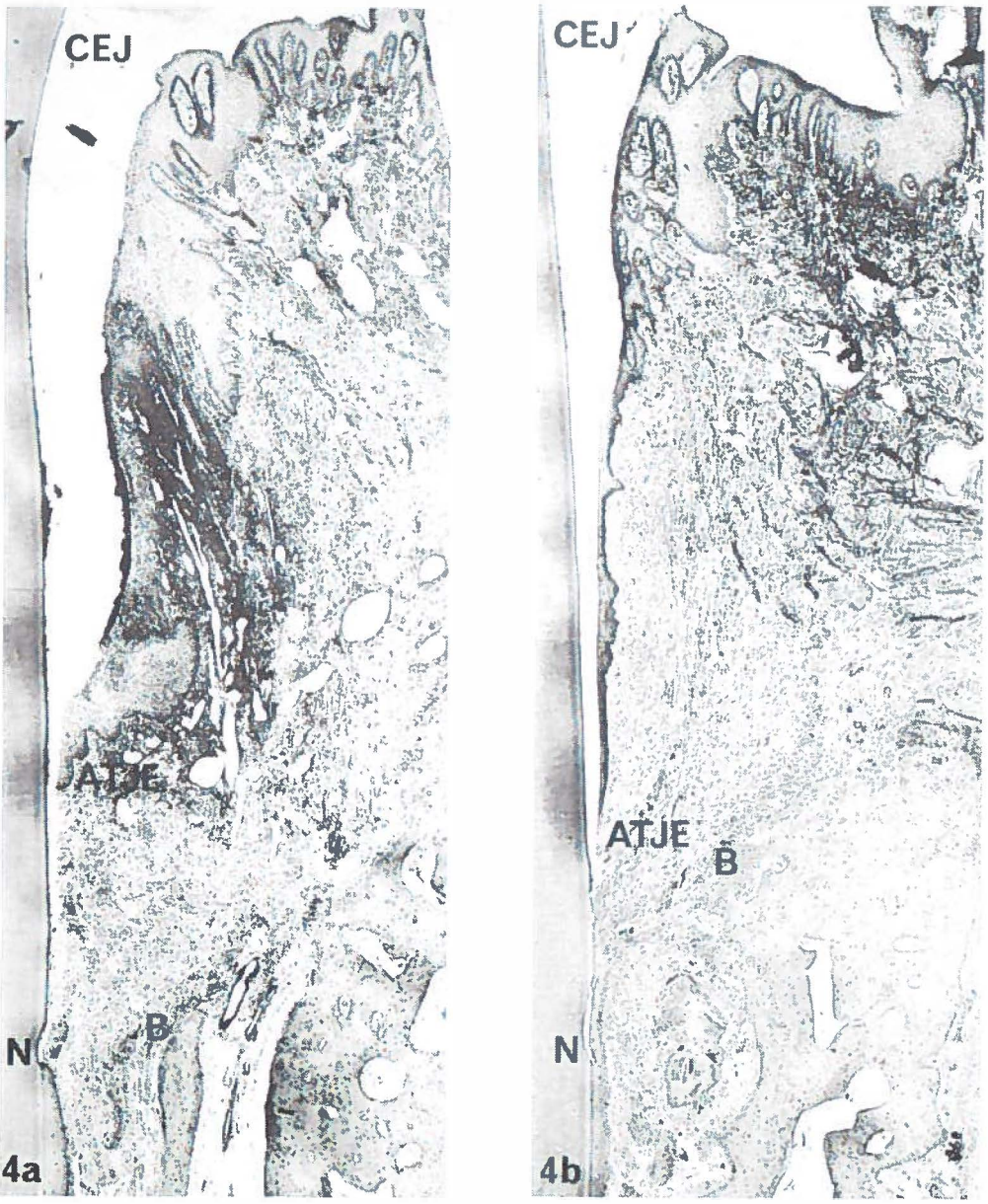


Fig. 4a. Control defect, 1 week after elastic band removal. Mesial aspect of I^2.

Fig. 4b. Experimental defect, 7 weeks after elastic band removal. Mesial aspect of ^2I. CEJ = cemento-enamel junction; ATJE = apical termination of junctional epithelium; B = most coronal level of alveolar bone opposite the root surface at a distance similar to that of the normal width of the periodontal ligament; N = notch. Original magnification X 40.

Table 1

Clinical results: individual recordings for probing depth and mean values ($\bar{x}$) and standard deviations (s.d.) for probing depths and gingival recessions

		Baseline		End active phase		Experimental phase (weeks after elastic removal)			
	mm	contr. left	exp. right	contr. left	exp. right	exp. 2	exp. 6	exp. 14	exp. 30
individual recordings for probing depths	1								
	2	○○○○○○	○○○○○○○						
		○○○○○○	○○○○○○○				○		
	3	○○○○○○○ ○○○○○○	○○○○○○○ ○○○		○○	○○○○	○○○○○	○○○○○	○
			○			○○○○	○○○○○	○	○○○
	4			○○	○	○○○○○○	○	○○○○	○
				○	○	○	○○	○○	
	5			○○○○○○ ○○	○○○○	○○	○○○○		○
				○○○○○	○○○○○○	○			
	6			○	○○○○○○ ○○○				
					○				
	7			○○○○○					
				○					
	8			○					
probing depth	$\bar{x}$	2.62	2.58	5.68	5.33	3.88	3.77	3.62	3.75
	s.d.	0.42	0.45	1.11	0.91	0.73	0.84	0.60	0.68
gingival recession	$\bar{x}$	—	—	2.03	2.33	2.41	2.02	2.24	1.91
	s.d.	—	—	1.12	1.26	0.77	0.66	1.01	0.93

The differences between mean probing depths at baseline and at the end of the active phase were significant ($p < 0.05$). The differences between mean probing depths at the end of the active phase and during all stages of the experimental phase were also significant ($p < 0.05$). Differences between mean probing depths during all stages of the experimental phase and baseline recordings remained significant ($p < 0.05$).

Table 2

Histometrical results: mean values and standard deviations in millimeters for the left (control) and the right (experimental) side of the jaw for each pair of dogs

dog nr.	weeks after elastic removal		loss of attachment (C.E.J.-A.T.J.E.)		bone level (C.E.J.-bone)		bone level (notch-bone)		width of connective tissue (A.T.J.E.-bone)	
	contr.	exp.	contr.	exp.	contr.	exp.	contr.	exp.	contr.	exp.
1&2	1	3	3.26±0.92	3.48±0.72	5.41±0.87	4.96±1.03	-0.80±0.46	-0.87±0.46	2.14±0.91	1.48±0.35
3&4	1	7	3.08±1.09	3.30±0.76	5.01±0.49	4.61±0.60	-0.61±0.27	+0.20±0.36	1.93±0.97	1.31±0.22
5&6	1	15	3.04±0.62	3.44±0.55	4.93±0.61	4.83±0.76	-0.34±0.36	+0.40±0.70	1.88±0.43	1.35±1.01
7&8	1	31	3.20±0.67	3.28±0.44	5.02±0.40	4.13±0.71	-0.62±0.45	+0.93±0.30	1.82±0.69	0.84±0.52

Chapter 6

HISTOPATHOLOGY OF ARTIFICIAL PERIODONTAL DEFECTS IN BEAGLE DOGS BEFORE AND AFTER LIGATURE REMOVAL

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Abstract. The aim of the present study was to investigate the histopathological characteristics of artificial periodontal defects before and after removal of ligatures. The defects were created in 12 young adult beagle dogs using cotton floss ligatures. The supracrestal dentogingival fibres were dissected around the 6 lower premolars ($_2P_2$, $_3P_3$ and $_4P_4$) after which closely adapted copper bands were cemented to the teeth. After 3 weeks these copper bands were replaced by subgingival cotton floss ligatures which were left in place for a further 11 weeks. The placement of ligatures around the different teeth and their removal prior to sacrifice was scheduled so, that it was possible to make histological observations of the periodontal defects both with ligatures in situ, and 1 day and 1, 3, 7, 15 or 31 weeks after their removal.

Defects with ligatures in situ showed histopathological characteristics of advanced chronic periodontitis. Removal of the ligatures and the subsequent production of a notch (to mark the bottom of the pocket) resulted initially in an exacerbation of the inflammatory process but 3 weeks after ligature removal, a distinct decrease in size of the infiltrated connective tissue (I.C.T.) could be observed especially in the deeper part of the lesion. The tissues of the periodontium showed signs of repair which became more pronounced with time. There was no indication that the lesion had become progressive in the remaining period of 7-31 weeks after ligature removal.

It is concluded that the maintenance of the established lesion is dependent on the persistance of subgingival plaque containing ligatures.

Introduction

Studies dealing with histopathological features of ligature placement around necks of teeth in dogs, monkeys and rats in order to produce experimental periodontal breakdown have been published frequently during the past decade. In dogs these

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studies were limited to the description of artificial periodontal defects *with ligatures in situ* (Lindhe & Svanberg 1974, Ericsson et al. 1975, Schroeder & Lindhe 1975, Lindhe & Ericsson 1978, Nyman, Schroeder & Lindhe 1979, Schroeder & Lindhe 1980) or *long time after ligature removal* (Johanson, Nilvéus & Egelberg 1978, Lindhe & Ericsson 1978, Hugoson & Schmidt 1978).

Recently data were presented from a *histometric* analysis of ligature-induced periodontal defects in beagle dogs during a period of 1-31 weeks after removal of the ligatures (Jansen, Van Dijk & Pilot 1982). It was concluded that the established loss of alveolar bone was reversible after ligature removal.

The present study was carried out to investigate the *histopathological* features of artificial periodontal defects before and at varying intervals after the removal of subgingival ligatures.

Material and Methods

Twelve beagle dogs, approximately 18 months old and weighing ± 15 kg. were used in this study. Throughout the experiment the dogs were fed on a diet which favours gross plaque accumulation on the teeth and no oral hygiene procedures were performed.

Periodontal defects were created as described earlier (Van Dijk 1979, Jansen & Pilot 1981): the supracrestal dentogingival fibres around 6 lower premolars ($_2P_2$, $_3P_3$, $_4P_4$) were cut to the level of the alveolar bone. Closely adapted copper bands were then cemented around the teeth to the full depth of the defects to prevent spontaneous healing. After 3 weeks the copper bands were replaced by cotton floss ligatures which were tied around the toothnecks and forced down subgingivally as far as possible. These ligatures were left in place for 11 weeks.

Immediately after removal of the ligatures a notch was made with a small excavator on the mesial and distal root surface. This notch was made as a reference point for histometrical analysis (Jansen et al. 1982). No notch however was made in dogs number 1 & 2 and in dogs nr 3 & 4 on the left side, to be able to evaluate the effect of the notching procedure.

The placement of ligatures in the different regions of the mouth and their removal prior to sacrifice were scheduled so that it was possible to make histological observations of the periodontal defects with ligatures in situ, and 1 day and 1, 3, 7, 15 or 31 weeks after their removal (Table 1).

The dogs were sacrificed using the perfusion technique described in detail by Jansen et al. (1982). Three hematoxilin-eosin stained paraffin sections of 8 μm in thickness and 40 μm apart were selected from the midsagittal region of each inter-

proximal block and analysed using either a light microscope (magnification x 200) or projected as colour slides (magnification x 70).

The following measurements were made:

- Length of the junctional epithelium: Value per defect is the mean of 3 sections assessed on colour slides.
- Size of the infiltrated connective tissue I.C.T.: colour slides were projected on a Ferranti-Cetec digitizer table, connected to a Digital P.D.P.-11/0.5 computer. The interdental papilla between 2 neighbouring teeth was divided into 2 portions of equal width, the base being the surface of the alveolar bone (Fig. 1). The size of the infiltrated part of the connective tissue in each portion was computed and expressed as a percentage of the total interdental connective tissue area. The value for a defect was expressed as the mean of the 3 percentages.

For the histological qualification of the specimens the following criteria were used:

- Presence and continuity of an epithelial lining of the periodontal defect.
- Type of an epithelial lining of the defect: Pocket epithelium (P.E.): thickened layers of epithelium with distinct rete peg formation not adhering to the tooth, or junctional epithelium (J.E.): epithelium adhering to the tooth.
- Types and localisation of inflammatory cells.
- Structure and organization of the connective tissue fibres at the root surface and in the supracrestal area.
- Presence and localization of lightly stained new cementum or cementoid on old cementum or dentin.
- Morphology of alveolar bone, estimated using polarised light, which discriminates between new fibrillar and old lamellar bone.
- Presence of osteoclasts on scaloped bone surfaces indicating bone resorption.
- Presence and localization of plaque or calculus.

Results

Extensive areas of root resorption were observed in 11 out of 132 defects. These were excluded from the present material because it was felt that the influence of root resorption might seriously hamper proper interpretation of the histopathological feature to be studied.

Histometrical results

Mean values for the length of the junctional epithelium are presented in Table 2. A distinct increase can be observed especially in the first 7 weeks after removal of the ligatures.

Mean values for the size of the I.C.T. are presented in Table 3. In the specimens from defects one day after ligature removal and the production of the notch, a distinct higher percentage of I.C.T. was found than for 0-day specimens. In those defects where no notch was produced (dogs 3 & 4, left side), such increase could not be observed. After 3 weeks the size of the I.C.T. had returned to approximately the level of the 0-day specimens. There was no distinct change during the remainder of the experiment.

Histological observations

A schematical representation of the histological changes during the observation period is presented in Figure 2.

0-day specimens: defects with ligatures in situ (Figure 3).

All 0-day specimens showed complete epithelialization. The epithelial lining consisted almost entirely of pocket epithelium with marked rete peg formation. The apical termination of the pocket epithelium was invariably located on cementum. The epithelium was often only a few cell layers thick and occasionally showed small areas of discontinuity (ulceration). The ligatures were surrounded by plaque, which was continuous with supragingival plaque in a coronal direction. The I.C.T. extended from the gingival margin close to the surface of the alveolar bone, but was separated from the latter by a zone of relatively intact supracrestal connective tissue in which scattered leucocytes were present. The I.C.T. consisted mainly of plasmacells and lymfocytes. However, polymorphonuclear leucocytes (P.M.N.'s) were present in large numbers in between the subgingival plaque and the pocket epithelium, in the widened intercellular spaces of the pocket epithelium and in and around the capillaries of the subepithelial plexus. The connective tissue attachment had lost its normal appearance in the area just apical to the apical termination of the pocket epithelium although remnants of collagen fibres remained inserted in the cementum. Further apically, near the bone crest, the fibre orientation showed its normal appearance. The bone was mature osteon in type. Sparse osteoclasts were invariably present on its surface and in the marrow spaces below the bone surface. The alveolar bone loss was horizontal except at the mesial aspect of $_{2}P$ and the distal aspect of $_{4}P$. In these areas the bone near the unaffected $_{1}P$ and $_{1}M$ extended more coronally leading to a hemiseptum.

1-day specimens + notch: 1 day after removal of the ligatures and the production of the notch (Figure 4a).

The notch was generally situated just coronal to the alveolar bone crest but far below the apical termination of the pocket epithelium. Dentin and cementum

fragments were frequently situated in its vicinity. The apical termination of the pocket epithelium showed signs of disruption by the notch instrument. The inflammatory infiltrate extended to the bone surface and consisted almost exclusively of PMN's surrounded by extravascular erythrocytes and strands of fibrin. Many more osteoclasts were present on the bone surface than was observed in the 0-day specimens. There were also more dilated blood vessels. The I.C.T. in the coronal part of the interdental papilla continued to be dominated by plasma cells and lymfocytes. No functional collagen fibre arrangement or fibre attachment to the cementum could be detected in the supracrestal area. There was no subgingival plaque present in any defect.

1-day specimens - notch: 1 day after removal of the ligatures but without the production of a notch (Figure 4b).

The defects were completely lined by pocket epithelium. The inflammatory infiltrate appeared more dense than in the 0-day specimens. The infiltrate in which PMN's could frequently be observed, was separated from the bone surface by collagen fibres although these appeared to have lost their normal integrity. No subgingival plaque could be detected.

1-week specimens (Figure 5).

In these specimens the inflammatory infiltrate still occupied almost the total supracrestal area. PMN's were no longer dominant but lymfocytes and plasma cells had taken their place. The epithelial lining of the defects was continuous and in most specimens junctional epithelium adhered to the cementum. No subgingival plaque was present. At the tooth side spicules of new bone could occasionally be observed. In the central part of the alveolar bone crest marrow spaces were in open contact with the supracrestal I.C.T. and also contained many inflammatory cells. Many osteoclasts could be observed, both on the bone surface as well as on the internal surface of the marrow spaces. There was still a complete loss of functional orientation of the connective tissue structures in the supracrestal area and in most specimens it was hard to detect any remaining fibre attachment to the teeth.

3-weeks specimens (Figure 6 a - b).

The overall picture of these specimens differed markedly from those already described. In the supracrestal area below the line connecting the apical terminations of the junctional epithelium of neighbouring teeth the inflammatory infiltrate had almost completely subsided, although scattered leucocytes remained. This area looked collagen rich - cell poor. In approximately half of the specimens isolated

areas of light staining cementoid, covered by numerous cementoblasts were found, not only in the notch area but also near the apical extension of the junctional epithelium as well as apical to the notch. In the region near the tooth spicules of fibrillar bone covered by numerous blastlike cells could be observed. No bone apposition could be seen in the central part of the alveolar bone crest and osteoclasts were few in number. In most specimens there was a functional orientation of collagen fibres to the root surface. In those areas where dentin had been exposed by the notching procedure and new cementum had not yet been deposited the fibres were often aligned parallel to the root surface. In all specimens a zone of junctional epithelium was present. In the coronal part of the lesion, beneath the pocket epithelium, a dense inflammatory infiltrate, consisting of mainly plasmacells and lymfocytes remained. Subgingival plaque could be detected in many specimens but only in the coronal part of the defect opposite pocket epithelium.

7-weeks specimens (Figure 7 a - b).

New bone formation and new cementum formation was present in the majority of specimens and was more pronounced than that found in the 3-weeks specimens. New bone formation extended coronal to the notch in many cases. In the central part of the alveolar bone crest little new bone formation could be observed. In these areas former marrow spaces were still in contact with supracrestal connective tissue. In those areas where new bone had formed opposite root surfaces on which new cementum was deposited the collagen fibres in between frequently showed the appearance of a well-oriented periodontal ligament. As a rule new cementum contained few cementocytes. Root fragments, when present, were consistently found to be incorporated by new cementum. In most specimens a long junctional epithelium was found. Beneath this epithelium scattered lymfocytes and plasma cells remained present but the dense inflammatory infiltrate consisting of mainly lymfocytes and plasma cells was confined to the coronal portion of the interdental papilla. In this area pocket epithelium with extensive rete peg formation and occasional ulcerations remained a consistant finding. Supra- and subgingival plaque could be observed in the coronal portion of all defects.

15- and 31-weeks specimens (Figures 8 & 9).

New alveolar bone formation was more pronounced than in the previous specimens. In many specimens new bone was found not only opposite the root surfaces but also in the central crestal region, demarcating the former open marrow spaces from the supra alveolar transseptal connective tissue area. Although the supra alveolar connective tissue was devoid of inflammatory cells and often dis-

played a normal transseptal fibre orientation, in the coronal marrow spaces inflammatory cells could be observed near large venules and capillaries in a minority of specimens. In the 31-weeks specimens the most coronal extension of the new bone was occasionally situated coronal to the apical termination of the junctional epithelium. Junctional epithelium was never found to be situated on new cementum. Severe marginal inflammation in the coronal region of the interdental papilla, was always present, as was subgingival plaque.

Discussion

The present study has shown that the placement of subgingival cotton ligatures, preceded by dissection of supracrestal dentogingival fibres and copper banding establishes defects which show histopathological features frequently described in association with naturally occurring chronic periodontitis, both in humans (Saglie, Johanson & Flǿtra 1975, Page & Schroeder 1976) and in dogs (Hull, Soames & Davies 1974, Lindhe, Hamp & Löe 1975, Hamp & Lindberg 1977, Page & Schroeder 1981). Longstanding ligature placement in dogs has also been reported to induce periodontal defects with histopathological features similar to those described here (Schroeder & Lindhe 1975, Ericsson et al. 1975, Lindhe & Ericsson 1978).

The onset of rapid experimental periodontal breakdown is believed to be associated with subgingival ligature placement and ulceration of the pocket epithelium, leading to an inflammatory infiltrate dominated by P.M.N.'s which in turn provokes high osteoclastic activity (Heijl, Rifkin & Zander 1976, Nyman, Schroeder & Lindhe 1979, Schroeder & Lindhe 1980). It may be assumed that in the initial phase of the present experiment the injurious procedures i.e. dissection of the dentogingival fibres, followed by placement of copper bands and the installation of subgingival ligatures have provoked a similar P.M.N.-osteoclast dominated process. Schroeder & Lindhe (1980) postulated that once the initial ulceration had been repaired the inflammatory reaction would become chronic in type, involving mainly plasma cells and showing low osteoclastic activity. Others have also established that periodontal breakdown is more pronounced immediately after ligature placement and then slows down at a later stage (Kennedy & Polson 1973, Lindhe & Ericsson 1978, Nyman et al. 1980). From the present observations in the 0-day specimens it seems that the presence of subgingival ligatures for a period of 11 weeks was long enough to establish a chronic non aggressive lesion.

Immediately following ligature removal and the production of the notch a marked change in size and composition of the I.C.T. was observed. The extensive P.M.N. response in the supracrestal connective tissue area in the 1-day specimens (+ notch)

was undoubtedly due to the injurious effect of the notching procedure, since the mere removal of ligatures resulted in less distinct changes (1 day specimens - notch). One week after ligature removal and notching the acute inflammation had changed into a mononuclear dominated type which still extended to the bone surface but in the subsequent period of 3-7 weeks the inflammation in the supracrestal part of the lesions subsided very rapidly. As a consequence repair of most of the tissues of the periodontium was observed.

The reason for the decrease of inflammation, in spite of the absence of oral hygiene procedures, obviously was the removal, although unintended, of the subgingival plaque as a result of ligature removal (and may be also as a result of the notching procedure). This is validated by the observation that in the 1-day as well as in the 1, 3 and 7 weeks specimens deep subgingival plaque was absent.

Similar results have been reported for ligature induced periodontal defects in monkeys (Polson, Kantor & Zander 1979, Adams, Zander & Polson 1979, Kantor 1980). When ligature removal was accompanied by active mechanical plaque removal, a reduction of inflammation in the supracrestal area, followed by a reorganisation of transseptal fibres and bone repair was observed. There seems to be general agreement that spontaneous healing after the removal of "ligatures" will not occur as long as plaque remains present in the artificial defect (Ellegaard et al. 1973, Caton & Zander 1975, Johanson, Nilvéus and Egelberg 1978, Lindhe & Ericsson 1978). Hugoson & Schmidt (1978) however reported radiographical and histological evidence of regeneration 6 months after silk ligature removal from experimental bifurcation defects in dogs. Although supragingival plaque was allowed to accumulate, these authors did not mention subgingival plaque which may have been unintendedly removed along with the ligatures.

These results, together with the observations in the present ligature-off specimens, permit the conclusion that the persistance of deep subgingival plaque adjacent to the apical termination of the pocket epithelium plays an important role in the maintenance of chronic experimental periodontitis.

However, it can not be excluded that the production of the notch has had an additional influence on the observed repair process, especially on the formation of new cementum. It is evident from earlier histometric data (Jansen et al. 1982) and from the observations in the present 1-day specimens that this notch was prepared, on average, just coronal to the alveolar bone, approximately 1 millimeter apical to the apical termination of the pocket epithelium, thereby separating the connective tisue attachment from the root surface. It may be that the new cementum formation in the attachment area is the result of this surgical separation and the subsequent

reuniting of these tissues: a conclusion which is in agreement with observations by Schaffer & Zander (1953) Jansen, Coppes & Verdenius (1955), Caton & Zander (1979) and Garrett et al. (1981). New cementum formation in notched areas has been reported frequently (Morris & Thompson 1963, Morris 1978, Listgarten 1972 and Frank et al. 1974), but the stimulating effect of the notch per se has only recently been questioned (Garrett et al. 1981). The finding that new cementum formation in the present study was not solely confined to the notch area is in full agreement with the results of Garrett and co-workers.

For a better understanding of the nature of the repair process, it should be noted that the area between the apical termination of the junctional epithelium and the bone crest at the completion of the experiment was originally occupied by an intact periodontal ligament. Cells residing the periodontal ligament are believed to possess good repair capacity, not only for the ligament itself but also for cementum and alveolar bone (Melcher 1969, 1976). It may be speculated that periodontal ligament cells remained present along the root surface during the experiment and served as a source for repair cementum and new bone formation. The observation that new bone was preferentially formed opposite the root surface during the early stages of repair substantiates this assumption.

Also the finding that root fragments in the notch area in the older specimens were invariably surrounded by a substance reminiscent of bone or cellular cementum, further indicates that cells of the connective tissue near the root surface possessed the capacity of transforming into cementoblasts and osteoblasts.

From the present observations it would appear that not only the connective tissue components of the periodontium, but also the pocket epithelium showed signs of repair, i.e. pocket epithelium becomes attached to the root surface previously exposed to plaque. The possibility that the junctional epithelium had migrated apically a short distance during the first few weeks after ligature removal, cannot however be excluded as it was not prevented from doing so by an organised connective tissue attachment. Since in no instance junctional epithelium was found on new cementum it may be concluded that the apical proliferation of junctional epithelium, if any, had stopped after 3-7 weeks. It seems very unlikely that in this short period of time an apical proliferation of junctional epithelium of approximately 1.3 millimeters could be established, the more so because from the histometrical data presented for these defects (Jansen et al. 1982) there appeared to be no apical migration of junctional epithelium during this period. Consequently it may be assumed that at least part of the pocket epithelium was transformed into junctional epithelium which became attached to the root surface previously exposed to plaque.

This finding suggests not only the absence of plaque on the root surface, but also the absence of other substances such as endotoxins which can inhibit epithelial readherance to the exposed cementum (Aleo et al. 1974, 1975, Stahl 1979). It may therefore be speculated that the exposure of cementum to the plaque endotoxins in the present defect was insufficient to effectively prevent epithelial re-adhesion.

Acknowledgments

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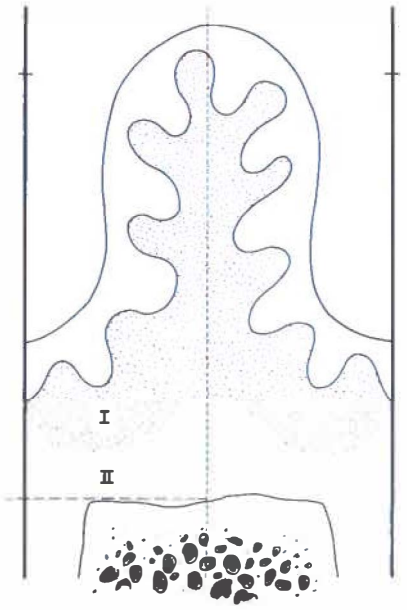


Fig. 1. Relative size of the infiltrated connective tissue area:

$$\text{I.C.T.} = \frac{\text{I}}{\text{I + II}} \times 100\ \%$$

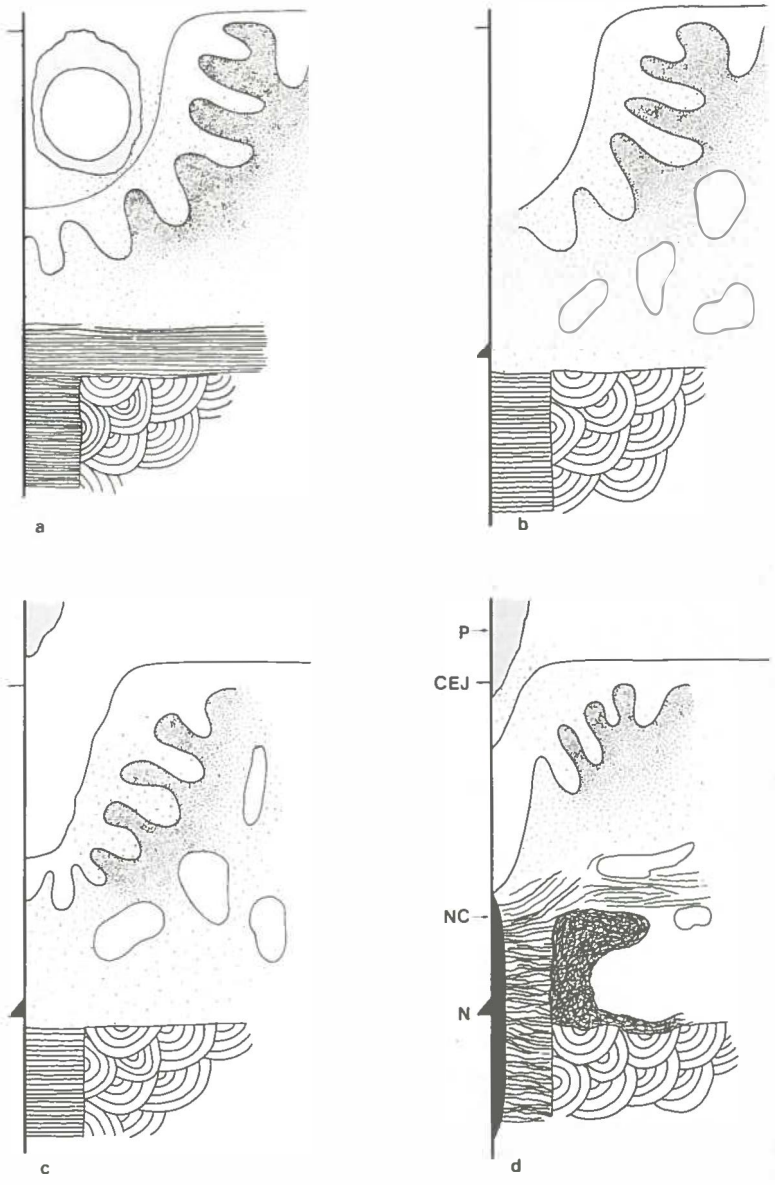


Figure 2. Schematic representation of defects at different stages of the experiment

2a. defect with ligature in situ,

2b. defect 1 day after ligature removal and the production of a notch,

2c. defect 1 week after ligature removal and the production of a notch,

2d. defect 7-31 weeks after ligature removal
P = plaque; N = notch; CEJ = cemento enamel junction; NC = new cementum.

Fig. 3. 0-day specimen, defect with ligature in situ. C.E.J. = cemento-enamel junction; L = ligature. Original magnification X 40.

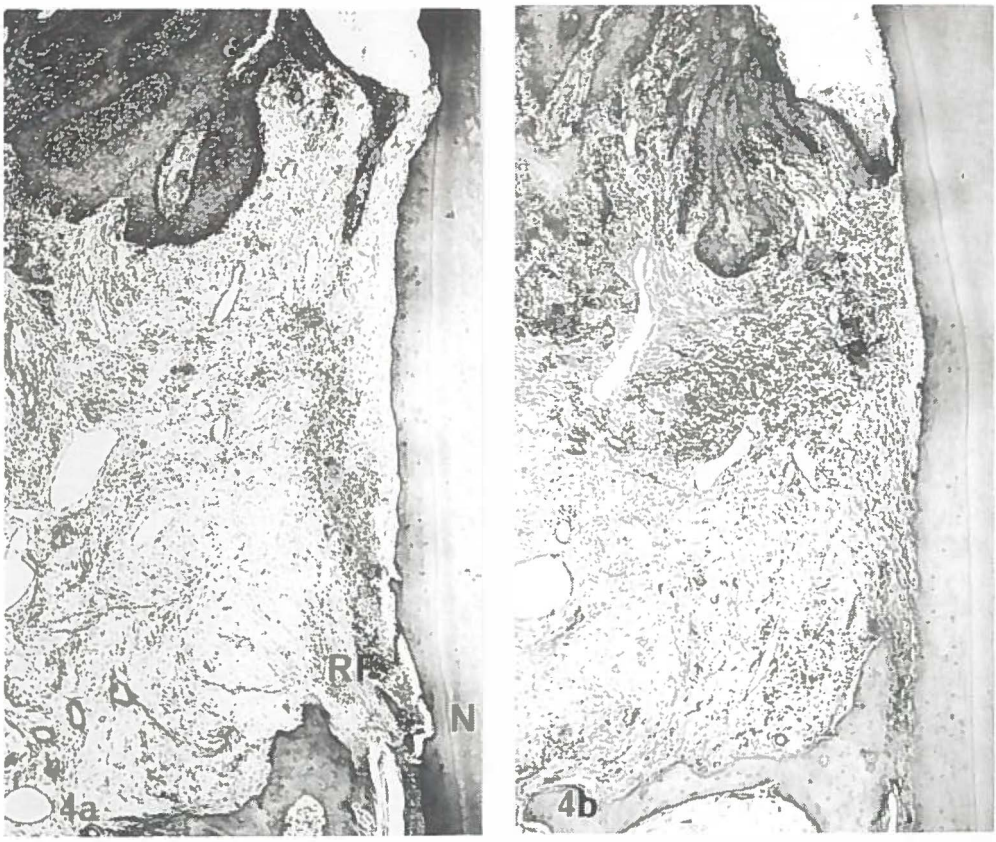


Fig. 4a. 1-day specimen, defect in which a notch (N) was prepared 1 day earlier. R.F. = root fragments

Fig. 4b. 1-day specimen, defect from which the ligature was removed 1 day earlier, but no notch was prepared. Original magnification X 64.

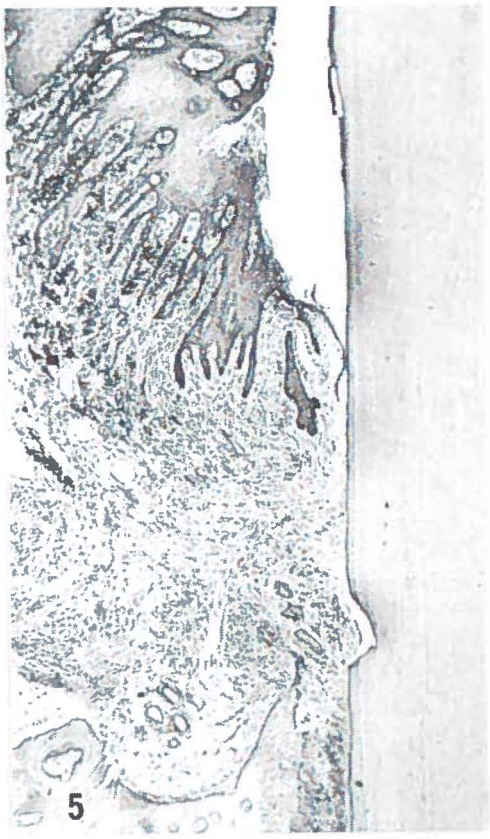
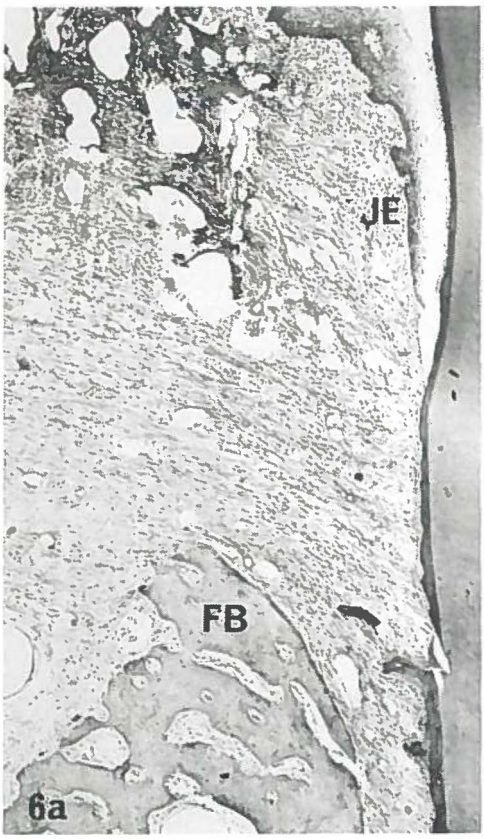


Fig. 5. 1-week specimen, note the absence of subgingival plaque. Original magnification X 40.



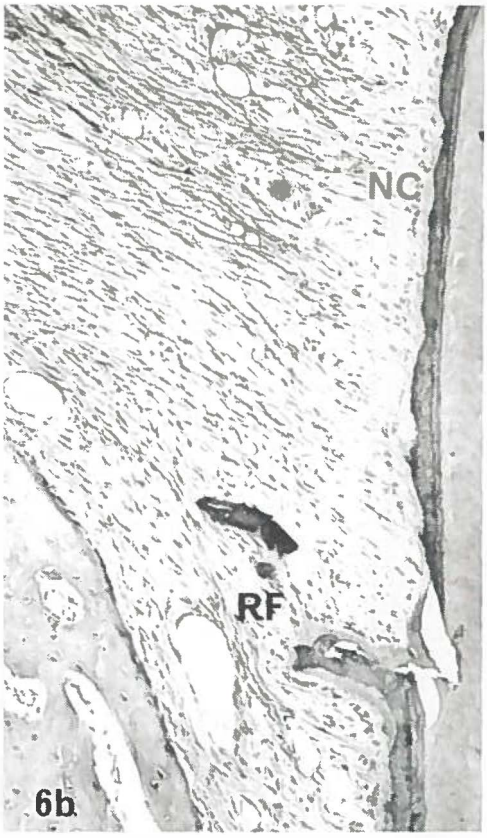


Fig. 6a. 3-weeks specimen. J.E. = junctional epithelium; F.B. = fibrillar bone. Original magnification X 64.

Fig. 6b. Higher magnification of the same specimen. N.C. = new cementum; R.F. = root fragments. Original magnification X 160.

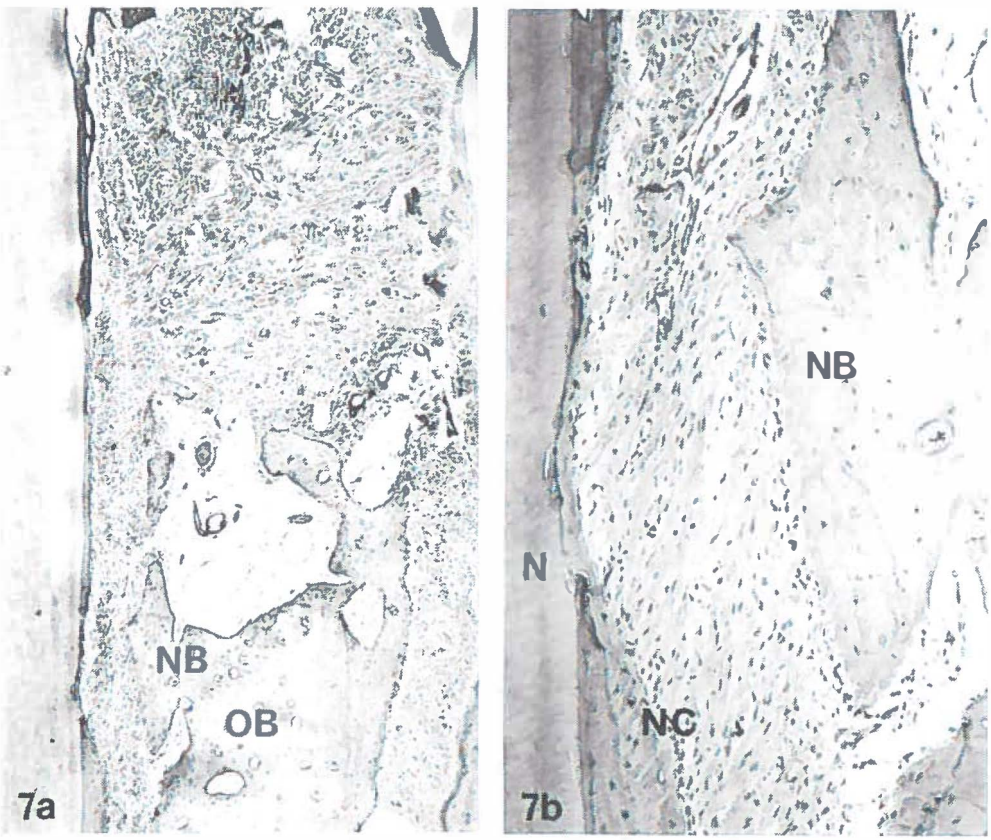
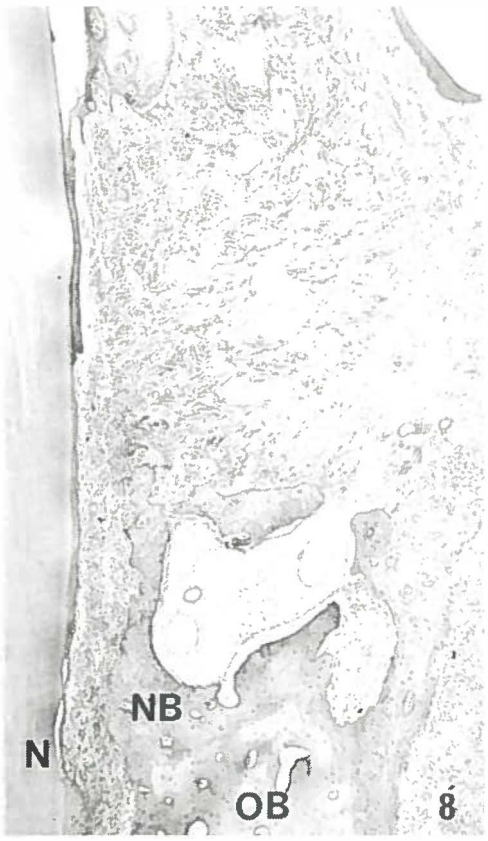


Fig. 7a. 7-weeks specimen. There is a distinct demarcation between the old osteon-type of bone (O.B.) and the new fibrillar bone (N.B.). Original magnification X 40.

Fig. 7b. Higher magnification (X 160) of the notch area (N). Note the functional orientation of the periodontal ligament fibres in between the new cementum (N.C.) and the new alveolar bone.



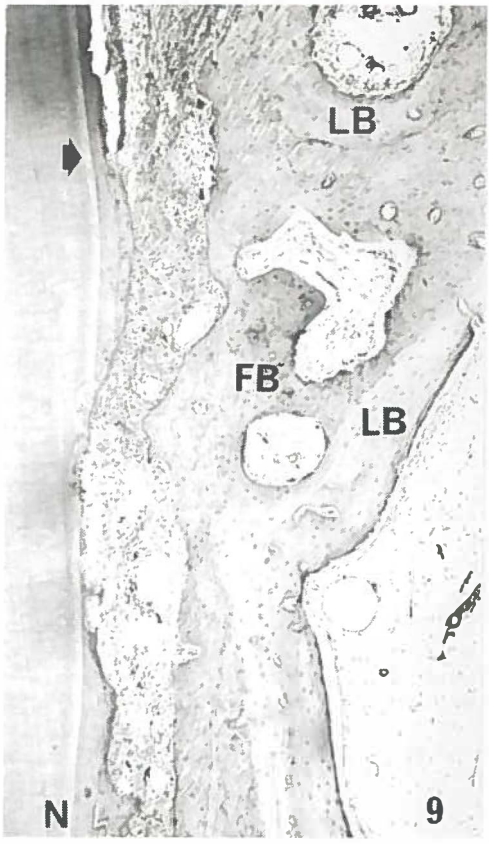


Fig. 8. 15-weeks specimen. N.B. = new alveolar bone. O.B. = old osteon-type of bone. Original magnification X 40.

Fig. 9. 31-weeks specimen. Extensive new bone formation coronal to the notch (N). Not only fibrillar type of bone (F.B.) is present but also lamellar new bone (L.B.) indicating that maturation of the bone had occurred. Junctional epithelium was present between the new bone and the root surface. The arrow indicates the apical termination of the junctional epithelium. Original magnification X 64.

Table 1

Material available for histological observations

dogs nr.	time between ligature removal and sacrifice		number of defects	
	right	left	right	left
1&2	0 days*	—	10	—
3&4	1 day	1 day**	11	10
5&6	3 weeks	1 week	10	9
7&8	7 weeks	1 week	9	9
9&10	15 weeks	1 week	10	9
11&12	31 weeks	1 week	10	11

In dogs nr. 1 & 2 no defects were created on the left side of the jaw. From a total of 132 possible defects 13 were not available for observation because the specimens were incorrectly sectioned. Another 11 defects were excluded because the roots of the teeth showed extensive resorption.

* ligatures in situ, no notch was made,

** in these defects no notch was made upon removal of the ligatures.

Table 2

Length of the junctional epithelium in millimeters.
Mean values and standard deviations of all
available defects at one observation time point.

	$\bar{x}\pm$s.d.
0 day	0.16±0.25
1 week	0.67±0.32
3 weeks	0.65±0.27
7 weeks	1.35±0.29
15 weeks	1.38±0.31
31 weeks	1.56±0.31

Table 3

Size of the I.C.T. as a percentage of the total connective tissue area.
Mean values and standard deviations of all available
defects at one observation time point.

	$\bar{x}\pm$s.d.
0 day	56.4%± 9.8
1 day (+ notch)	87.2%±11.5
1 day (- notch)	65.6%±15.7
1 week	76.2%±13.1
3 weeks	55.4%±13.8
7 weeks	49.8%±12.9
15 weeks	54.2%±12.6
31 weeks	42.4%±13.5

Chapter 7

HISTOLOGICAL OBSERVATIONS ON ELASTIC BAND-INDUCED PERIODONTAL DEFECTS IN BEAGLE DOGS

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Abstract. Elastic band induced periodontal defects around upper incisor teeth of beagle dogs were evaluated for their histopathological characteristics before and after elastic band removal. Defects with *elastic bands in situ* were frequently characterized by incomplete epithelialization, the presence of an acute inflammatory connective tissue infiltrate and extensive areas of root resorption. After *removal* of the *elastic bands* the periodontal tissues showed distinct signs of repair. It was concluded that the frequent occurrence of root resorption represents a complicating factor in the application of this model.

Introduction

Elastic band placement has been reported to be successful in creating deep, non-reversible periodontal defects in Rhesus monkeys (Caton & Zander 1975). Recently, it was demonstrated that in dogs a modification of this method produced deep periodontal defects. However, upon elastic band removal, decreasing probing depths and alveolar bone repair were reported (Jansen 1982[a]). Therefore, the aim of the present investigation was to evaluate the *histopathological* characteristics of these defects before and after removal of the elastic bands.

Material and Methods

The defects were created around 4 contralateral upper incisors ($^{3}I^{3}$ and $^{2}I^{2}$) in 12 beagle dogs as described earlier (Jansen 1982[a], chapter 5). Immediately after removal of the elastic bands a notch was made on the mesial and distal root surfaces to serve as a reference point for the histometrical assessments.

Histopathological observations were made on defects with elastic bands in situ, and 1 day and 1, 3, 7, 15 or 31 weeks after their removal (Table 1). Three hematoxylin-eosin stained paraffin sections of 8 μm thickness and 40 μm apart were

selected from the mid-sagittal region of each interproximal block. These sections were analysed as described in detail by Jansen (1982[b]) (chapter 6) for: length of the junctional epithelium, size of the infiltrated connective tissue (I.C.T.), quality of the epithelial lining of the pocket, type and localization of inflammatory cells, structure and organisation of the supracrestal connective tissue, presence and localization of new cementum, and presence of new alveolar bone.

As described earlier the distal aspects of $^{3}\underline{1}^{3}$ were excluded from the material in the clinical and histometrical results. Therefore their histopathological characteristics will not be described here.

Observations

The most striking feature was the occurrence of extensive areas of root resorption in 12 out of 19 defects on the mesial aspect of $^{3}\underline{1}$. No such resorption was observed on the mesial or distal aspect of the second incisors.

Elastic band-containing defects mesial to $^{3}\underline{1}$ (8 weeks after elastic band placement) showed definite signs of a destructive inflammatory process (Fig. 1). On the surface of the resorptive lacunae many cemento- and dentino-clasts could be observed and many osteoclasts were present on the alveolar bone surface. The apical portions of the defects were not epithelialized and the inflammatory infiltrate which extended to the bone surface consisted mainly of polymorphonuclear leucocytes. Although this acute inflammatory reaction inevitably affected the total interdental area, it did not result in a horizontal breakdown of the periodontal tissues between the adjacent incisors (Fig. 1).

The elastic band-containing defects at the mesial aspects of $^{2}\underline{1}$ showed the histopathological characteristics of naturally occurring periodontitis, very similar to those described for the ligature-containing defects (chapter 6) (Fig. 2). Since these defects were created next to the unaffected first incisors this resulted in vertical bone loss.

Distinct repair phenomena were observed in the periodontal tissues in the deeper part of most of the lesions *after elastic band removal*, very similar to those described for ligature-induced defects after ligature removal (chapter 6). The size of the I.C.T. decreased within 3-7 weeks (Table 2) and as a result the collagen fibres reorganised. During the same period the lenght of the junctional epithelium increased (Table 3). New cementum was deposited on the root surfaces and new fibrillar bone was found on top of pre-existing lamellar bone. Resorptive concavities in the mesial root surfaces of $^{3}\underline{1}$ were repaired by new cementum formation and filled with non-inflamed connective tissue (Fig. 3). Ankylosis was never observed. At

the mesial aspects of ^{2}I bone repair resulted in partial filling of the vertical bony defect.

Discussion

The mechanisms possibly underlying the repair process after elastic band removal are assumed to be similar to those discussed in chapter 6, in association with ligature removal, and will not be discussed here again.

The frequent occurrence of root resorption in defects at the mesial aspect of the third incisors (63%) is probably the result of abcess formation as a result of deep elastic band placement. After initial dissection of the supracrestal dentogingival fibres the elastic bands were first placed around the third incisors and subsequently around the second incisors. Since the narrow interdental area between the second and third incisors did not provide enough space for 2 elastic bands to be situated at the same level, bands at the mesial aspects of $^{3}I^{3}$ were situated far more apically than those at the distal aspect of $^{2}I^{2}$ (chapter 5). The latter bands probably obstructed the entrance of the deeper defects mechanically, thereby preventing a normal flow of inflammatory products.

Root resorption has been reported earlier in association with ligature placement in dogs. Schroeder & Lindhe (1975, 1980) described the phenomenon of root resorption extensively but did not report on its prevalence. Jansen (1982[b]) reported of 8% root resorption after subgingival ligature placement around the premolars of beagle dogs.

In the monkey model, using the same technique for creating defects, abcess formation or root resorption has not been reported (Caton & Zander 1975, 1979, Caton & Nyman 1980, Caton, Nyman & Zander 1980). The discrepancy may be explained by the difference in anatomy between the Rhesus monkey and the beagle dog, the former having a wider interdental area between adjacent incisors than the latter. Root resorption obviously represents a complicating factor if the aim of the experiment is to establish a simplified model for studying treatment procedures.

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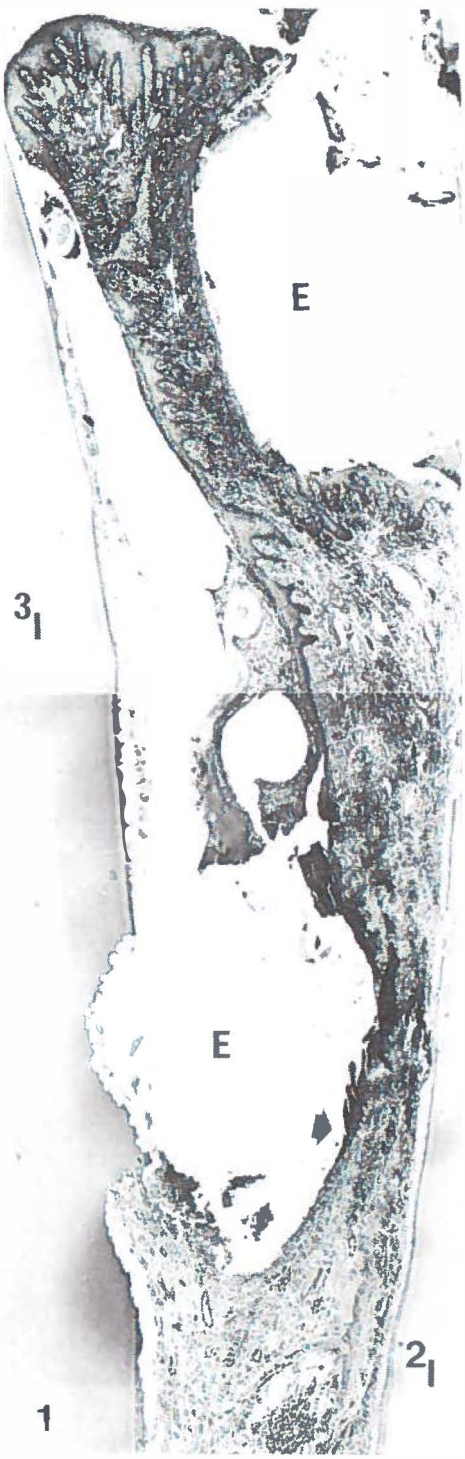

Fig. 1. Representative section of a 0-day specimen (elastic bands in situ) showing abcess formation in the interdental area between ^{3}I and ^{2}I. The deepest defect at the mesial aspect of ^{3}I is not epithelialized (arrow indicates the apical termination of the pocket epithelium). There are many dentoclasts present on the root surface in the resorptive lacunae. Note that in spite of the acute destructive inflammatory proces the periodontal tissues at the mesial aspect of ^{2}I remained situated at a more coronal level. E = elastic band. Original magnification 30 X.

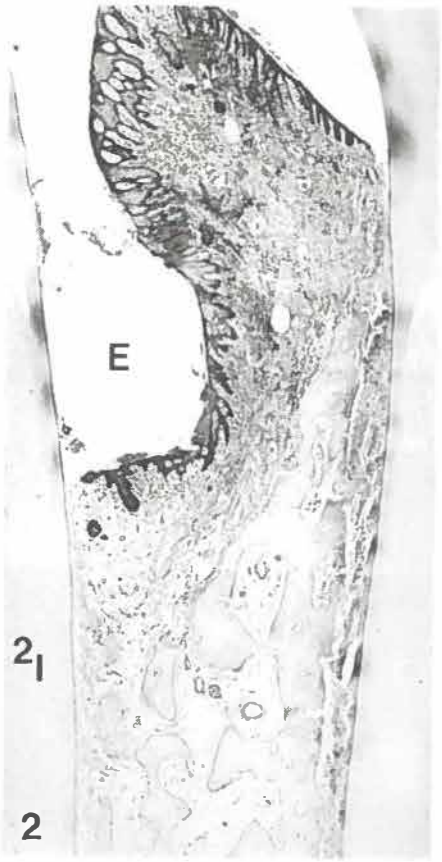

Fig. 2. Representative section of an elastic band-containing defect at the mesial aspect of ^{2}I. The defect is completely epithelialized. The I.C.T. is separated from the bone surface by a zone of relatively intact connective tissue. The inflammatory cells were mainly plasma cells and lymfocytes. At the mesial aspect of the non-ligated ^{1}I no loss of attachment and no alveolar bone loss had taken place. As a result a vertical bony defect was established. E = elastic band. Original magnification 30 X.

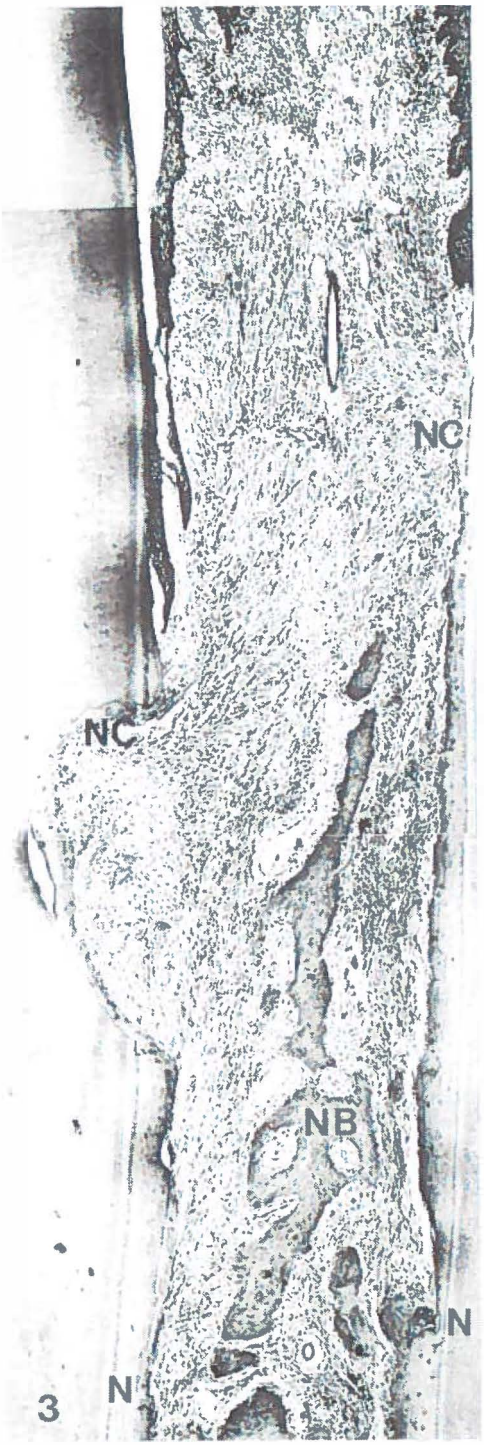

Fig. 3. Representative section of an interdental area between ^{3}I and ^{2}I, 15 weeks after elastic band removal. On both tooth surfaces new cementum (NC) was deposited and new alveolar bone was formed coronal to the notches (N). The resorptive area is filled with non-inflamed, well-structured connective tissue. NB = new bone. Original magnification 30 X.

Table 1

Material available for histological observations

dogs nr.	time between elastic removal and sacrifice		number of defects	
	right	left	right	left
1&2	0 days*	—	6	—
3&4	1 day	1 day**	5	4
5&6	3 weeks	1 week	5	5
7&8	7 weeks	1 week	6	4
9&10	15 weeks	1 week	4	5
11&12	31 weeks	1 week	5	5

In dogs nr. 1 & 2 no defects were created on the left side of the jaw. From a total of 66 possible defects 12 were not available for observation because the specimens were incorrectly sectioned.

* ligatures in situ, no notch was made

** in these defects no notch was made upon removal of the ligatures.

Table 2

Size of the I.C.T. as a percentage of the total connective tissue area. Mean values and standard deviations of all available defects at one observation time point.

	$\bar{x}$±s.d.
0 day	80.4%±10.6
1 day (+ notch)	96.9%± 3.7
1 day (- notch)	92.5%± 7.4
1 week	85.4%± 8.7
3 weeks	52.0%±14.5
7 weeks	35.4%±14.0
15 weeks	44.6%± 8.7
31 weeks	38.2%±11.4

Table 3

Length of the junctional epithelium in millimeters. Mean values and standard deviations of all available defects at one observation time point.

	$\bar{x}$±s.d.
0 day	0.12±0.15
1 week	0.57±0.25
3 weeks	0.94±0.09
7 weeks	2.06±0.58
15 weeks	1.06±0.68
31 weeks	1.38±0.44

Chapter 8

GENERAL DISCUSSION

Abstract. The histological and clinical changes observed during the *evaluation phase* are discussed in this chapter. This phase lasted for 31 weeks after completion of the *active phase* of defect formation. Furthermore, the implications of these changes are discussed and the features of the artificial periodontal defect, found at the end of the evaluation phase, are summarized. Finally, considerations on the future use of the model are presented.

The evaluation phase.

Periodontal lesions with the following characteristics were obtained at the end of the active phase (the start of the evaluation phase

- probing depths of approximately 4 millimeters for premolars and 5.5 millimeters for incisors (chapters 3 & 5),
- loss of connective tissue attachment and alveolar bone loss of 2-2.5 millimeters for premolars and 3-4 millimeters for incisors (chapters 4 & 5),
- histopathological features of slowly progressing chronic periodontitis (chapters 6 & 7) with the exception of the incisor region where periodontal abcesses and root resorption were frequently found (chapter 7).

Histological changes. It became apparent that healing was occurring during the evaluation phase. Histologically this was characterized by decreased inflammation in the apical part of the lesion, most probably as a result of the co-incidental removal of the subgingival plaque with the ligatures (chapter 6). The connective tissue area, formerly occupied by inflammatory cell infiltrate became reorganised with the formation of well-aligned collagen bundles, new bone formation and new cementum deposition. A long epithelial adhesion to previously exposed cementum was formed. However, it was also shown that the established apical positioning of the junctional epithelium was not reversible, indicating that no new attachment (connective tissue fibres inserted into new cementum, deposited on previously exposed cementum) was formed during the evaluation phase.

Repair or regeneration?

According to the definitions originally formulated by Gillman (1961) and re-emphasized by Melcher (1969, 1976) and Stahl (1979) regeneration is defined as: a biologic

process by which the architecture and function of lost tissue is completely renewed. Repair is defined as: a biologic process by which the continuity of lost tissues is restored by new tissues which do not replicate the structure and function of the lost tissues. Although these definitions have primarily been used to describe tissue restoration after surgery, they may equally well be applied to restoration of tissues, lost as a result of artificial periodontal inflammation. According to these definitions the healing process after the induced periodontal breakdown in the present experiments included both regeneration and repair. In the most apical parts of the former lesions new cementum was deposited on old (but not previously exposed) cementum. New bone was formed opposite this new cementum together with a functionally oriented periodontal ligament connecting these two structures. These phenomena indicate regeneration, because such a biologic entity was present before the artificial periodontal breakdown. All healing processes that took place coronal to the regenerated tissues may then be called repair, including the formation of a long epithelial adhesion (Fig. 1).

The term spontaneous healing should be reserved for healing which occurs without any intervention. Since it has been concluded that the above mentioned processes were the result of ligature removal and, in part, the result of the production of a notch in the rootsurface (chapters 6 & 7) the term spontaneous healing is not appropriate here.

Clinical changes. Healing was also suggested by a reduction in probing depths (chapters 3 & 5). Since it was shown that the established apical positioning of the junctional epithelium was not reversible during the evaluation phase, the reduced probing depths were not a reflection of new connective tissue attachment. The possibility that the decreased probing depths were the result of further gingival recession can also be excluded. The gingival margin tended on the contrary to shift in a coronal direction after ligature and elastic band removal. It can thus be concluded that the histologically observed healing of the periodontal tissues (chapters 6 & 7) influenced the probing conditions resulting in more resistance to the probe than in the severely inflamed tissues found at the start of the evaluation phase. The results of chapter 2 confirm this assumption. In that chapter it was found that clinical probing at the end of the active phase leads to overestimation of the pocket depth because the probe tip passes the coronal border of the connective tissue attachment. This level was more accurately indicated during the evaluation phase after a certain degree of healing had been demonstrated.

Implications of the observed healing on the use of artificial periodontal defects. In chapter 1 three requirements were formulated for artificial periodontal defects.

- the defects should not heal spontaneously after their establishment,
- size and morphology should be suitable for testing the results of treatment procedures,
- the creation of the defects should be both, reproducible and predictable.

In view of the observed results, the first requirement should be redefined as follows: artificial periodontal defects should not be used in studies aimed at evaluating the effect of treatment procedures until the potential healing process has stopped.

The experimental design presented here did not allow us to determine exactly when this point in time had been reached. It seemed that most of the healing occurred within 7 weeks. During this time the size of the infiltrated connective tissue decreased rapidly and the long junctional epithelium was established. However, bone repair and maturation of the healing tissues seemed to continue during the remainder of the evaluation period, although no major histopathological differences were observed between 15 and 31 weeks after ligature or elastic band removal. On the basis of these observations it can be concluded that the present defects were certainly not stable within 7 weeks; the use of such a defect during this period should therefore be avoided. It may be safer to include a resting period of 3 months, although there is always the risk that even after this length of time an ongoing process of bone repair and tissue maturation will affect the conclusions drawn from treatment experiments.

The size and morphology of the defects were influenced by the healing process. Bone repair in the defects, which were initially characterized by vertical alveolar bone loss (P_2 mesial, P_4 distal and I_2 mesial) and an even bone apposition in the remaining areas resulted in defects being ultimately characterized by a horizontal level of the alveolar bone. The depth of the defects, assessed clinically, was 3-3.5 millimeters in the premolar region and 3.5-4.0 millimeters in the incisor region. In chapter 2 it was shown that the probe tip rather accurately indicated the coronal level of the connective tissue attachment in this phase. It should however, be noted that generally the apical third of the defect was occupied by epithelial attachment.

In order to ensure an efficient use of the experimental animal model, it is necessary to produce artificial defects *reproducibly* (values for contralateral sides in the same animal should not differ significantly) and *predictably* (probing depths values should fall within a pre estimated range of measurements).

From the clinical results in chapter 3 it appeared that cotton floss *ligature-*

induced defects satisfactorily meet these requirements. No significant differences were found between the mean values for probing depths in contralateral areas, in spite of occasional large variations between dogs and within individual dogs. These variations gradually disappeared within 3 months after ligature removal and experimental defects, that fell within the depth category of 3.0-4.5 millimeters were predictably created.

From the results in chapter 5 it appeared that defects in the upper incisor area can also be produced rather reproducibly, because the differences in probing depths between contralateral elastic induced defects in the same dog were small. As was shown for the ligature-induced defects, the wide variations in probing depths between dogs, present just after creation of the defects gradually disappeared during the evaluation phase.

Considerations on the future use of the model.

In conclusion, some final comments on the use of this dog model should be made. Three different aspects, related to the characteristics of the model at different stages of the experiment, can be distinguished.

Model with active periodontitis: At the end of the active phase a slowly progressing inflammatory process is available for study purposes. This process continues as long as the ligatures remain in situ. Two points are worthy of note:

Firstly, *deep* pockets were obtained, with both experimental methods, in the premolar and the incisor region. So far only models with *marginal* artificial periodontitis around single teeth have been reported in the literature. The subgingival bacterial flora in the deep pockets, found in this experiment, may be different from the bacterial flora in more direct contact with the oral environment in marginal periodontitis and consequently the tissue reaction may also be different. The model presented in this thesis can therefore be regarded as a valuable addition to those currently being used to study active periodontitis. It can be used for evaluating a variety of factors which are thought to influence inflammatory processes e.g. hormones, enzymes or therapeutic drugs. Co-factors in the progression of periodontal disease, such as trauma from occlusion, can also be studied in this model.

Secondly, periodontal abcesses and root resorption were seen so frequently in the incisor region, that it might be assumed that these can be induced with a fair degree of predictability. Although the abcess formation may be regarded as an unwanted side effect, it provides a model which may be of value for studying the influence of subgingival micro-organisms on ulcerated soft tissues. Furthermore, it

could be applied in the study of factors involved in the process of root resorption.

Model during healing: After the ligatures were removed, at the end of the active phase, a process of healing started which lasted for at least 15 weeks. During this phase, the model provides an opportunity of studying the healing of active periodontitis as well as factors which may modify this healing process. The production of a notch to indicate the apical extension of the defects provided indispensable information in the histometrical analysis. Since it was concluded that this procedure may also have contributed to some aspects of healing (new cementum deposition) the production of a notch should be avoided if the model is to be used solely to evaluate healing procedures.

Model with stable periodontal defects: Most, if not all, the healing occurred during the first 3 months of evaluation resulting in a relatively *stable defect*. Any residual healing thereafter can be balanced out if an adequate split-mouth experimental design is used.

Defects within a predictable range of probing depths were obtained. The perenninal problem of aberrantly reacting dogs (in this material 3 out of the 12 dogs) can be controlled by frequent clinical measurements.

It should be noted that after 3 months the lesions were confined to the soft tissues. Infrabony defects were no longer present. This phenomenon limits the application of this dog model to investigations related to suprabony disease processes.

The defects obtained after healing showed the histological appearance of a periodontal lesion after initial periodontal therapy (scaling, subgingival cleaning). This might seem a restriction, but it could also be regarded as a positive starting point for testing surgical treatment procedures, because these procedures are usually performed after initial therapy.

The morphology of the canine dentition, in particular the absence of contact points, is markedly different from the human dentition. Treatment modalities, which have been comprehensively described for the human dentition, may therefore not be applicable to the dog. Furthermore, artificial defects, 3.0 to 4.0 millimeters in depth, though relatively deep for the dog, are much shallower than pockets over 6 millimeters in depth, which are usually the object of surgical treatment in humans. Because of this, *quantitative* results cannot be directly extrapolated to the human situation. It is beyond doubt, however, that the dog model provides an excellent opportunity for studying qualitative *biological processes* initiated by treatment

procedures.

It would be interesting to know when and under what conditions the defects, considered stable after initial healing, change into progressive lesions similar to naturally occurring periodontitis. Future research on this model should be directed towards investigating methods of accelerating and monitoring this process.

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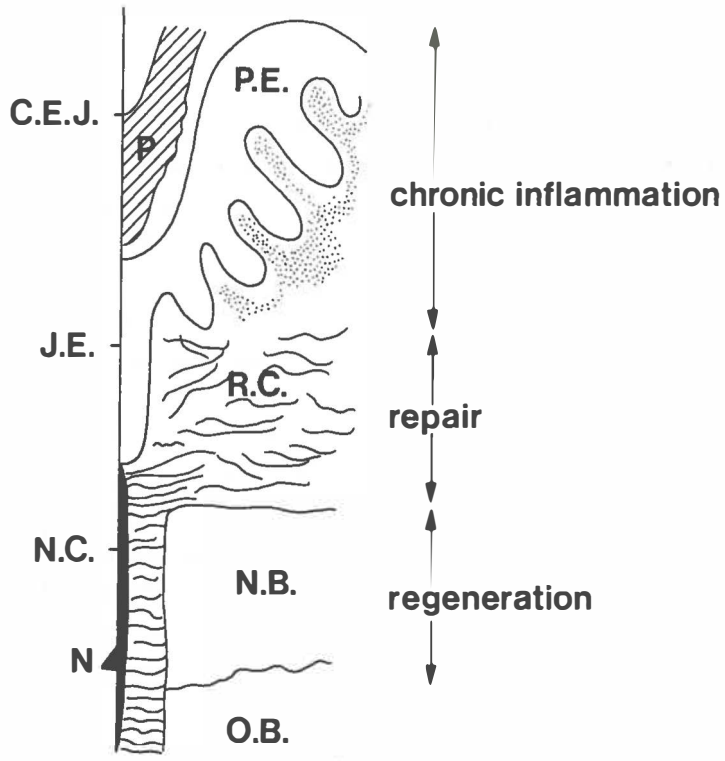

Fig. 1. The established periodontal defect.
- an apical area in which the lost periodontal tissues have *regenerated* through the deposition of new cementum, new alveolar bone and a periodontal ligament inbetween;
- a narrow zone between the coronal extension of the new alveolar bone and the apical termination of the junctional epithelium, where *repair* in the form of new cementum deposition and connective tissue fibre attachment has taken place;
- a wide zone between the apical termination of the junctional epithelium and the transition from junctional epithelium to pocket epithelium in which the former periodontal tissues are *repaired* by a long epithelial attachment to the root surface with well-aligned connective tissue bundles adjacent;
- a coronal zone between the transition of junctional epithelium and the gingival margin in which plaque is present on the root surface and the adjacent marginal gingiva is chronically inflamed.

C.E.J. = cemento-enamel junction; J.E. = junctional epithelium; N = notch; N.C. = new cementum; N.B. = new bone; O.B. = old bone; P = plaque; P.E. = pocket epithelium; R.C. = reorganised connective tissue.

Chapter 9

SUMMARY

Artificial periodontal defects in experimental animals have been widely used in the past to study the effects of periodontal treatment procedures.

Since the ultimate goal of animal experiments is the extrapolation of the results to the human situation, the artificially created periodontal defects should resemble the naturally occurring periodontal lesions found in man, as closely as possible. The features of naturally occurring periodontitis are described in chapter 1. A very important requirement is that the artificial defect, once established, should not heal spontaneously since this is not found in man. Furthermore spontaneous healing would obviously interfere with results obtained from experimental healing procedures. It was concluded from a review of the literature (chapter 1) that the creation of deep non-reversible artificial periodontal defects, especially in dogs is not without its problems. The process of artificial periodontal breakdown in dogs is frequently accompanied by gingival recession resulting in extremely shallow pockets, which are, for obvious reasons, rarely the object of periodontal treatment procedures. On the other hand, when deep periodontal defects are created signs of reversibility have been reported.

Preliminary clinical and histological observations on ligature or elastic band-induced periodontal defects in dogs indicated that spontaneous partial reversibility indeed occurred after ligature or elastic band removal. Probing depths were found to have decreased and there was evidence of new cementum and new bone formation. The exact nature of these processes could not however be determined from these observations.

A series of studies was therefore designed to answer the following questions:

- do the clinical and histological changes following the creation of artificial periodontal defects reflect spontaneous healing and if so of what type (new attachment, repair, regeneration),?
- what are the consequences of these phenomena on the application of the dog model in experiments aimed at evaluating the effect of treatment procedures ?

The experiments were performed on 12 beagle dogs. Two different methods of creating artificial defects were evaluated in different areas of the mouth.

- Around the upper and lower premolars (^{1}P, ^{2}P, ^{3}P, $_{2}P_{2}$, $_{3}P_{3}$, $_{4}P_{4}$) the dento-gingival fibres were dissected and closely adapted copper bands were then

cemented to the full depth of the defects. After 3 weeks the copper bands were replaced by cotton floss ligatures, which were forced down as deeply as possible. These subgingival ligatures were left in place for a further 11 weeks.

- Around 4 contralateral upper incisors ($^{2}I^{2}$, $^{3}I^{3}$) the dentogingival fibres were dissected and orthodontic elastic bands placed into these deepened sulci under tension. These bands then slipped subgingivally and remained in place for 8 weeks.

The phase in which the periodontal defects were created was termed the *active phase* (14 weeks for the premolar region and 8 weeks for the incisor region). The experimental design allowed for clinical and histological observations before and immediately after the creation of the defects and during the evaluation of the defects up to 31 weeks after ligature and elastic band removal *(evaluation phase)*.

Results of the histological evaluation of probe penetration during the clinical assessment of periodontal attachment levels in these artificial periodontal defects are presented in chapter 2. Gutta-percha points similar in shape to thin periodontal probes, were gently inserted into the defects around the upper premolar teeth, both immediately after the termination of the active phase and during the evaluation phase. Histological specimens with these "probes" in situ were histometrically analysed for the distance from the probe tip to the apical termination of the junctional epithelium (attachment level). It was found that at the end of the active phase the tips of the "probes" passed the apical termination of junctional epithelium by approximately 0.5 millimeter. A more accurate assessment of the apical termination of the junctional epithelium was found during probing in the evaluation phase (3-31 weeks after ligature removal). It was concluded that the extent of probe penetration in artificially induced periodontal defects is dependent upon the degree of inflammation of the periodontal tissues.

Results of probing depth measurements in the artificial periodontal defects in the lower premolar area are presented in chapter 3. Mean values for probing depths at the end of the active phase were approximately 4 millimeters. At the end of the evaluation phase these values had decreased and varied from 3.0-3.5 millimeters. Attention was also focussed on the aspect of the predictability and reproducibility of this technique for creating defects. The magnitude of the standard deviations of the mean probing depths and the differences between probing depths in contralateral areas were used as indicators of predictability and reproducibility. The results revealed no significant differences between the mean values for probing depth in contralateral areas at the end of the active phase, in spite of occasional large

variations between dogs and between the left and right sides of the jaws in individual dogs. During the evaluation phase standard deviations of mean values became much smaller. It was concluded that artificial periodontal defects with probing depths of 3.0-3.5 millimeters can be created predictably provided that the defects are allowed to heal for at least 3 months.

The histometrical data of defects in the lower premolar region, analysed for reversibility of the apical positioning of the junctional epithelium and of alveolar bone loss during the evaluation phase, are presented in chapter 4. The results of defects in the right sides of the jaws at 3, 7, 15 or 31 weeks after ligature removal were compared to contralateral defects in the same dogs examined 1 week after ligature removal. It was found that, in contrast to alveolar bone, the apical positioning of the junctional epithelium was not reversible after ligature removal. The bone level was related to both the cemento-enamel junction and to a reference point (notch) in the root surface. In the defects examined 15 and 31 weeks after ligature removal the mean values for bone loss were smaller than those found in the contralateral defects 1 week after ligature removal. Indeed in the 1 and 3 week specimens the bone was situated apical to the notch whilst in the 7, 15 and 31 week specimens it was situated coronal to the notch, suggesting that the alveolar bone height tended to increase with time.

Results of the clinical and histometrical analysis of the elastic band-induced periodontal defects around the upper incisor teeth are presented in chapter 5. Immediately after elastic band removal the mean values for probing depth were approximately 5.5 millimeters. Histometrical assessments revealed a loss of attachment of approximately 3.0 millimeters. The differences in probing depth between contralateral defects *in the same dog* were small indicating a high level of reproducibility. However, occasional wide variations in probing depth and histometrical dimensions *between* dogs were found. Values for probing depths decreased by approximately 1.5 millimeters after elastic band removal, leaving moderately deep defects of approximately 4 millimeters. Histometrically there was no evidence that the apical positioning of the junctional epithelium was reversible after elastic band removal. Alveolar bone repair could however be demonstrated in the 7, 15 and 31 week specimens.

The histopathological changes which occurred during the evaluation phase in the ligature-induced periodontal defects in the lower premolar region are described in

chapter 6. At the end of the active phase (defects with ligature in situ for 11 weeks) the defects showed the features of slowly progressing chronic periodontitis. The ligatures were surrounded by plaque and separated from the gingival connective tissue by inflamed pocket epithelium. The infiltrated connective tissue, which consisted mainly of plasma cells and lymfocytes, extended from the gingival margin to close to the alveolar bone surface but was separated from the latter by a zone of relatively intact connective tissue. Sparse osteoclasts were present on its surface. A notch was prepared in the root surface upon ligature removal just coronal to the level of alveolar bone. This procedure resulted in tissue destruction and a polymorphonuclear leucocyte dominated inflammatory response.

Within 3-7 weeks after ligature removal distinct signs of healing became apparent in the periodontal tissues: a decrease in size of the infiltrated connective tissue, the formation of a long junctional epithelium on the previously exposed root surface, deposition of new cementum on old (but not previously exposed) cementum and alveolar bone repair. Maturation of these healing tissues and further bone repair was observed in the specimens obtained 15 and 31 weeks after ligature removal. It was suggested that this healing was the result of the coincidental removal of the subgingival plaque, when the ligatures were removed, and in part as a result of the notching procedure. It was therefore concluded that the maintenance of the established lesion is dependent on the persistance of subgingival plaque-containing ligatures.

The histopathological characteristics of the elastic band induced periodontal defects in the upper incisor region, both before and after band removal, are described in chapter 7. Generally the changes which occurred during the evaluation phase were similar to those described in the previous chapter for ligature-induced defects. However, in contrast to these, a very destructive process of periodontal breakdown (periodontal abcess formation) was consistantly observed at the mesial aspect of the third incisors. This tissue destruction also affected the root surfaces and resulted in extensive areas of root resorption. In the specimens obtained in the second half of the evaluation phase these areas of root resorption had been repaired by new cementum deposition and the formation of a well-structured non-inflamed connective tissue. It was suggested that the periodontal abcesses were the result of placing 2 elastic bands in the narrow interdental area between adjacent incisors.

The histological and clinical changes observed during the evaluation phase are discussed in chapter 8. The implications of these changes for the use of this type of

defect were considered and the features of the artificial periodontal defects found at the end of the evaluation phase were summarised. Finally considerations on the application of the present model for future experiments were given.

SAMENVATTING

Kunstmatige parodontale defecten bij proefdieren worden veelvuldig gebruikt om de uitwerking van behandelmethoden in de parodontologie te bestuderen.

Het uiteindelijk doel van dier-experimenteel onderzoek is het extrapoleren naar de situatie bij de mens. Kunstmatig gecreëerde parodontale defecten moeten daarom zoveel mogelijk gelijkenis vertonen met de parodontale afwijking zoals die veelvuldig bij mensen aangetroffen wordt. De verschijnselen bij het ontstaan en voortschrijden van parodontale afwijkingen worden beschreven in hoofdstuk 1. Een zeer belangrijke eis is dat een eenmaal tot stand gebracht kunstmatig defect niet uit zichzelf geneest omdat dit bij de mens ook niet het geval is. Bovendien zal een genezing van invloed zijn op de resultaten bij experimenten waarin genezing bestudeerd wordt. Uit het literatuuroverzicht in hoofdstuk 1 wordt geconcludeerd dat het creëren van diepe, irreversibele parodontale defecten, met name bij honden niet zonder problemen verloopt omdat tijdens het proces van parodontale afbraak veelvuldig een recessie van de gingiva optreedt. Het resultaat is zeer ondiepe pockets, die om voor de hand liggende redenen zelden het doel zijn van parodontale behandeling. Voorts wordt, wanneer men er wèl in slaagt diepe parodontale defecten te verkrijgen, in de literatuur melding gemaakt van reversibiliteit.

Ook uit eigen ervaringen met parodontale defecten bij honden, gecreëerd met behulp van ligaturen of elastieken, bleek dat er spontaan een gedeeltelijk herstel optrad na het verwijderen van de ligaturen of elastieken. De gemeten pocketdiepte nam af en er werd nieuw cement en nieuw bot waargenomen. Deze waarnemingen konden echter niet adequaat worden geïnterpreteerd.

Derhalve is een serie onderzoekingen gestart om antwoord te kunnen geven op de volgende vragen:

- Treedt er na het creëren van kunstmatige parodontale defecten spontane genezing op, klinisch en/of histologisch? Als dit zo is, wat voor type genezing is het (nieuwe vezelige aanhechting, reparatief proces, regeneratie) ?
- Wat zijn de consequenties voor het toepassen van de hond als dier-experimenteel model waar het gaat om het evalueren van behandelingsprocedures?

De experimenten werden uitgevoerd op 12 beagle honden. Twee verschillende methoden om parodontale defecten te creëren werden geëvalueerd in verschillende orale gebieden van de hond.

- De dento-gingivale vezels rond de premolaren in boven- en onderkaak (^{1}P, ^{2}P, ^{3}P, $_{2}P_{2}$, $_{3}P_{3}$, $_{4}P_{4}$) werden doorgesneden en nauwkeurig passende koperbanden werden met behulp van cement vastgezet tot op de bodem van het defect. Na 3 weken werden de koperbanden vervangen door katoenligaturen, die zo ver mogelijk naar de diepte geduwd werden. Deze subgingivale ligaturen werden 11 weken op hun plaats gelaten.
- De dento-gingivale vezels rond 4 bovenincisieven ($^{2}I^{2}$, $^{3}I^{3}$) werden doorgesneden en orthodontische elastieken werden in de verdiepte sulcus geduwd. De aldus subgingivaal gesitueerde elastieken bleven ter plaatse gedurende 8 weken.

De fase waarin de parodontale defecten werden gecreëerd, werd de *actieve fase* genoemd (14 weken voor de premolaarstreek en 8 weken voor de incisiefstreek). Het onderzoeksprotocol voorzag in klinische en histologische waarnemingen vóór en onmiddellijk na het creëren van de defecten en gedurende een periode tot 31 weken na het verwijderen van de ligaturen of elastieken *(evaluatie fase)*.

Resultaten van de histologische beoordeling van de penetratie van de pocketmeter in de weefsels tijdens de pocketdieptemetingen bij deze kunstmatige defecten worden gepresenteerd in hoofdstuk 2. Gutta-percha points, in vorm gelijk aan de gebruikte dunne pocketmeter, werden in de defecten geplaatst rondom de premolaren in de bovenkaak: onmiddellijk na het creëren van de defecten en gedurende de evaluatie fase.

Histologische preparaten met deze "pocketmeters" in situ werden histometrisch geanalyseerd wat betreft de afstand van de punt van de pocketmeter tot aan het apicale einde van het verbindingsepitheel (niveau van aanhechting). Aan het einde van de actieve fase bleken de punten van de "pocketmeters" tot voorbij het apicale einde van het verbindingsepitheel gekomen te zijn en wel ongeveer 0,5 millimeter. Gedurende de evaluatie fase (3-31 weken na verwijderen van het ligatuur) lag de "pocketmeterpunt" echter dicht in de buurt van het apicale einde van het verbindingsepitheel. Conclusie: de mate waarin de pocketmeter doordringt in kunstmatig tot stand gebrachte parodontale defecten is afhankelijk van de mate van ontsteking in het parodontium. Daarmee moet rekening gehouden worden bij de beoordeling van de metingen in het eigenlijke onderzoek.

Resultaten van pocketdieptemetingen bij kunstmatige parodontale defecten in de

premolaarstreek van de onderkaak worden gepresenteerd in hoofdstuk 3. De gemeten pocketdiepte aan het einde van de actieve fase bedroeg gemiddeld ongeveer 4 millimeter. Aan het eind van de evaluatie fase was deze waarde afgenomen en varieerde van 3-3,5 millimeter. Een ander punt van aandacht was de mate van voorspelbaarheid en reproduceerbaarheid van deze manier om defecten te creëren. De grootte van de standaard deviatie van de gemiddelde diepte en de verschillen tussen diepten in contralaterale gebieden van de tandboog werden gebruikt als indicatoren voor voorspelbaarheid en reproduceerbaarheid. De resultaten lieten geen significante verschillen zien tussen de gemiddelde waarden in contralaterale gebieden van de tandboog aan het eind van de actieve fase, ondanks de nu en dan grote variaties tussen honden onderling en tussen de linker en rechter-zijde van de kaak in één afzonderlijke hond. Gedurende de evaluatie fase werden de standaard deviaties van de gemiddelden snel kleiner. Hieruit werd geconcludeerd dat kunstmatige parodontale defecten met pocketdiepten van 3-3,5 millimeter voorspelbaar gecreëerd kunnen worden, vooropgesteld dat een genezingsperiode van 3 maanden in acht wordt genomen.

De histometrische gegevens van de defecten in de premolaarstreek van de onderkaak worden gepresenteerd in hoofdstuk 4. Dat betrof de analyse of de apicale verplaatsing van het verbindingsepitheel en het verlies van alveolair bot al dan niet reversibel was gedurende de evaluatie fase. De resultaten voor defecten in de rechter kaakhelften op de tijdstippen 3, 7, 15 of 31 weken na het verwijderen van de ligaturen werden hiertoe vergeleken met contralaterale defecten bij dezelfde honden 1 week na verwijderen van de ligaturen. De apicaalwaartse verplaatsing van het verbindingsepitheel bleek niet reversibel na verwijdering van de ligaturen. Het botniveau werd gerelateerd aan zowel de glazuur-cementgrens als aan een referentiepunt (notch) in het wortelоppervlak. Bij de defecten die 15 en 31 weken na verwijderen van de ligaturen onderzocht werden, waren de gemiddelde waarden voor botverlies kleiner dan bij contralaterale defecten 1 week na ligatuur verwijdering. Verder was in de histologische preparaten van de 1e en 3e week waar te nemen dat het bot apicaal van de notch gelegen was, terwijl het botniveau in preparaten van de 7e, 15e en 31e week coronair van de notch lag, de indruk gevend dat de hoogte van het alveolair bot met het verstrijken van de tijd toenam.

Resultaten van de klinische en histometrische analyse van de met behulp van elastieken gecreëerde defecten rond de bovenincisieven worden gepresenteerd in hoofdstuk 5. Onmiddellijk na het verwijderen van de elastieken was de gemiddelde pocket-

diepte ongeveer 5,5 millimeter. Histometrische bepalingen gaven een verlies aan aanhechting te zien van ongeveer 3 millimeter. De verschillen in pocketdiepten tussen contralaterale defecten *bij dezelfde hond* waren klein, hetgeen een grote mate van reproduceerbaarheid betekende. Soms werden echter aanzienlijke variaties in pocketdiepte en histometrische dimensies *tussen honden* aangetroffen, hetgeen aangaf dat de methode hier niet goed voorspelbare resultaten oplevert. De pocketdiepten namen met ongeveer 1,5 millimeter af na het verwijderen van de elastieken, uiteindelijk resulterend in matig diepe defecten van ongeveer 4 millimeter. Histometrisch was niet aan te tonen dat de apicaalwaartse verplaatsing van het verbindingsepitheel reversibel was na verwijdering van de elastieken. Herstel van alveolair bot werd echter wel aangetoond in de preparaten van 7, 15 en 31 weken na verwijdering van de elastieken.

De histopathologische veranderingen die plaats vonden in de met behulp van ligaturen tot stand gebrachte defecten worden beschreven in hoofdstuk 6. Aan het einde van de actieve fase (defecten met ligaturen in situ gedurende 11 weken) vertoonden de defecten de kenmerken van een langzaam voortschrijdende chronische parodontitis. De ligaturen waren omgeven door plaque en gescheiden van het bindweefsel door ontstoken pocketepitheel. Het ontstoken bindweefsel dat voornamelijk plasmacellen en lymfocyten bevatte, reikte van de rand van de gingiva tot het alveolaire bot. Het grensgebied met het bot vertoonde relatief intact bindweefsel. Enkele osteoclasten waren op het botoppervlak aanwezig. Onmiddellijk na het verwijderen van de ligaturen werd een "notch" geprepareerd in het worteloppervlak juist coronair van het alveolaire bot. Deze handelwijze resulteerde in beschadiging van weefsel en een ontstekingsinfiltraat voornamelijk bestaande uit neutrofiele granulocyten. Binnen 3-7 weken na verwijdering van ligaturen waren duidelijke tekenen van genezing waar te nemen in de parodontale weefsels: afname van de hoeveelheid ontstoken bindweefsel, vorming van een verlengde epitheliale aanhechting aan worteloppervlak dat in aanraking was geweest met de mondholte, afzetting van nieuw cement op oud cement (dat overigens niet in aanraking was geweest met de mondholte) en vorming van nieuw alveolair bot. Een verdere genezing van deze weefsels en het alveolaire bot werd histologisch waargenomen 15 en 31 weken na verwijdering van de ligaturen. Deze genezing was wellicht het gevolg van de toevallige eliminatie van subgingivale plaque tengevolge van het verwijderen van de ligaturen en voor een gedeelte het gevolg van het aanbrengen van de "notch". Daarom werd geconcludeerd dat het in stand houden van de chronische laesie afhankelijk is van het aanwezig blijven van de ligaturen die subgingivale plaque ter plaatse houden.

De histologische karakteristieken van parodontale defecten bij bovenincisieven gemaakt met behulp van elastieken zijn beschreven in hoofdstuk 7. Over het algemeen waren de veranderingen tijdens de evaluatie fase gelijk aan die welke in het vorige hoofdstuk beschreven zijn voor defecten met behulp van ligaturen. Opmerkelijk was echter een proces van extreme parodontale afbraak (met abcesvorming) dat steeds aanwezig was mesiaal van de derde incisieven. Deze weefseldestructie tastte ook de worteloppervlakken aan, resulterend in uitgebreide gebieden met wortelresorptie. Uit de histologische preparaten van de tweede helft van de evaluatie fase bleek dat deze gebieden met wortelresorptie hersteld waren door afzetting van nieuw cement en door vorming van een goed georganiseerd gezond bindweefsel. Gesuggereerd werd dat de parodontale abcessen veroorzaakt werden door het aanbrengen van 2 elastieken in de nauwe interdentale ruimte van de dicht aan elkaar grenzende incisieven.

De histologische en klinische veranderingen gedurende de evaluatie fase worden bediscussiëerd in hoofdstuk 8. De consequenties van deze veranderingen voor het gebruik van dit soort defecten worden behandeld en er wordt een overzicht gegeven van de eigenschappen van de kunstmatige parodontale defecten aan het einde van de evaluatie fase. Tenslotte worden overwegingen besproken ten aanzien van de toepassing van het besproken model voor verdere onderzoeken.

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